REVISTA ESPAÑOLA DE Quimioterapia

SPANISH JOURNAL OF CHEMOTHERAPY

ISSN: 0214-3429
Volumen 33
Número 2
Abril 2020
Páginas: 87-175

Publicación Oficial de la Sociedad Española de Quimioterapia

REVISTA ESPAÑOLA DE
Quimioterapia

Revista Española de Quimioterapia tiene un carácter multidisciplinar y está dirigida a todos aquellos profesionales involucrados en la epidemiología, diagnóstico, clínica y tratamiento de las enfermedades infecciosas

Fundada en 1988 por la Sociedad Española de Quimioterapia

Indexada en
Science Citation Index Expanded (SCI),
Index Medicus (MEDLINE),
Excerpta Medica/EMBASE,
Índice Médico Español (IME),
Índice Bibliográfico en Ciencias de la Salud (IBECS)

Secretaría técnica
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Facultad de Medicina
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28040 Madrid
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Disponible en Internet:
www.seq.es

Sociedad Española de Quimioterapia

Publicidad y Suscripciones
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Publicación que cumple los requisitos de soporte válido

ISSN
0214-3429

e-ISSN
1988-9518

Depósito Legal
M-32320-2012

Maquetación
Vic+DreamStudio

Impresión
España

Esta publicación se imprime en papel no ácido.
This publication is printed in acid free paper.

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Quimioterapia

Sumario

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000/ES/19-05//14469

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April 2020

Official journal of the Spanish Society of Chemotherapy

Review

Revista Española de Quimioterapia
doi:10.37201/req/107.2019

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John Donne, Spanish Doctors and the epidemic typhus: fleas or lice?

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Article history
Received: 14 December 2019; Accepted: 16 January 2020; Published: 7 February 2020

ABSTRACT

We describe the infections that appeared in the life and work of John Donne (1572-1631), the English metaphysical poet, mainly the exanthematic typhus that suffered and gave arise to his work *Devotions upon emergent occasions, and several steps in my sickness*. We discuss the vector of transmission of this disease, in comparison of other infections in that period, that Donne´s scholars have related to the flea without mentioning the body louse, the true vector of the exanthematic typhus. Likewise, we mention the exanthematic typhus´s symptoms in his *Devotions* in comparison with the Luis de Toro´s or Alfonso López de Corella´s works, Spanish doctors in those times and the first doctors in write books about the disease, and the singular treatment of pigeon carcasses on the soles of the feet in English Doctors but not in Spanish Doctors.

Key-words: Exanthematic typhus, lice, Devotions, John Donne, Spanish doctors

John Donne, médicos españoles y el tifus epidémico: ¿pulgas o piojos?

RESUMEN

Se describen las infecciones que aparecieron en la vida y la obra de John Donne (1572-1631), el poeta metafísico inglés, principalmente el tifus epidémico que padeció y que dio lugar a su obra "*Devotions upon emergent ocassions, and several steps in my sickness*". Discutimos el vector transmisor de la enfermedad, en comparación de otras infecciones en ese periodo, que los estudiosos de Donne han relacionado a las pulgas y sin mencionar el piojo del cuerpo que es el verdadero vector del tifus epidémico. Además, mencionamos los síntomas de la enfermedad en su obra "*Devotions*" en comparación con los trabajos de Luis de Toro o Alfonso López Corella, médicos españoles de su tiempo y los primeros en escribir los tratados sobre la enfermedad, y el tratamiento singular de las carcasas de palomas en las palmas y plantas de los pies en los médicos ingleses pero no presente en los médicos españoles.

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INTRODUCTION

The first three treaties on epidemic or exanthematic typhus were from the Spanish doctors Alfonso López de Corella (c. 1519-1584), Luis Mercado (c. 1520-1606) and Luis de Toro (c. 1532-1591), printing in Zaragoza, Valladolid and Burgos, respectively. As Arrizabalaga remarks: "*behind the word and the label of epidemic typhus there are several entities with which they were confused*" [1], for example the distinction between typhoid fever and exanthematic typhus occurred already in modern times.

In this review, we describe the infections that appear in the life and work of Donne, fundamentally the exanthematic typhus that suffered and gave arise to his work *Devotions upon emergent occasions, and several steps in my sickness* and we discuss the vector of transmission of this disease that Donne´s scholars have related to the flea without mentioning the body louse and as a source of contagion through the dresses of his time. Likewise, we mention the exanthematic typhus´s symptoms in his *Devotions* in comparison with the Luis de Toro´s or Alfonso López de Corella´s works and the singular treatment of pigeon carcasses on the soles of the feet in English Doctors but not in Spanish Doctors.

John Donne (1572-1631). John Donne has been defined as a metaphysical poet, denomination coined by his enemies [2],

and is known above all for his quotes as *"No man is an island, entire of itself; every man is a piece of the Continent, a part of the main... "* and "*Any therefore never send to know for whom the bell tolls; it tolls for thee".* Contemporary of Shakespeare, who is believed to be also crypto-Catholic, and the Quixote of Cervantes is a writer who was little understandable, close to the baroque poetry of Luis de Góngora, and therefore he was postponed for a long time. Also, he changed from Catholicism to Anglicanism, at a time that being of this religion in England was a very great danger of death, and of writing erotic and love poems to become a cleric and dean of St Paul's Cathedral in London. He has been described as a flatterer and adventurer who participated in his youth in the attack of the Earl of Essex against Cadiz (Spain) in 1596. Also, in some of his works such as the "*Elegy to his mistress going to bed*" censored and, that was not published until 1669, a pornographic Donne and full of eroticism is displayed [2].

In the poem "*Whispers of Immortality*", TS Eliot places Donne in the same category as John Webster (1580-1633), those writers with a depressive disposition. The melancholy was a typical vision of the 17th century as in the book of Robert Burton (1577-1640), *Anatomy of the Melancholy* (1621), and Webster in the Elizabethan Age [3]. There is an obsession in Donne for mortality in all his works, the result of his time: at that time mortality, especially in newborns, was very high (five out of his children died), a certain depression of his personality and his concerns by lack of money, the existence of epidemics such as plague, typhus, smallpox and cholera, the executions and funerals that were public. The culture of melancholy was prevalent in that period [3].

INFECTIONS AND JOHN DONNE

The infections prevalent in Donne's time were typhus, dysentery and smallpox [4], but plague and other infections were present in Donne´s life in addition to the epidemic typhus:

a) Pharyngotonsillitis. Throughout his life, Donne had repeated attacks of intermittent mild fever that have been labeled as probable episodes of pharyngotonsillitis [3].

b) Plague. Donne attributes it to vapors and humidities of the earth and the flea says that "*although it does not kill, it produces all the damage it can*". One of Donne's most popular erotic poems is *The flea*, due to the frequency of fleas in the Renaissance and the appearance of the plague. In this poem, the flea, it is a sexual metaphor. The argument is the refusal of the woman to have sex and how to convince her to make love, if a flea takes blood from the woman and after the man, both bloods are mixed in the flea, why not do it in the same way in the sexual act and its consummation as the image of the flea and the blood?. Also, in the original English, the word *maidenhead* in the poem means hymen so the poem implies that the woman is a virgin. It is believed that he wrote it when he was young and was studying law and that he did it to impress his male classmates. The poem was not published until two years later, in 1633, of Donne's death [5].

c) Greatpox. In his poem *The aparition* says [6]:

And then poor aspen wretch, neglected thou
Bath´d in a cold quicksilver sweat wilt lie
A verier ghost than I ...
(ll. 11–13)

(pobre álamo tembloroso,
yacerás bañada en un frio sudor de mercurio,
más fantasmal que yo)

Mercury (quicksilver) was a treatment for greatpox, or misnamed currently syphilis, here of a woman called "*false vestal*" indicating that she carries a sexually transmitted infection, and ends the poem sinisterly [5]:

since my love is spent,
I'had rather thou should'st painfully repent,
Than by my threat'nings rest still innocent.
(15–17)

(puesto que mi amor ya no existe,
me gustaría que te arrepientas con dolor,
más que a que por miedo seas inocente).

d) Exanthematic typhus. Donne already had had an encounter with the disease 30 years earlier, in 1593, the typhus had killed his brother Henry in prison after having housed a Catholic priest. He refers to this event and exposes it in *Expostulations* XVIII. In the biography that accompanies the edition of *One Hundred Poems* in Spanish (100 poemas en español, Editorial Pretextos), it is said that he died of plague in jail [7], but at that time typhus was more typical in prisons.

EXANTHEMATIC TYPHUS AND DEVOTIONS

a) Chronology. On November 22, 1623, Donne presides over a trial and is his last appearance before becoming ill on November 23-24. Donne suffers from the first symptoms of an infection that most scholars think it was an exanthematic typhus, some scholars think it could be a flu, tuberculosis or recurrent fever and that in any case the nature of their disease would be unproven [8a, 8b]. The most likely diagnosis by the symptoms, in any case, was typhus in an epidemic that devastated London that year and that killed around 8,000 people and that closed the Parliament from September 4 to February 15, 1624 [9]. At that time Donne was dean of St. Paul's Cathedral, he had already written most of his poetry and sermons.

The disease prostrates him about 20 days and writes one of his most famous books: *Devotions upon emergent occasions, and several steps in my sickness*, [10] which is

divided into 23 parts or chapters that are each of them subdivided into the *Meditations*, *Reconventions* and *Prayer* sections, the three emblematic parts of the Holy Spirit, but also the internal structure in 3 parts of each chapter should be read as the morning, late entries and night of a medical history: *Meditations* represent the symptoms collected from each day in a rational and scientific way, at noon (*Reconventions*) the dissatisfaction appears and with the increase of the fever the protests appear with biblical references, so the section *Prayer* is the shorter of the three [11]. It is partly a personal diary, meditation and prayer and is one of the most accurate, and in the first person, examples of the literary description of a disease [12]. It is a metaphorical construction and the analogy of the disease of the body and the disease of the soul [13] with precise observations of his illness, the treatments applied and his recovery. In the 14 days of the disease progress, descriptions of the symptoms that match an exanthematic typhus are made. In addition, his recovery was slow, about 3 months since he does not give another sermon until March 28 on 1624 in St. Paul´s Cathedral [13].

b) Doctors who treated John Donne. Since the reign of Henry VIII, there was an act that said that medicine could not be practiced in London unless the doctor was reviewed and endorsed by the Bishop or Dean of St. Paul's Cathedral, so Donne knew his doctors [14]. Donne's known doctors, and who had a relationship with William Harvey, were Simeon Fox and William Clement [3]. Those who assisted him in his illness were first his friend Simeon Foxes and due to the seriousness of the picture the doctor of King James I, Theodore Turquet de Mayerne. Mayerne introduced the calamine lotion, lotio nigra (lotion used for syphilitic and scrofulous ulcers) into the pharmacopoeia of his time, and an early form of laudanum. He wrote after treating Donne, a treatise in Latin *Ad febram purpurean about typhus* (the typhus was called purple fever) [15]. This supports the fact that Donne's disease was an exanthematic typhus since at that time the typhus was well known to doctors and previously Fracastoro in his book *De Contagione* in 1545 classifies pestilent fevers and gives the first description of typhus.

c) Denomination of the disease. The body louse is known to transmit the trench fever by *Bartonella quintana*, the recurrent fever by *Bartonella recurrentis*, the exanthematic typhus by *Rickettsia prowazekii* and the plague by *Yersinia pestis*. Exanthematic typhus (the etymology means: smoke, fog, senselessness or stupor caused by fever), has been closely linked to epidemics in wars since time immemorial. Typhus, as a clinical concept, appears in the work of Persian doctors, *'Homay-e mohregheh'* (typhic fever) and the clinic is described in the book *al-Hāwi, Qānun fi´l -tebb* (Canon of medicine) by Abu Bakr Mohammad Ibn Zakariyā Rāzi and in the *Dakira-ye kvārazmšāhi* (Treasure of the kvārazmšāh) by Esmāil Jorjāni. The latter one, he describes the symptoms and rash as well as its mortality and recommends washing the patient's body with cold water, calling to the typhus *mohregheh* and to typhoid *motabhbegheh* fever. Several fevers including typhus are also described in the oldest treatise on Persian medicine written about 983: *Hedāyat al-mota´allemin fi´l-tebb* by Akawayni Bokāri [16].

In the 16th century in Spain, the word typhus had several denominations: "*punticular"* or "*lenticular"* fever (derived from lentil due to the size of the spots on the skin), "*pulicularis"* (derived from fleas), "*aphid"*, "*tabardillo"*, "*tabardete"*, "*pintas*" (denominations by the common people) or "*tabardillo pintado (painted)"* described by the doctor Luis del Toro in 1557 [17]. The Spanish doctors who studied the typhus at this time were: Alfonso López de Corella and Luis de Luis Toro, both in 1574, and Luis Mercado, in 1586. Alfonso López de Corella (1513-1584), published an important and original text dedicated to exanthematic typhus, called by the Spanish Renaissance doctors "*morbus lenticularis*", "*tabardillo*", or "*pintas*", for dermatological lesions "*similar to flea bites*". This text appeared under the title of: *De morbo pustulato sive lenticulari, quem nostrates Tabardillo appellant liber unus, atque de Galeni Placitis liber alter, quo omnibus fere medicis qui praedictum auctorem hucusque impugnarunt respontur* ...[18].

The word "*tabardillo*" (1570) derives from the eruption of spots that covers the entire body like a tabard [19] and De Covarrubias in his *Treasury of the Castilian or Spanish language* (1611) [20] says that it is named after the Latin "*Tabes"* that it means rot, because the blood rots or corrupts [19]. An excellent review of the term "*tabardillo*" is collected by Jon Arrizabalaga and it is out of the scope of this review [1].

It is however, Luis de Toro who makes a reliable description of the symptoms considering himself the first and most important doctor of the "*tabardillo"* [17]. De Toro describes the pustules as "*almost never bloom from the beginning (of fever), but on the fourth, fifth, sixth, seventh day, and even later*", also "*the spots that are seen never accuse any detectable tumor to the touch, but they are as if someone stained the meat with ink dots* ". As a measure to avoid it already describes that "*you must run away from the dresses and shirts of the sick*".

Gregorio Marañón (1887-1960) made a fairly precise description of the clinical picture: he observes that the picture begins with 3 days of progressively ascending fever that ends with a sudden crisis, after 2 to 4 weeks of feverish picture. There is an intense headache and conjunctival injection. The rash appears from the third to the sixth day with a rash (skin rash), first congestive and then petechial (live red spot, similar to the flea bite, which does not disappear at the pressure of the finger), and even hemorrhagic. Marañón says that there is the tremor in the hands and the frequent "*typhoid state*" of the patients. Complications include myocarditis, neurological manifestations, and involvement of the parotid glands. The duration of the disease is 14 to 16 days. Towards the 10th day is the crucial moment of the disease, either the patient worsens with presentation of coma and consecutive death, or begins to improve in a definitive way [21].

The typhoid state also was known as the "*new fever*", "*Irish fever*" or "*flea bite fever*", the name of exanthematic typhus was given by the French doctor Boissier De Sauvages in 1760. The rash of typhus was differentiated of the typhoid

by Huxham in 1739 and subsequently Gerhard, histologically, based on the absence of ulcers in Peyer's corpuscles during the 1836 Philadelphia epidemic.

SOURCE AND TRANSMISSION OF DONNE'S TYPHUS: FLEAS OR LICE?

The exanthematic typhus is produced by the bacterium *Rickettsia prowazekii* and transmitted by the body louse (*Pediculus humanus var. corporis*), in exceptional cases there have been cases transmitted by the head louse (*Pediculus humanus var. capitis*). In his book *Devotions*, Donne talks about the flea as the cause of his illness. Although typhus transmission by fleas is possible by aerosols, this pathway is very rare and exceptional since the body louse is the main vector involved and it is suspected, although is controversial, that in some cases also ticks. At that time, the fleas were causing the bubonic plague produced by *Yersinia pestis* and was associated with the transmission of typhus, curiously today it is known that the plague can also be transmitted by the body louse [22]. Spanish Renaissance doctors, as we had indicated above, comment that dermatological lesions are "*similar to flea bites*", hence the confusion with the typhus vector. In a molecular study, three scenarios of plague transmission in Europe have been established between the 14th and 19th centuries: the first would be the classical dissemination of rodents and fleas, the second by humans who spread pneumonic plague through coughs and the third that has been seen to be the most likely is that lice and fleas spread the plague and did not depend so much on the increase in rats [23]. In the time of Donne, fleas could easily coexist with the patients (in an average of 6 fleas) with the body louse that remain infectious for a period of 3 days [23].

On the contrary, the possibility that fleas transmit exanthematic typhus is unlikely, although fleas can transmit other *Rickettsias* (murine typhus for example), and is recognized as a transmission mechanism only to the body louse when it is epidemic and in cases of recrudescence (Brill-Zinser disease) is transmitted by humans. Only in the eastern United States has been seen transmission, by lice and fleas, between flying squirrels (*Glaucomys volans*) although this mechanism is not clear [22].

None of the scholars on Donne, and included in the bibliography of this work, although they mention the possible infections that could be the cause of Donne's clinical picture described in his *Devotions*, refers to the possibility of transmission by the body louse and is assumed that the transmission is by fleas, the prevailing idea is that period, something that is, as we indicate, quite unlikely. In one study, the author indicates that typhus was often confused with the plague and that "*in fact, there is a strong analogy between the two diseases, both derived from a similar source, mainly rats and infestation with fleas...*" [11]. The presence of two coincident and overlapping epidemics occurred in Andalucia between 1569 and 1570: in Seville and Puerto de Santa María, people died of "*landres*" or plague and in areas such as Bazan of "*modorra*" or typhus [24]. In the same way, both overlapping diseases could be coexisting in the Donne´s period.

As we indicated, the epidemiology the source and the vector, are different in the two diseases although they are associated with common hygienic and sanitary conditions. Typhus manifests itself in a cold season, Donne had the disease in November, the time of more cases due to wear more clothes. Therefore, the source of contagion was in the case of typhus the dresses worn by people with body lice in addition to fleas. Samuel Pepys (1633–1703) in his diaries [25] says that he did not wash more than exceptionally, but in February 1664 his wife did it and discovered the pleasures of the bathroom and did not allow him to enter on the bed until he did the same, which took 3 days to complete. He washed his feet every few weeks or when he was going to enjoy a night of sexual rejoicing, curiously his wife died of exanthematic typhus. Hygiene was poor at that time and caused diseases such as typhus. It is a Bohemian doctor, Tobias Coberus, who makes the first description (1606) in which he relates the abundance of lice with the disease in his treatise *Observations castrensium et ungaricarum* [26].

Donne only mentions lice in the *Sermons* [27]:

God punished the Egyptian with little things: with hailstones, and frogs, and grasshoppers; and Pharaoh´s conjurers, that counterfeited all Moses´ greater works, failed in the least, in the making of lice.

Alfonso López Corella, in his work [18], tells that it is due to the bite of some insects (he talks in general of insects not just fleas) and called the disease "*tabardillo*", "*aphid*", "*tabardete*", "*punticular*" and "*tuberquillo*".

TYPHOID CLINIC AND TREATMENTS IN DEVOTIONS

Donne´s symptoms appear as indicated on November 23-24, 1623 (Table 1), the disease appears as a sudden fever with frontal headache [28]. A symptom of typhus was a persistent and high annoying tinnitus with vertigo and a feeling of weight or head load.

Luis de Toro [17] tells that it starts with great laxity of the whole body and then there is heaviness and pain between the scapulae. The face becomes very hot, the eyes are injected with blood and tear incessantly. There is a vehement headache; the pulse becomes large, as in pleurisy; they feel a serious weight in the lumbar region; they sleep little and uneasily: most of the time they have sleeplessness and delirium; the urine is very ingrown and murky ... inextinguishable thirst, anxiety, nausea, vomiting, roughness and blackness of the tongue. If the dominant mood was melancholy, the patients had, in addition to what was indicated, sad delusions, the dream was very disturbed, fear, sadness and fainting ... It produces atrocious delusions, in other fatiguing vigils; some leave them deaf; to other dumb, some comatose and stunned or convulsed and shaky.

Although Donne's red urine (*Meditation I*) may have been a hematuria due to the disease, he may be referring to

Table 1 **Clinical course of epidemic typhus and in Devotions and Luis de Toro´s description**

Disease course	John Donne´s Devotions	Luis del Toro´s descriptions
Incubation period: 10-14 days (1 week)		
1st day	*Devotions* I, II y III:	
Chills and fever	Yes (Fever 40ºC at 3rd day)	Yes
Fever		
Headache	Yes	Yes
Myalgia		Yes
Flashing lights (phosphenes)	Yes	Yes (eyes are injected with blood and tear incessantly)
Face flushing		Yes
Hematuria	Yes	Yes (urine is red and cloudy)
Loss of appetite		
Sleeplessness	Yes (*Meditation* XV)	Yes
4th-7th (5th) Day		
Rash: axillary and upper trunk non- confluent centrifuge, pink that does not disapperar under pressure In days: maculopapular, dark, petechiae and confluent and invades the whole body except face, palms, and soles	November 26-27 (*Meditations* XIII)	Yes
Deafness and tinnitus	Yes	Yes
Nonproductive cough		
Radiology pulmonary inflitrates		
Meningism		
Confusion		
Delirium	Yes	Yes
Comma		Yes
2 weeks (12th-14th days)		
Fever ends in lysis	December 6 (*Devotions* XIX and *Meditation* XX)	
Recover mental disturbance		
Typhus mortality:		
<20 years: <5%		
40 years: 10-15%		
50 years: 50%		
>60 years in general fatal		
2-3 months	*Meditations* XXI	
Convalescence	*Devotions* XXI (cups and bleeding treatment)	

the first of the 10 plagues in Egypt and speak metaphorically. And hydrops of the heavens in *Meditation X* can refer to the retention of urine in the typhoid or a metaphor. In *Meditation III* that speaks through stones, it can refer to talking with pustules in the mouth and pharynx that occur as clinical manifestations in typhus or refer to Demosthenes speaking with stones to practice. The suffocation, in *Meditation XII,* may be the pulmonary phlegm and cough that accompanies typhus. As Frost says [15], symptoms are screened with symbols and rhetoric remedies.

In delirium, the patient speaks loudly incessantly, singing, making noises night and day. Dr. John Amstrong, in the early nineteenth century, suffered a typhus attack and says that during the illness he wanted to collect all the neurological symptoms that appeared [29]. Also it is known that a rare complication of typhoid fever, caused by *Salmonella* spp., are psychiatric manifestations.

The treatment followed the principles of Galenic and Paracelsian medicine, was symptomatic and supportive, maintaining nutrition and fluid balance, preventing heart failure, mitigating fever and the consequences of delirium [8]. The treatment would be 5 indications [18]: the regime, the cooking of the moods, the evacuation, the revulsion and the repair of forces. Patients who took more food healed better and convalesced sooner than those undergoing a debilitating diet. Of the purgatives, he prefers drastic ones to the simple and soft ones, for example the cooking of "*albérchigo*" leaves. Bleeding only in case of need and not always in all cases and should be proportionate to the disease and the forces of the sick.

The strangest treatment was the application of pigeon carcasses on the soles of the feet, this practice is cited by several English writers: John Webster in his work *The Duchess of Amalfi* (Act III, scene 1, 11.45-50) [30]:

Bosola: I would sooner eat a dead pigeon taken from the soles of the feet of one sick of the plague than kiss one of you fasting.

This work was printed in 1623, the same year of Donne's disease but which was already written in 1614 and in *The Diary of Samuel Pepys*. Thomas Lodge also cites the pigeon carcasses in his "*Plague Treaty*" of 1603 and is used by Shakespeare's own son-in-law, doctor John Hall when he had a fever in 1632 [15].

Luis de Toro or Luis Mercado, who used or wrote in his book the most animals and his products in his time, does not mention the use of pigeons in the same manner as in England´s doctors [31]. Finally, Corella quotes instead an oil from Florence: "*Now there is also a certain secret oil, brought from Florence, with which they say they should anoint the palms and soles of the feet, and also the wrists and the region that is next to the heart, I certainly believe that this is the oil that Mathiolo from Siena describes against poisons, but, in truth, it is too warm, so if it is a burning fever, it should be tempered with some cold medicine. But in the absence of this oil, scorpion oil in which blessed thistle, decree and scorzone would have been useful. Well, yes, to attract the humors from the inside outwards in the burning fever, Aecio praise the oil or water, in which nitro had been poured, it should not be denied that to seek a similar action the predicted oil must be useful. Add the fact that, by a certain antipathy, he opposes poison. " (" Ahora también se tiene por gran secreto cierto óleo, traído desde Florencia, con el que dicen se deben ungir las palmas y las plantas de los pies, y también las muñecas y la región que está junto al corazón. Ciertamente creo que se trata del oleo que contra los venenos describe Mathiolo de Siena; pero, en verdad, éste es demasiado cálido, por lo cual, si se trata de una fiebre ardiente, se debería atemperar con algún medicamento frío. Pero a falta de este aceite, será útil el aceite de escorpiones en el que se hubiese echado cardo bendito, dictamo y escorzonera. Pues si, para atraer los humores desde el interior hacia el exterior en la fiebre ardiente, Aecio alaba el aceite o el agua, en la que se hubiese vertido nitro, no se debe negar que para procurar una acción similar ha de ser útil el oleo predicho. Añade el hecho de que, por cierta antipatía, se opone al veneno.")* [18].

FUNDING

None to declare

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/085.2019

Should we leave the paper currency? A microbiological examination

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Article history
Received: 23 October 2019; Revision Requested: 4 December 2019; Revision Received: 6 December 2019; Accepted: 7 January 2020; Published: 17 February 2020

ABSTRACT

Objetives. Pathogens can be transmitted to banknotes due to the personal unhygienic habits. The aim of study was to find the possible pathogens on the banknotes circulating in the market and also to present their antibacterial resistance and their various virulence factors using genotypic and phenotypic methods.

Material and methods. A total of 150 samples of banknotes were randomly collected between August 2017 and March 2018. VITEK systems were used for identification and antimicrobial susceptibility testing respectively. Antimicrobial resistance genes (*mecA*, *van*, extended-spectrum β-lactamase [ESBL] and carbapenemases) and staphyloccoccal virulence genes (staphyloccoccal enterotoxins [SEs], *pvl*, and *tsst-1*) were determined using with real-time PCR.

Results. *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Enterococcus* spp., Gram-negative enteric bacteria, non-fermentative Gram-negative bacteria and *Candida* spp. were detected 48%, 54.7%, 56%, 21.3%, 18.7%, and 4%, respectively. Methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and ESBL producing Gram-negative were found 46.8%, 1.3%, and 28.7%, respectively. *Pvl*, *tsst-1*, and SEs genes were found in a 2.8/4.9%, 1.4/1.2%, and 100/87.8% of the *S. aureus*/CoNS strains, respectively. The *sea* gene was found the most common enterotoxigenic gene. bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}1}$, bla_{KPC}, and $bla_{OXA\text{-}48}$ were found 55.8%, 46.5%, 41.2%, 18.6%, 18.6%, and 18.6%, respectively in Gram-negative strains.

Conclusion. These results is very important to highlight hygienic status of paper currencies. This can be considered as an indication that banknotes may contribute to the spread of pathogens and antimicrobial resistance. Therefore, we may need to start using alternative products instead of banknotes.

Key-words: Paper currency; Bacterial contamination; Antimicrobial resistance genes; Staphyloccoccal enterotoxins

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¿Deberíamos dejar de usar los billetes? Análisis microbiológico

RESUMEN

Objetivo. Los patógenos se pueden transmitir a los billetes debido a los hábitos antihigiénicos personales. El objetivo del estudio fue buscar los posibles patógenos en los billetes que circulan en el mercado y también observar su resistencia antibacteriana así como sus diversos factores de virulencia utilizando métodos genotípicos y fenotípicos.

Material y métodos. Se recogieron al azar un total de 150 muestras de billetes entre agosto de 2017 y marzo de 2018. Se utilizaron los sistemas VITEK para la identificación y las pruebas de sensibilidad a los antimicrobianos, respectivamente. Los genes de resistencia a los antimicrobianos (*mecA, van*, betalactamasas de espectro ampliado [BLEA] y carbapenemasas) y los genes de virulencia estafilocócica (SE, pvl y tsst -1) se determinaron mediante PCR a tiempo real.

Resultados. Se detectó la presencia de cepas de *Staphylococcus aureus*, *Staphylococcus* coagulasa negativos (SCN), *Enterococcus* spp, bacterias gramnegativas, bacterias gramnegativas no fermentativas y *Candida* spp en un 48%, 54,7%, 56%, 21,3%, 18,7% y 4% de los billetes, respectivamente. Se observó la presencia de *S. aureus* resistente a meticilina, *Enterococcus* resistentes a vancomicina y gramnegativos productores de BLEA en un 46,8%, 1,3% y 28,7%, respectivamente. Los genes Pvl, tsst-1 y SE se encontraron en un 2,8/4,9%; 1,4/1,2% y 100/87,8% de las cepas de *S. aureus*/SCN, respectivamente. El gen *sea* fue el gen enterotoxigénico más frecuente. Los genes

bla$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-1}$, *bla*$_{KPC}$, y *bla*$_{OXA-48}$ se encontraron 55,8%, 46,5%, 41,2%, 18,6%, 18,6%, y 18,6%, respectivamente en cepas gramnegativas.

Conclusión. Estos resultados son muy importantes para resaltar el estado higiénico de los billetes. De este modo, los billetes pueden contribuir a la propagación de patógenos y de la resistencia a los antimicrobianos. Por lo tanto, es posible que debamos comenzar a utilizar productos alternativos a los billetes.

Palabras clave: Papel moneda; Contaminación bacteriana; Genes de resistencia, Antimicrobianos; Enterotoxinas estafilocócicas

INTRODUCTION

The hygienic status of banknotes has been a topic of speculation since the late 1800s [1]. *In vitro* culture studies have established that microbial contamination of paper currency is widespread, and that money represents an important human-microbe interface. Microbial contamination of paper money can ocur by money counting machines, atmosphere, dust, soil, storage process, during usage or production process [2]. Contamination during use is most often caused by handwashing after the toilet or false hand washing, by saliva counting, coughing and sneezing in hands. As a result, paper money is contaminated with microorganisms from the human hand, mouth and even in the gastrointestinal tract microbiota. As a result of the exchange of these contaminated banknotes among people, microorganisms begin to spread, contributing to the spread of both antibiotic resistance and many virulence factors and they pose a risk to public health [2, 3]. Researches show that the most common microorganisms carried with paper money were enteric bacteria such as *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter* spp. and other non-fermentative Gram-negative bacilli, *Staphylococcus aureus* and other various Gram-positive cocci and various types of fungus such as *Candida* spp., *Aspergillus* spp., *Penicillium* spp. [2]. Humans are the most important source of *Staphylococcus* spp, especially *S. aureus and S. epidermidis* but also *S. hominis, S. haemolyticus, S. saprophyticus, S. capitis, S. warneri, S. simulans* and *S. cohnii*. The pathogenic capacity of these *Staphylococcus* spp. that can be easily transmitted to paper money is attributed to a combination of invasive properties, production of extracellular factors (like toxins) and antibiotic resistance. Staphylococcal toxins with superantigens characteristic include Panton-Valentin Leucocidin (PVL), toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETA to ETD) and staphylococcal enterotoxins (SEs) [4]. Staphylococcal food poisoning (SFP) is caused by the ingestion of food containing SEs produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly *S. aureus*, although other CPS strains, such as *S. hyicus*, may also be enterotoxigenic [4, 5]. Recently, the enterotoxigenic potential of coagulase-negative staphylococci (CoNS) species in food poisoning has also been recognized [5-8]. There are various publications which investigated the microorganisms carried by currency banknotes [1-3, 9, 10]. However, there is limited number of studies on the dissemination of antibiotic resistance by paper money in the literature. At the same time, it has been determined that there are no studies investigating the species of staphylococci that can be carried by paper money and investigating the important virulence factors of staphylococci such as PVL, TSST-1 and SEs.

This study was planned in order to determine the microorganisms that can be transported with Turkish currency banknotes in Istanbul and to determine their role in the spread of antibiotic resistance and the potential effects of money on the spread of toxin genes by investigating the toxin genes of staphylococci.

MATERIAL AND METHODS

Bacterial isolates. A total of 150 samples of Turkish banknotes involving six denominations (5, 10, 20, 50, 100 and 200), 25 samples each, were randomly collected from hospital cafeteria, canteen of medical faculty, supermarkets near the hospital and restaurants, banks, buyers in open-air markets, and filling-stations in Istanbul, the most populated in Turkey from August 2017 to August 2018. The banknotes were obtained by using aseptic sampling method and banknotes were placed in a sterile polyethylene bag. The bag was sealed and the individual was given a replacement banknote, then all the collected samples were taken to the medical microbiology laboratory at the Medical School in Istanbul. Each banknote was placed in 10-mL of thioglicolat broth and shaken for 5–10 min on and subsequently incubated at 35-37°C for 48 hours. For isolation of bacteria, a sterile, cotton-tipped swab was introduced in the incubated thioglicolat broth and was then inoculated onto blood agar plates, Chromagar methicillin-resistant *S. aureus* (MRSA) and MacConkey agar plates and incubated at 35-37°C for 48 hours. For routine identification procedures automatized systems VITEK MS (BioMerieux, France) was used [4, 11]. For identification of fungi, a loopful of incubated nutrient broth was inoculated onto Sabouraud dextrose agar plates and incubated at 22-25°C for 48–72 hours. Identification of fungal isolates was based on growth characteristics and the lacto-phenol cotton blue reaction [4]. The isolates were stored separately in tryptic soy broth medium with 15% glycerol at –80°C for further phenotypic and genotypic analysis.

Phenotypic antibiotic susceptibility patterns of the ısolates. Phenotypic antimicrobial susceptibility testing was performed by VITEK 2 Compact (BioMerieux, France), and interpretation was done according to EUCAST-2016 guidelines [11]. MRSA isolates were defined as MRSA using a cefoxitin 30-µg disk screening test and PCR (for *mecA* gene). *S. aureus* ATCC 25923 was used as quality control [11].

Suspected isolates of *Enterococcus* spp. were screened for vancomycin resistance. The concentration of vancomycin in vancomycin screening agar was 6 mg/L. A swab which was dipped in a suspension of the isolate and then was deposited as a spot on the agar surface and it was incubated for 24 hours at 35°C. Any growth after 24 hours was interpreted as vancomy-

cin resistance [4,11]. For quality control, was used *Enterococcus faecalis* ATCC 29212 as a susceptible control and *Enterococcus faecium* ATCC 51299 as a resistant control.

Isolates of Gram-negative bacilli were inoculated on MH-agar plates. Discs containing respectively ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg) and aztreonam (30 µg) disks were placed 20 mm (center to center) away from a disc containing a 20 µg amoxicillin/10 µg clavulanic acid disk before overnight incubation at 37°C. Extended-spectrum β-lactamase (ESBL) production was considered positive when the clavulanate mediated enhancement of the activity of an indicator drug produced a keyhole effect and regarded as a phenotypic confirmation of the presence of ESBL [11].

M**olecular detection.** Template DNA was prepared by a simple and rapid boiling procedure from suspension of *S. aureus* colonies [12]. DNA was collected and stored at -20°C until real-time PCR runs.

a) Molecular detection of staphyloccoccal *mecA* genes. Real-time polymerase chain reaction (PCR) was used for detection of *mecA* (table 1). As positive controls, *S. aureus* ATCC BAA-41 was used. Light Cycler 480 Probe Master kit (Roche Diagnostics GmbH, Mannheim, Germany) was used with these primers and probes on Light Cycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany) instrument according to the manufacturer's instructions [12]. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 45 cycles, of 10s at 95°C, 30s at 55°C, 1s at 72°C.

Molecular detection of *van* genes in *Staphylococcus* spp., *Enterococcus* spp., and ESBL genes in Gram-negative strains. Primers of *vanA, vanB, vanC1, vanC2-C3 gene*s for *Staphylococcus* spp. and *Enterococcus* spp. and beta lactamase & carbapenemase (bla_{CTX-M1}, bla_{CTX-M2}, bla_{KPC}, bla_{OXA-48}, bla_{SHV} and bla_{TEM}) genes for Gram-negative strains were provided from Integrated DNA Technologies (IDT, Coralville, IA) (table 1) [13-16]. Light Cycler 480 Sybr Green I Master kit (Roche Diagnostics GmbH, Mannheim, Germany) was used with these primers on Light Cycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany) instrument according to the manufacturer's

Table 1 **Primers used for *mecA*, van, ESBL and carbapenemase genes presence in the real-time PCR assay**

Name	Name	Oligonukleotid sequence	Ref.
mecA primers for *Staphylococcus*	MecA F	5-GGCAATATTACCGCACCTCA-3	McDonald et al, 2005 [12]
	MecA R	5-GTCTGCCACTTTCTCCTTGT-3	McDonald et al, 2005 [12]
	MecA probe	5-FAM- AGATCTTATGCAAACTTAATTGGCAAATCC-Tamra-3	McDonald et al, 2005 [12]
van gene primers for *Enterococcus* spp. and *Staphylococcus* spp.	vanA F	5-AATACTGTTTGGGGGTTGCTC-3	Khan et al, 2005 [13]
	vanA R	5-CTTTTTCCGGCTCGACTTCCT-3	Khan et al, 2005 [13]
	vanB F	5-GCGGGGAGGATGGTGCGATACAG-3	Khan et al, 2005 [13]
	vanB R	5-GGAAGATACCGTGGCTCAAAC-3	Khan et al, 2005 [13]
	vanC1 F	5-TTGACCCGCTGAAATATGAAGTAA-3	Khan et al, 2005 [13]
	vanC1 R	5-TAGAACCGTAAGCAAAAGCAGTCG-3	Khan et al, 2005 [13]
	vanC2-C3 F	5-GCATGGCAAATACGGGGAAGAT-3	Khan et al, 2005 [13]
	vanC2-C3 R	5-CATGGCAGGATAGCGGGAGTGA-3	Khan et al, 2005 [13]
ESBL and carbapenemase genes primers for Gram-negative bacilli	blaCTX-M-1	5-GCGTGATACCACTTCACCTC-3	Copur et al, 2013 [14]
		5-TGAAGTAAGTGACCAGAATC-3	
	blaCTX-M-2	5- TGATACCACCACGCCGCTC-3	Copur et al, 2013 [14]
		5-TATTGCATCAGAAACCGTGGG-3	
	blaKPC	5- CGTTCTTGTCTCTCATGGCC-3	Poirel et al, 2004 [15]
		5- CCTCGCTGTGCTTGTCATCC-3	
	blaOXA-48	5- TTGGTGGCATCGATTATCGG-3	Poirel et al, 2004 [15]
		5- GAGCACTTCTTTTGTGATGGC-3	
	blaSHV	5- ATGCGTTATATTCGCCTGTG-3	Copur et al, 2013 [14]
		5-TTAGCGTTGCCAGTGCTC-3	
	TEM	5- AGTATTCAACATTTYCGTGT-3	Copur et al, 2013 [14]
		5- TAATCAGTGAGGCACCTATCTC-3	

Table 2 **Frequency distribution [%] of microorganisms isolated from paper currencies**

Microorganisms	Paper currencies [n=25 each other]						Total [n=150]
	5£	10£	20£	50£	100£	200£	
Bacillus spp.	20	17	14	14	12	13	90 (60%)
Corynebacterium spp.	4	4	1	2	1	2	14 (9.3%)
Staphylococcus aureus	14	12	10	11	10	15	72 (48%)
Coagulase negative staphylococci (CoNS)	21	17	15	14	9	6	82 (54.7%)
Streptococcus spp.	1	1	0	0	0	0	2 (1.3%)
Micrococcus spp.	2	1	1	0	0	0	4 (2.7%)
Enterococcus spp.	8	18	12	15	11	20	84 (56%)
Neisseria spp.	1	1	1	0	0	0	3 (2%)
Escherichia coli	2	0	0	0	0	2	4 (2.7%)
Enterobacter cloacae	10	2	1	1	1	0	15 (10%)
Pantoea agglomerans	1	0	3	3	2	0	9 (6%)
Klebsiella pneumoniae	2	1	0	0	1	0	4 (2.7%)
Klebsiella oxytoca	1	1	0	0	0	0	2 (1.3%)
Pseudomonas aeruginosa	4	2	2	1	1	0	10 (6.7%)
Pseudomonas putida	2	2	0	0	1	1	6 (4%)
Acinetobacter baumannii complex	9	3	1	0	0	2	15 (10%)
Candida spp.	0	0	3	2	0	1	6 (4%)
Total	102	82	64	63	49	62	422

instructions. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 35 cycles of amplification; 10s at 95°C, 30s at 52°C, 1s at 72°C and melting curves; 5s at 95°C, 60s at 65°C, and 97°C cont. reading). *E. faecium* ATCC 51559, *E. faecalis* ATCC 51299, *E. gallinarum* ATCC 49573, and *E. casseliflavus* ATCC 25788 strains were used as a positive control for *vanA, vanB, vanC1,* and *vanC2-C3* genes respectively. *K. pneumoniae* ATCC 700603 and *E.coli* ATCC 25922 were also used as a control of beta lactamase and carbapenemase genes.

b) Molecular detection of SEs, pvl and tsst -1 genes. Real-time polymerase chain reaction (real-time PCR) was used for detection of specific genes to confirm their identities (such as SEs, *pvl*, and *tsst-1* gene) via the primers previously described Peck et al [17]. Light Cycler 480 Sybr Green Master kit (Roche Diagnostics GmBH, Mannheim, Germany) was used with these primers on Light Cycler 480 II (Roche Diagnostics GmBH, Mannheim, Germany) instrument according to the manufacturer's instructions. 0.5 uM primers were added in reactions of final concentrations. Real-time PCR profile was used; denaturation step at 95°C for 10 min, followed by 40 cycles, of 10s at 95°C, 30s at 55°C, 1s at 72°C and melting curves; 5s at 95°C, 60s at 65°C, and 97°C cont. reading). As positive controls, *S. aureus* ATCC 13565 (*sea, sej*), *S. aureus* ATCC 14458 (*seb*), *S. aureus* ATCC 19095 (*sec, seh*), *S. aureus* ATCC 23235 (*sed, seg, sei*), *S. aureus* ATCC 27664 (*see*), *S. aureus* ATCC 25923 *(pvl*), *S. aureus* ATCC 51650 (*tsst-1*) were used. As a nontoxigenic control *S. aureus* ATCC 6538 was used.

RESULTS

Of the 150 samples of Turkish currency banknotes on which bacteriological analysis was conducted, 81% were found to be contaminated with several microbial species. The spectrum of microbial species were detected at rates of; *S. aureus* 48% (46.8% MRSA and 1.2% MSSA), CoNS 54.7%, *Enterococcus* spp. 56%, enteric bacteria 21.3%, non-fermentative Gram-negative bacteria 18.7% and *Candida* spp. 4%. A wide distribution of pathogens occurred from the different points included (table 2). The highest microbial contamination was obtained in the Turkish currency banknotes from the hospital cafeteria, followed by the cafeteria of medical faculty students. Others were with order supermarkets and restau-

Table 3 **Identification of ESBL and carbapenemase genes in Gram-negative bacilli**

Bacteria (number of isolates/EBSL positive)					
E. coli (n=4/3)	*K. pneumoniae* (n=4/4)	*E. cloacae* (n=15/10)	*P. agglomerans* (n= 9/4)	*A. baumannii* (n=15/12)	*P. aeruginosa* (n=10/7)
TEM + KPC + SHV (1 strain)	CTX-M-1 + OXA 48 (1 strain)	TEM (1 strain)	SHV (1 strain)	CTX-M-1 + OXA-48 (1 strain)	CTX-M-1 + KPC (1 strain)
CTX-M-2 + TEM + SHV (1 strain)	CTX-M-2 + KPC (1 strain)	CTX-M-2 (1 strain)	CTX-M-2 + TEM + SHV (2 strains)	CTX-M-2 + OXA-48 (1 strain)	CTX-M-1 + TEM + SHV (1 strain)
CTX-M-2 + TEM + OXA-48 + SHV (1 strain)	TEM + OXA-48 (1 strain)	CTX-M-2 + TEM (2 strains)	CTX-M-1 + CTX-M-2 + TEM + SHV (1 strain)	CTX-M-2 + TEM (3 strains)	CTX-M-2 (1 strain)
	TEM + OXA-48 + SHV (1 strain)	CTX-M-1 + TEM + SHV (1 strain)		KPC (2 strains)	CTX-M-2 + TEM (2 strains)
		TEM + SHV (2 strains)		OXA-48 + SHV (2 strain)	TEM + OXA-48 (1 strain)
		CTX-M-2 + TEM + SHV (2 strains)		SHV (3 strains)	KPC (1 strain)
		CTX-M-1 + CTX-M-2 + SHV (1 strain)			

rants around the hospital, banks, buyers in open-air markets and filling-stations. In the Turkish currency banknotes, the most intensive bacterial contamination was found in 5£, followed by 10£, 20£, 50£ and 100£, respectively. When looking at 200£banknotes, the contamination rate was found to be higher than 100£. The species of *Staphylococcus* spp. 154 produced in the highest proportion were *S. aureus* 48% and CoNS 54.7%. The distribution of CoNS were *S. epidermidis* 46.7%, *S. haemolyticus* 20%, *S. hominis* 12.2%, *S. capitis* 11%, *S. warneri* 4.9%, *S. lugdunensis* 3.7%, *S. caprae* 2.4% and *S. saprophyticus* 2.4%. The *mecA* gene was observed in 90.3% of *S. aureus* and in 73% of CoNS isolates. When the antibiotic resistance of *Staphylococcus* spp. were examined; the resistance rates in MRSA strains were erythromycin 66.7%, clindamycin 22.2%, gentamicin 16.7%, trimethoprim+sulfamethoxazole (SXT) 16.7%; In *S. epidermidis*, erythromycin 34.3%, clindamycin 17.2%, gentamicin 5.9%, ciprofloxacin 5.9% and SXT 5.9%; in *S. haemolyticus* erythromycin 72.2%, clindamycin 44.4%, tigecycline 38.9%, ciprofloxacin 38.9% and linezolid 38.9%; in *S. hominis* erythromycin 16.2% and SXT 16.2%; in *S. capitis* gentamicin 20%. None of the staphylococci strains were found to have quinupristin/dalfopristin and vancomycin resistance. The rate of multi-drug resistance (resistance to more than three antibiotics-MDR) was found as 40.3%.

The second most frequently isolated 84 *Enterococcus* spp. (56.7%) was the distribution of bacteria in the species *E. faecium* 35 (41.7%), *E. faecalis* 8 (9.5%), *E. casseliflavus* 21 (25%) and other *Enterococcus* spp. 10 (11.9%), respectively. Vancomycin resistance was determined by both phenotypic and genotypic methods in two origins, one *E. faecium* and one *E. casseliflavus* (2.4%). The resistance gene was *vanA*. Other vancomycin resistance genes were not detected. Enteric bacteria isolated from banknotes were 21.3%. *Enterobacter cloacae* was the first line of enteric bacteria with 46.9%. The others were *Pantoea agglomerans* 28.2%, *E. coli* 12.5%, *K. pneumoniae* and *K. oxytoca* 12.5%, respectively. When the antimicrobial resistance in enteric bacteries was examined ampicillin was found to be with the highest resistance rate as 81%. Resistance rates to other antibiotics were determined as follows: ceftazidime 75%, cefuroxime and cefuroxime + axetil combination of 65.6% cefoxitine 62.5%, cefepime 78%, ceftriaxone 9.4%, ertapenem, meropenem, imipenem 12.5%, amikacin 25%, gentamicin 22%, ciprofloxacin 40.6%, tigecycline 3% trimethoprim-sulfamethoxazole 25%, colistin 6.3%. MDR in enteric bacteria was 40.6%. ESBL enzyme genes were found to be 66.7% in enteric bacteria (table 3). Non-fermentative Gram-negative rods isolated from banknotes were 18.7%. Among the non-fermentative bacteria, *Acinetobacter baumannii* complex ranked first with 53.6%. The others *were P. aeruginosa* 35.7%, *P. putida* 10.7% and *P. stutzeri* 7.2% respectively. Antimicrobial resistance rates of *Pseudomonas* spp. were as piperacillin 50%, piperacillin+ tazobactam 40%, ceftazidime 40%, ceftriaxone 30%, imipenem 10%, amikacin 20% and ciprofloxacin 30%. Antimicrobial resistance rates of *Acinetobacter baumannii* complex were as piperacillin 53.3%, piperacillin+ tazobactam 40%, ceftazidime 66.7%, ceftriaxone 33%, imipenem 26.7%, amikacin 33% ve ciprofloxacin 46.7%. MDR was 60% in *P. aeruginosa* and 76% in *A. baumannii* complex. ESBL enzyme genes were found to be 65.6% in enteric bacteria and 76% in non fermentative Gram-negative bacteria. The distribution by species was *E. coli* 75%, *K. pneumoniae* 100%, *E. cloacae* 66.7%, *P. agglomerans* 44.4%, *P. aeruginosa 70%* and *A. bau-*

Table 4 Status of *mecA* and toxin genes of 154 *Staphylococcus* spp. isolates

	Number of positive isolates							
Bacterial isolates (n)	*mecA*	Pvl	Tsst-1	SEs	Only one toxin gene	Multiple toxin gene	Toxigenic	Non-toxigenic
S. aureus (72)	65	2	1	72	1	71	72	0
CoNS (82)	60	4	1	74	3	71	74	8
S. epidermidis (35)	29	1	0	35	1	34	35	0
S. haemolyticus (17)	8	1	0	12	1	11	12	5
S. hominis (10)	9	1	0	10	1	9	10	0
S. capitis (9)	7	0	0	9	0	9	9	0
S. warneri (4)	3	1	1	4	0	4	4	0
S. lugdunensis (3)	2	0	0	2	0	2	2	1
S. caprae (2)	1	0	0	1	0	1	1	1
S. saprophyticus (2)	1	0	0	1	0	1	1	1
Total (154)	125	6	2	146	4	142	146	8

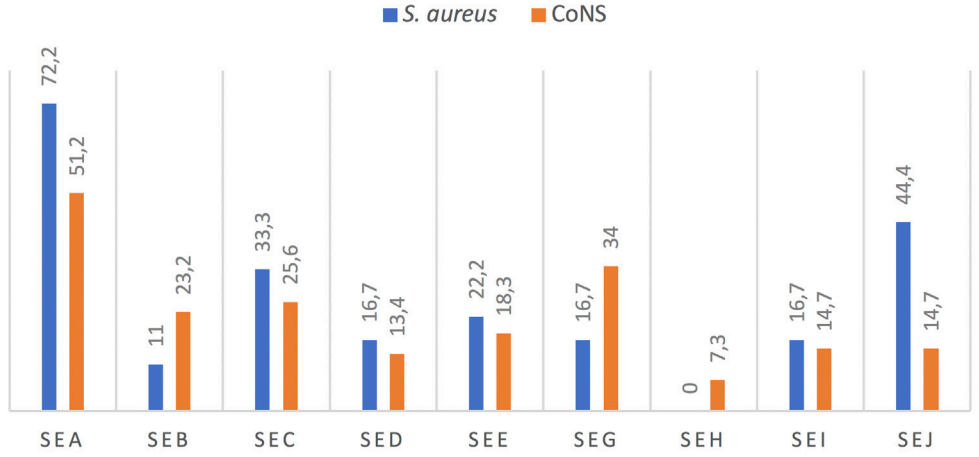

Figure 1 Distribution of SEs genes in *S. aureus* and CoNS

mannii 80% (table 3). CTX-M type ESBL enzyme genes were found to be 43.8% in enteric bacteria and 40% in non fermentative Gram-negative bacteria. The distribution by species was *E. coli* 50%, *K. pneumoniae* 50%, *E. cloacae* 46.7%, *P. agglomerans* 22.2%, *P. aeruginosa* 50% and *A. baumannii* 33.3%. In our study, bla_{KPC} was found as 6.2 % in enteric bacteria and as 12 % in non-fermentative bacteria. The distribution by species was *E. coli* 25%, *K. pneumoniae*, 25%, *P. aeruginosa* 20% and *A. baumannii* 13.3%. OXA-48 enzyme genes were found to be 12.5 % in enteric bacteria and 20% in non-fermentative Gram-negative bacteria. The distribution by species was *E. coli* 25%, *K. pneumoniae* 75%, *P. aeruginosa* 10% and *A. baumannii* 26.7%. The availability of toxin genes were 100% in *S. aureus*, 100% in *S. epidermidis*, 70.6% in *S. haemolyticus*, 66.7% in *S. lugdunensis*, 50% in *S. caprae and* 50% in *S. saprophyticus*. The distribution of toxin genes were *pvl* 2.8%, *tsst-1* 1.4% and SEs 100% in *S. aureus, pvl* 4.9%, *tsst-1* 1.2% and SEs 87.8% in CoNS (table 4). The distribution of SEs genes in *S. aureus* were as *sea* 72.2%, *seb* 11%, *sec* 33.3%, *sed* 16.7%, *see* 22.2%, *seg* 16.7%, *sei* 16.7% and *sej* 44.4%, the *seh* gene was not found. The distribution of SEs genes in CoNS were as *sea* 51.2%, *seb* 23.2%, *sec* 25.6%, *sed* 13.4%, *see* 18.3%, *seg* 34%, *seh* 7.3%, *sei* 14.7% and *sej* 14.7% (figure 1). Comparing with that of CoNS, the *sea* gene was found statistically significantly high in *S. aureus* strains ($p<0.05$) and comparing with that of *S. aureus* strains, the *seb*, *seg and seh* genes were found statistically significantly high in CoNS strains.

DISCUSSION

Paper currencies are objects capable of absorbing, harboring and transmitting infectious microorganisms [2]. Researches show that the microbial load on banknotes varies according to the banknotes, seasons, stored under varying environmental conditions, the age of banknotes, the local community microbiota, the general hygiene level, and the general hygienic conditions [3, 9, 10]. The amount of bacterial contamination on currency varies widely between countries. Previous studies have revealed that 70-97% of banknotes harbor various bacteria and viruses on the surface in different nations such as the United States, Mexico, China, India, Saudi Arabia, Sudan, Pakistan, Brasil etc, [9, 10, 18, 19, 20]. In our study, Turkish currency banknotes on which bacteriological analysis was conducted, 81% were found to be contaminated with several microbial species. Our results show that similar results were obtained in previous studies. Numerous studies have shown that cotton-based banknotes have more microbial loads than polymer-based ones [3, 10].

Vriesekoop et al. reported that comparison of cotton-based banknotes of countries such as China, Ireland, The Netharlands, Nigeria, United Kingdom and the United States, as well as the polymer-based banknotes of countries such as Australia and New Zealand. They found that cotton-based banknotes had much more bacterial loading than polymer-based banknotes [3]. The bacterial load evaluated as 81% in Turkish banknotes can be explained by the fact that they are based on cotton. Some studies showed that, the longer the paper currencies remain in circulation, the more chance there is for them to become contaminated, and lower-denomination notes receive the most handling because they are exchanged more frequently [2, 3]. According to our results also showed that health centers and health center workers and people who stay here play an important role contributing to the bacterial contamination. Many previous studies also claimed similar results [2, 3, 9, 10]. Many bacteria have been isolated from banknotes in studies from Turkey, China, Philippines, India, Saudi Arabia, Mexico, New Zealand, Australia, Canada, USA and Europe. It was also reported that *S. aureus, E. coli, Klebsiella* spp. and *Enterobacter* spp. were identified from these countries' banknotes [9, 10]. *Staphylococcus* spp. present in the nose often contaminate hands, fingers, faces, and nasal carriers which can easily become skin carriers [4]. In general, there was no obvious difference in survival between multiresistant and susceptible *S. aureus* strains. *S. aureus* (including MRSA) survive for 7 day -7 months on dry surfaces [21]. In our study, *Staphylococcus* spp. were the most isolated bacteria. Previous studies have also determined that there is a high number of *Staphylococcus* spp. on banknote, however, most studies did not identify *Staphylococcus* spp. Our study was the first research to identified *Staphylococcus species* unlike other researches on this topic. Methicillin resistance is an important consideration in all *Staphylococcus* spp., especially *S. aureus.* Global transmission of MRSA has been the subject of many studies [3, 22]. In recent study, it was determined that the rate of methicillin resistant *S. aureus* was 90.3% and methicillin resistant in CoNS is 73.2%. The highest antibiotic resistance in staphylococcus was erythromycin (72.2%), and clindamycin (44.4%) resistance in *S. haemolyticus*; gentamicin resistance (20%) in *S. capitis*. Tigecycline (38.9%), ciprofloxacin (38.9%) and linezolid (33.3%) resistance were found only in *S. haemolyticus* strains. None of the staphylococci strains had resistance to quinupristin/dalfopristin and vancomycin. The rate of MDR was found as 40.3%. Recently, many published studies reported that *E. faecium* infections are increasing worldwide [4]. In our country, the rates of *E. faecium* and *E. faecalis* were determined to be 15 - 50% and 52 - 85%, respectively [23, 24]. In previous researches, *Enterococcus* spp., which can be found without losing their vitality for 4 months in inanimate environments [21].

Many Gram-negative species, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia marcescens*, or *Shigella* spp. can survive on inanimate surfaces even for months. Overall, Gram-negative bacteria have been described to persist longer than Gram-positive bacteria [3, 21]. Humid conditions improved persistence for most types of bacteria, such as *Salmonella typhimurium, P. aeruginosa, E. coli* or other relevant pathogens [2, 9, 21, 25]. In previous studies, reported that *Enterobacteraceae* members are 13%-55.5% range of the paper currencies and the most frequently isolated enteric bacteria was *E. coli* (19.4-48.14%) [2, 3, 9]. Antimicrobial resistance is a global phenomenon that has resulted in high morbidity and mortality as a result of treatment failures and increased health care costs. Research has shown that contaminated fomites in general and paper currency in particular, plays a key role in the spread of bacterial infections with antimicrobial resistance [2, 3, 25]. Heshiki et al. [22] in a metagenomic study showed that the antimicrobial resistance genes on banknotes were significantly higher (4.86 times more) than environmental samples such as water, air, soil and dust.

Emergence of glycopeptide resistance causes more severe prognosis, higher mortality, and recurrence in enterococcal infections. The most common type of enterococcal vancomycin resistance is high-level resistance associated with acquisition of the *vanA* and *vanB genes*, typically observed in *E. faecium* and *E. faecalis* isolates [4]. Conversely, the *vanC* genotype is associated with constitutive low-level vancomycin resistance and is intrinsic to *E. gallinarum* and *E. casseliflavus* [4]. In our study, vancomycin resistance was determined by both phenotypic and genotypic methods in two isolates (2.4%), these were one *E. faecium* and one *E. casseliflavus.* The resistance genes were *vanA.* Other vancomycin resistance genes were not detected.

Resistance mediated by ESBLs includes all penicillins, cephalosporins (including third-generation cephalosporins) and aztreonam. Since plasmid-mediated ESBLs were first detected in a *K. pneumoniae* isolate in 1983 in Germany [26]. A new non-TEM non-SHV ESBL was isolated in Germany, in 1989, in a strain of *E. coli* called CTX-M because of its preferential activity on cefotaxime rather than ceftazidime [27]. Over the past 20 years, some *Enterobacteriaceae* mainly *E. coli, K. pneu-*

moniae, and *Proteus mirabilis* have demonstrated acquisition of plasmids secreting ESBL [28]. In our study, the rates of ESBL enzyme genes were found to be high as 65.6% in enteric bacteria and as 76% in non-fermentative Gram-negative bacteria. CTX-M type ESBL enzyme genes were found to be 43.8% in enteric bacteria and 40% in non-fermentative Gram-negative bacteria. Carbapenemases in *Enterobacteriaceae* are mainly found in *K. pneumoniae*, and to a much lesser extent in *E. coli* and other enterobacterial species, with a higher prevalence in southern Europe and Asia than in other parts of the World [28]. The first OXA-48 carbapenemase was identified in 2001 from a K. pneumoniae isolate obtained from a urine specimen collected in Istanbul, Turkey [15]. Shortly thereafter there was an outbreak of OXA-48 producing *K. pneumoniae* isolates reported in Istanbul in 2006 [29]. In our study, OXA-48 enzyme genes were found to be 12.5% in enteric bacteria and 20% in non fermentative Gram-negative bacteria. *Staphylococcus* spp. are also capable of producing "distant" diseases, which are mediated by the secretion of toxins and these toxins can be produced directly by bacteria that colonize the skin or mucosa or *indirectly* by microorganisms that colonize food, beverages and fomites [4, 30]. Bacteriological studies about banknotes, have included no analysis of the toxin genes (*pvl, tsst-1* and SEs). 95.4% of *Staphylococcus* spp. that are analyzed from our study were determined to possess toxin genomes. The distribution of these toxin genomes was as follows: 3.9% *pvl,* 1.3% *tsst-1* and 98.4% SEs. There was no toxin genomes in the rest of the *Staphylococcus* spp. (5.2%). PVL is a cytotoxin that causes tissue necrosis and leukocyte destruction. This linkage to virulent strains suggests its capability of causing deadly infections in healthy people [4]. Toxic shock syndrome (TSS) is a life-threatening illness characterized by high fever, erythematous rash with subsequent desquamation of the skin, shock, and multiple organ involvement [4, 31]. In our study, it was possible to detect 1.3% of tsst-1 genomes from our isolated banknotes.

Six enterotoxins serotypes (*sea* to *see* and *seh*) have been involved in most of the *Staphylococcus* poisoning outbreaks worldwide [31]. In our study, it was indicated that 94.8% of *Staphylococcus* spp. have SEs genomes. *S. aureus* and CoNS strains can encode more than one enterotoxin gene simultaneously; over 50% of the isolates assessed showed this property [8]. All *S. aureus* strains were carried at least one SEs gene and the combination *sea+sei, sea+sec+sei, sea+sed+sej* was the most frequent. CoNS strains were positive SEs genome 90.2% and the combination *sea+sej, sea+sec+sej, sea+seg+sej, sea+sed+seg+sei* was the most frequent. *sea* is one of the most frequently observed enterotoxins, although the literature shows highly variable results in the prevalence of *S. aureus* enterotoxin genes, depending on the kind of food and the biovar investigated [4, 31]. Compared to CoNS strains, *sea* genes were statistically significantly higher in *S. aureus* strains ($p<0.05$). Compared to *S. aureus* strains, *seb, seg* and *seh* genes were statistically significantly high in CoNS strains ($p<0.05$). On the other hand, the *seh* gene was detected at a rate of 7.3% in CoNS strains, although there was no in the *S. aureus* strains.

Several factors in the spread of pathogen and potential pathogenic bacteria, as well as antimicrobial resistance and virulance genes such as SEs, community and hospital enviroments, animal products and the environmental compartment are important. Results of this study in terms of demonstrating that paper curriencies or banknotes circulating in society can potentially mediate the transport of microorganisms among people and poses a risk to public health and it is also very important to highlight the need for proper hygienic practices for maximally reducing the spread of disease-causing pathogens. This can be considered as an indication that banknotes may contribute to the spread of pathogens and antimicrobial resistance. In this study, it was aimed to pay attention to hand hygiene for reducing the microbial load on the currencies and the necessity of producing these banknotes with maintain less bacteria such as plastic etc. instead of cotton. In addition, our study has been the first research to identified staphylococcus species and its virulance genes unlike other researches on this topic.

FUNDING

None to declare

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/090.2019

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Human Papillomavirus in the region of La Ribera-Valencia: Present and future

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Article history
Received: 11 November 2019; Revision Requested: 4 December 2019; Revision Received: 4 December 2019; Accepted: 16 December 2019; Published: 21 February 2020

ABSTRACT

Introduction. Human Papillomavirus (HPV) is the main cause of cervical cancer. The etiology and effects derived from this infection are set by molecular techniques and cytological diagnosis, respectively. In the present study, data obtained by an opportunist screening of cervical cancer in La Ribera region are revised and related statistically.

Material and methods. Data considering different variables such as age, degree of lesion, HPV type detected and number of virus in coinfection were collected from 1,372 HPV positive cytology samples. HPV detection was carried out by means of three molecular techniques and the degree of lesion was analyzed by cytological diagnosis (Bethesda). In order to determine the relationship between different selected variables, several statistical analyses were performed.

Results. Only degree of lesion variable showed a direct relationship with the rest of variables, increasing with aging process, viral oncogenicity, presence of at least one high-risk virus and with the fact of being mono-infected. The probability of presenting a higher-level degree of lesion multiplied by 28.4 when high-risk HPV was detected in mono-infection.

Conclusions. HPV molecular detection is the most suitable technique to perform a cervix cancer primary screening for the management of women with negative cytological diagnose. The number of detected types is statistically related to the degree of lesion. The establishment of a properly regulated screening to identify HPV infection, and therefore, of cervical cancer risk, is essential.

Key-words: Human Papillomavirus, Cervical Cancer, Cancer Screening.

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Virus del papiloma humano en la comarca de La Ribera-Valencia: Presente y futuro

RESUMEN

Introducción. El virus del papiloma humano (VPH) es la principal causa de cáncer cervical. La etiología y los efectos derivados de esta infección se establecen mediante técnicas moleculares y diagnóstico citológico, respectivamente. En el presente estudio, los datos obtenidos por un cribado oportunista de cáncer cervical en la comarca de La Ribera se revisaron y se relacionaron estadísticamente.

Material y métodos. Se recopilaron datos que incluyeron diferentes variables como la edad, el grado de lesión, el tipo de VPH detectado y el número de virus en coinfección de 1.372 citologías positivas para VPH. La detección del VPH se realizó mediante tres técnicas moleculares y el grado de lesión se analizó mediante diagnóstico citológico (Bethesda). Para determinar la relación entre las diferentes variables, se realizaron varios análisis estadísticos.

Resultados. Sólo la variable del grado de lesión mostró una relación directa con el resto de variables, aumentando con el proceso de envejecimiento, la oncogenicidad viral, la presencia de al menos un virus de alto riesgo y el hecho de estar mono-infectado. La probabilidad de presentar un mayor nivel de lesión se multiplicó por 28,4 cuando se detectó VPH de alto riesgo en la mono-infección.

Conclusiones. La detección molecular del VPH es la técnica más adecuada para realizar un cribado primario del cáncer de cuello uterino para el manejo de mujeres con diagnóstico citológico negativo. El número de tipos detectados está estadísticamente relacionado con el grado de lesión. El establecimiento de un cribado regulado adecuadamente para identificar la infección por VPH y, por lo tanto, del riesgo de cáncer cervical, es esencial.

Palabras clave: Virus del Papiloma Humano, Cáncer de Cuello Uterino, Detección Precoz del Cáncer.

INTRODUCTION

Human Papillomavirus (HPV) belongs to the family *Papovaviridae*, subfamily *Papillomaviridae*. This virus is tissue-specific and infects both the cutaneous and mucosal epithelia. Its genome is divided into: an E region, of early expression encoding various structural proteins (E1-E7); an L region, of late expression, which encodes the capsid proteins (L1 and L2); and a regulatory, non-coding region (RNC/LCR), located in the 5'-direction [1].

HPV types have been classified by their tissue tropism (mucous or cutaneous types), as well as their oncogenic potential [High (HR) and Low-Risk (LR) types]. The International Agency for Research on Cancer, defines 16 High-Risk HPV (HR-HPV), associated with cancer in humans (types 16, 31, 33, 35, 52, 58, 73,18, 39, 45, 59, 68, 51 53, 56, 66) [2]. HPV-16 and HPV-18 types are responsible globally for 71% of cervical cancer cases [3].

Based on the antigenic and variable region L1 genomic sequence, coding for the major protein of the capsid, about 200 types have been identified and characterized, defined as those that include more than 1% difference in their nucleotide sequence. Phylogenetically, below in the scale of HPV types, lineages and sublineages are found, which differ by 1% and between 0.5-0.9% in their genomic sequence, respectively [2]

HPV infection is one of the most common sexually transmitted diseases in the world, as well as the main cause of cervical cancer, with a higher incidence in developing countries [4]. Most infections (70-90%) are asymptomatic and resolve spontaneously in 1-2 years. The disease degree transmission, the asymptomatic infection development and the poor immunological response, are directly related to the virus success in its replicative process.

HR-HPV persistent infection, defined as the presence of an HPV specific type in clinical samples separated between 6 months and 1 year, is an indispensable condition for progression to infiltrating carcinoma [5]. Likewise, the sequence of events caused by HPV infection is gradual and detectable by cytology with Papanicolaou staining. The control of the disease is possible through primary (vaccination) and secondary (detection of HPV and cytological diagnosis) preventions, so global eradication of cervical cancer (the only type of cancer with this future forecast) is feasible, due to knowledge of the disease cause, development and treatment. This possible eradication can be achieved by raising the need to control the population infection through vaccination and well-established screenings. The population screening should be well structured, a target population should be identified, appropriate detection techniques should be chosen, and the time intervals should be defined according to age parameters.

As a primary control of the infection, a global vaccination strategy against HPV was launched ten years ago. Nowadays, the definitive results derived from this strategy are not available since the population to which it was directed, has just been incorporated into the population screening of individuals susceptible to prevention. In Spain, National Health Service recommendations for HPV secondary prevention have been published, consisting of a women population screening between 25 and 65 years of age [6].

Currently, cervical cancer screening is opportunistic and performed by cervico-vaginal cytology as a screening technique, with a variable periodicity of 1 to 3 or 5 years [7]. In its latest revision (https://bethesda.soc.wisc.edu), The Bethesda System substitutes the term Cervical Intraepithelial Neoplasia (CIN) for Squamous Intraepithelial Lesion (SIL), with two categories: Low-grade (LSIL) and High-grade (HSIL) [8]. The WHO IARC (World Health Organization International Agency for Research on Cancer) reviewed in its monographs program the scientific evidence in relation to the HPV test as a primary screening technique and concluded [9], like other studies [10], that enough evidence in relation to viral detection tests was found, presenting at least the same results as Papanicolaou staining. According to controlled randomized trials, screening based on the detection of HPV DNA allows an early diagnosis of high-grade cervical neoplasia due to its high sensitivity. In addition, this approach may be more effective in the prevention of cervical cancer since, being a less specific technique than cytology, its negative predictive value is very high [11, 12].

HPV infections can be caused by a single type of virus (mono-infection) or by several at the same time (poly-infection). The effect of one or the other in the development of cervical cancer [13, 14], and the same prognosis of mono and poly-infections are controversial aspects.

The aims of this study were: to describe the distribution of the different HPV genotypes and their relationship with the different degrees of cervical pathology (cytology in liquid medium) in the female population selected by opportunistic screening criteria between 2012 and 2017 in Health Department 11 of the Valencian Community; to determine the relationship between the five variables studied for the total sample: cytological diagnosis, age, viral oncogenicity, detection of high-risk virus (HR-HPV) only and mono / poly-infection; and, to describe three molecular techniques comparing their results, among them and with the total samples.

MATERIAL AND METHODS

Cases and population. The series consisted of 3,541 cytology samples including a women population between 25 and 65 years, that underwent HPV detection and morphological study with Papanicolaou staining from liquid cytology (ThinPrep) classified according to Bethesda criteria. 1,372 samples (38.75%) positive for at least one HPV type corresponded to the number of cases in the study. All cases belong to the opportunistic screening program for cervical cancer from 2012 to 2017 in the Department of Health 11 of Valencian Community that serves 46 municipalities with a total population in 2017 (www.ine.es) of 298,182 inhabitants (149,984 women and 148,288 men).

Definitions

Case: positive cytology for HPV detection, regardless of whether it belongs to the same patient in clinical samples separated over time.

Degree of lesion: each of the morphological diagnostic categories (Bethesda classification).

Low-grade lesion: on the previous scale, from NORMAL to LSIL included.

High-grade lesion: on the aforementioned scale, from ASC-H (Atypical Squamous Cells, cannot exclude HSIL) to ADENOCARCINOMA.

Viral oncogenicity: ability of each type of HPV to favor progression towards adenocarcinoma [high-risk / low-risk HPV (HR-HPV / LR-HPV)].

Mono-infection: infection caused by a single type of HPV regardless of its viral oncogenicity.

Poly-infection: infection of more than one HPV type at the same time regardless of its viral oncogenicity.

HPV molecular detection. The detection of HPV DNA in the period was carried out by three different molecular methodologies based on the Polymerase Chain Reaction (PCR). In a first phase, a nested PCR was performed (technique 1) using as external primers MY109 and MY110 and, as internal GP5 + and GP6 +, that amplify a region of the L1 gene. The positive amplifications were sequenced for typing by the dideoxynucleotide triphosphate terminators method and subsequently, the editing with the software ChromasPro (Version 1.7.4 Technelysium Pty Ltd) and the comparison with the sequences of the NCBI database by means of BLAST (Basic Local Alignment Search Tool - NCBI - NIH), were carried out. The second phase, in addition to the previous one, included another Molecular Biology technique based on hybridization with probes (HPV Direct Flow CHIP Kit, MASTER DIAGNOSTIC (technique 2); this method can distinguish up to 36 HPV types. The negative results by this technique were processed by the nested PCR methodology described above due to its higher sensitivity. And in the third phase, the Anyplex II HPV 28 Detection Kit (Seegene) (technique 3) was used, which simultaneously detected, genotyped and semiquantified in two qPCR reactions, the 28 most prevalent high and low-risk HPV types, using the DPO system and melting curve analysis using TOCE technology.

Prior to the three techniques application, DNA extraction and quantification processes were implemented. The extraction was executed following the supplier's instructions using the Maxwell® 16 FFPE Plus LEV DNA Purification Kit (Promega). DNA concentrations and purity were measured by spectrophotometry at 260 and 280 nm with Nanodrop2000. In techniques 1 and 2, the presence of amplification for subsequent sequencing or hybridization, respectively, was previously detected through an automatic capillary electrophoresis system [QiaXcel Advanced (QIAGEN)].

Statistical analysis. In an electronic database, the following variables were included for each patient: quantitative age; qualitative age in 4 groups (23-35 / 36-45 / 46-55 / > 56);

Table 1 Descriptive Age (qualitative)

N	Minimum	Maximum	Mean	Standard Deviation
1,372	25	65	37.433	9.207

Table 2 Descriptive Qualitative Variables

Variables	Levels	N	%
Age 4 groups	23-35	661	48.2
	36-45	404	29.4
	46-55	254	18.5
	>56	53	3.9
	Total	1,372	100
Age 2 groups	≤ 30	388	28.3
	>30	984	71.7
	Total	1,372	100
'Papanicolaou' result 6 categories	ADENOCARCINOMA	2	0.1
	HSIL	260	19.0
	ASC-H	43	3.1
	LSIL	570	41.5
	ASC-US	311	22.7
	NORMAL/INFLAMMATION	186	13.6
	Total	1,372	100
'Papanicolaou' result 5 categories	ADENOCARCINOMA/HSIL	262	19.1
	ASC-H	43	3.1
	LSIL	570	41.5
	ASC-US	311	22.7
	NORMAL/INFLAMMATION	186	13.6
	Total	1,372	100
Technique	Technique 1	174	12.7
	Technique 2	571	41.6
	Technique 3	627	45.7
	Total	1,372	100
High/Low HPV risk	Low-Risk (LR)	193	14.1
	High-Risk (HR)	1179	85.9
	Total	1,372	100
Mono/poly HPV	Mono	782	57.0
	Poly	590	43.0
	Total	1,372	100
HR/LR Mono/Poly HPV infected	HR Mono-infected	606	44.2
	HR Poly-infected	573	41.8
	LR Mono-infected	176	12.8
	LR Poly-infected	17	1.2
	Total	1,372	100

HSIL: High-grade Squamous Intraepithelial Lesion; (LSIL): Low-grade Squamous Intraepithelial Lesion; ASC-H: Atypical Squamous Cells, cannot exclude HSIL; ACS-US: Atypical Squamous Cells of Undetermined Significance

qualitative age in 2 groups (≤ 30 / > 30); results of Papanicolaou staining in 6 groups (ADENOCARCINOMA / HSIL / ASC-H / LSIL / ASC-US (Atypical Squamous Cells of Undetermined Significance) / NORMAL or INFLAMMATION); results of Papanicolaou staining in 5 groups (ADENOCARCINOMA or HSIL / ASC-H / LSIL / ASC-US / NORMAL or INFLAMMATION); high / low-risk HPV: to create this variable, a cytology sample was considered to show an HPV high-risk result if at least one high-risk virus was presented and, a low-risk result, if any high-risk virus was not detected; mono / poly-infection: to create this variable, mono-infected was considered when only one virus was presented and, poly-infected, when more than one virus was detected, regardless of its oncogenicity; and, combination of high / low-risk HPV and mono / poly-infected results (high-risk HPV and poly-infected / high-risk HPV and mono-infected / low-risk HPV and poly-infected / low-risk HPV and mono-infected). In tables 1 and 2, the descriptive analysis of the variables is shown.

A descriptive analysis of the study variables was carried out; the qualitative variables were described by frequencies and percentages and, the quantitative variables, by dispersion measures (minimum, maximum, mean, standard deviation). A bivariate analysis was performed between the variables 'Papanicolaou staining', 'high/low-risk HPV' and 'mono/poly-infection' results with age (age variable was analyzed in a quantitative and qualitative way). In addition, a bivariate analysis was carried out in which the 'Papanicolaou staining' result was compared with the 'high/low-risk HPV' and 'mono/poly-infection' results. The qualitative variables were compared using the Chi square test, the quantitative variables with two levels, using the Student's T test and the quantitative variables with more than two levels, using the ANOVA and the post hoc Tukey tests to detect between which levels the differences were statistically significant. Odds Ratio (OR) and 95% confidence intervals were calculated by adjusting simple logistic regression models. A confidence level of 0.05 was considered for the results interpretation. A virus type descriptive analysis was accomplished in each of the 'Papanicolaou staining' results categories; specifically, the number of patients belonging to each virus as well as to each virus combination found, were described. For the most frequent viruses (16, 18, 31, 33, 42, 51, 53, 56, 66, 45, 58, 59 and 68) a dichotomous variable "The patient presents the virus (yes / no)" was created; this variable took the value 'yes' when the virus studied was detected in the cytology sample and takes the value 'no' when the virus studied was not. Subsequently, the dichotomous variables "The patient presents the virus (yes / no)" were compared with the 'Papanicolaou staining' results by the Chi square statistical test.

RESULTS

Distribution. The distribution of HPV types in this series is detailed in figure 1. In this study, the most frequent genotypes were 16, 31, 33, 53, 51 (HR types) and 42 (LR type). 71.7% of the total cases (n = 1,372) corresponded to 30-years-old women or older. Infection induced by at least one HR-HPV was observed in 85.9% of the samples studied, whereas a 14.9% of the cases suffered from a LR-HPV infection. 57% of cases belonged to mono-infection.

Relationship between the different studied variables

a) General situations. The diagnostic distribution of the 1,372 cases (positive HPV), according to Bethesda system, is shown in table 3. A multivariate study showed that only the variable "degree of lesion" (Papanicolaou test), of all the variables studied, presented a statistically significant direct relationship with each other. Thus, the degree of lesion increased with age, viral oncogenicity, the presence of at least one high-risk virus and with being mono-infected ($p < 0.007$, $p < 0.001$, $p < 0.001$, $p < 0.05$, respectively).

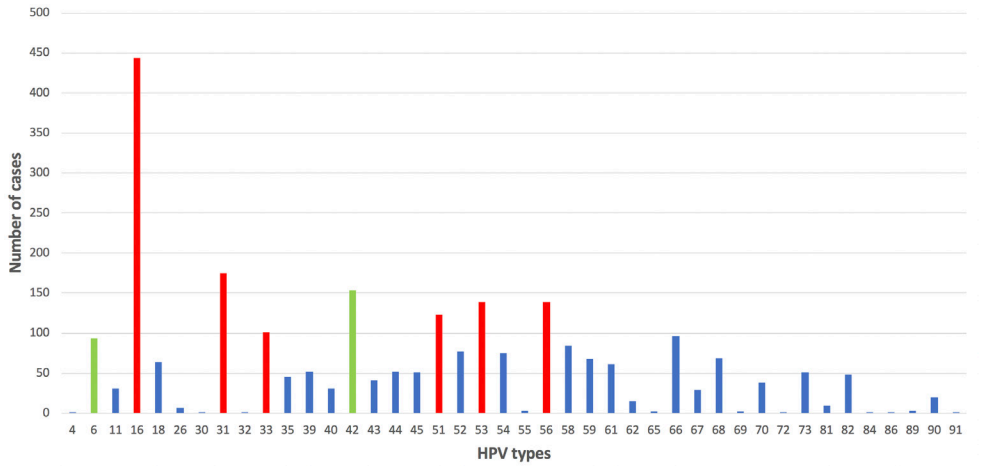

Figure 1 Distribution of HPV types.

Bar color red: most common HR-HPV types; bar color green: most common LR-HPV types; bar color blue: rest of HR/LR types.

Table 3 Diagnostic distribution of the 1,372 cases (positive HPV), according to Bethesda system

Lesion	ADENOCARCINOMA	HSIL	ASC-H	LSIL	ASC-US	NORMAL
%	0.1	19	3.1	41.5	22.7	13.6

HSIL: High-grade Squamous Intraepithelial Lesion; LSIL: Low-grade Squamous Intraepithelial Lesion; ASC-H: Atypical Squamous Cells, cannot exclude HSIL; ACS-US: Atypical Squamous Cells of Undetermined Significance.

Table 4 Frequency distribution of lesions with the different variables

	Age (years)	% HR-HPV	HR Mono N (%)	HR Poly N (%)	Mono/poly-infection
ADENOCARCINOMA		100	160 (61.07)	99 (37.79)	HR-mono
HSIL	>30	98.8			
ASC-H		88.4	16 (37.21)	22 (51.16)	HR-poly
LSIL		85.1	222 (38.95)	263 (46.14)	HR-poly
ASC-US	≤30	82.6	147 (47.27)	110 (35.37)	HR-mono
NORMAL		75.3	61 (32.8)	79 (42.47)	HR-poly

HSIL: High-grade Squamous Intraepithelial Lesion; LSIL: Low-grade Squamous Intraepithelial Lesion; ASC-H: Atypical Squamous Cells, cannot exclude HSIL; ACS-US: Atypical Squamous Cells of Undetermined Significance.

Table 5 Distribution of HR-HPV types in mono/poly-infection and the most frequent combinations, according to the cytological diagnosis.

Cytological diagnosis	Most frequent HPV in mono-infection	Most frequent HPV in poly-infection	Most frequent combination
HSIL	16 (61.4%)	16 (27.1%)	16 +33
LSIL	16 (25%)	16 (11.4%)	16+56,16+33
ASC-US	16 (33%)	31 (19.6%)	none
NORMAL	16 (27.9%)	53 (7.7%)	none

HSIL: High-grade Squamous Intraepithelial Lesion; LSIL: Low-grade Squamous Intraepithelial Lesion; ACS-US: Atypical Squamous Cells of Undetermined Significance.

In this series, LSIL was more frequent in the group ≤ 30 years, and HSIL in > 30 years. High-grade lesions showed a higher frequency of HR-HPV. When the infection was solely due to HR-HPV, the risk of presenting a high-grade lesion with respect to NORMAL citology was 28.4 times higher. Thus, the risk of displaying a high-grade lesion if the HPV corresponded to high-risk was multiplied by 28.4. In addition, if poly-infection existed, the probability of obtaining a LSIL than NORMAL cytological result was multiplied by 2 (with data from technique 3) (OR = 2.16) (CI 1.36-3.44) ($p < 0.05$).

The frequency distribution of lesions with different variables is shown in table 4.

b) Particular situations. Related to viral genotype, HPV 16 was associated more frequently with HSIL / LSIL ($p < 0.001$) than with the other Papanicolaou staining diagnoses; the risk of submitting a high-grade lesion was almost 10 times higher. In this study, the relationship between detecting an HPV 16 and the degree of lesion presented the following OR with respect to NORMAL cytology: HSIL (OR = 9.24), ASC-H (OR = 3.27), ASC-US (OR = 1.76) and LSIL (OR = 1.71). HPV 42 was linked with a higher frequency of ASCUS and NORMAL results ($p = 0.001$). If virus 42 was detected, the risk of ASC-H, ASC-US, HSIL and LSIL was less than being NORMAL (OR = 0.46, OR = 0.86, OR = 0.24, OR = 0.96, respectively). HPV 56 was more frequently associated with LSIL diagnosis than with the rest of diagnoses ($p = 0.001$). The risk of being LSIL versus being NORMAL was 1.89 times higher. HPV 66 was more frequently related to LSIL, being less frequent ASC-H and HSIL for this genotype ($p = 0.004$).

Table 5 shows the distribution according to the cytological diagnosis of HR-HPV types in mono and poly-infection, as well as the most frequent combinations.

Comparison amongst the three techniques. As explained previously, three techniques were employed for the detection of HPV: 45.7% was determined by technique 3, 41.6% by technique 2 and 12.7% with technique 1.

a) Relationship between the degree of lesion and infection with HR-HPV. In the total samples, the probability of presenting a high-grade lesion is multiplied by 28.4 times when the infection is caused by HR-HPV. It's multiplied by 18.75 times with technique 1; by 32.4 in technique 2, and with technique 3, HR-HPV was detected in 100% of HSILs.

b) Relationship between the degree of lesion and mono or poly-infection, regardless of its oncogenic potential. With the total samples, significant differences between both variables occurred ($p = 0.007$); regarding to the degree of lesion, mono-infection and poly-infection were not comparable. From ASC-US to LSIL, the most frequent situation was poly-infection, while in high-grade lesions, mono-infection.

All the results obtained were mono-infections applying technique 1. Therefore, no relationship could be established; by applying technique 2, no significant differences existed relating both variables; and with technique 3, significant differences between both variables happened ($p = 0.00009$), with the same considerations applied for the total of samples.

c) Mono or poly-infected with HR-HPV or LR-HPV only. In the total of samples, HR-HPV in mono or poly-infection was related to a value of $p < 0.001$; this relation was not observed in the case of

LR-HPV. These same results were obtained by applying technique 3 ($p < 0.001$). In technique 1, only mono-infections were found, so no relationship could be established; with technique 2, no statistical difference was found in the relationship of being HR-HPV / LR-HPV mono or poly-infected with the cytological study result.

DISCUSSION

The detection of HPV DNA in opportunistic screening cytology samples from Health Department 11 of the Valencian Community was carried out during a four-year period with three different PCR-based techniques. Data in this study, unlike others, were based on opportunistic and non-population screening. Thus, relative quantities to the total of the series were described and the terms "detection rates" or "frequencies" were used, against incidence or prevalence.

Of the total cases, three out of four (71.7%) corresponded to women over 30 years of age; in this age group, at least one HR-HPV was detected in 9 out of 10. The high rate of HR-HPV (85.9%) in this series is probably related to the easy and fast transmission of the virus itself and could suggest a colonization process rather than infection. In addition, only one in five cases progressed to lesion greater than HSIL. Thus, according to published data, a regression rate of 77.8% was observed [15]. Consistent with cytological diagnoses distribution (table 3), 13.6% of them would not have been detected taking cytology as a primary screening test, being NORMAL, but detecting at least one HPV by PCR techniques. High-grade lesions were more frequent in women over 30 years of age than in younger women (88.4-100% vs. 75.3-85.1%; table 4), which is related to the logical temporal evolution of the lesions.

The probability of presenting a high-grade lesion was 28.4 times higher when a HR-HPV is detected in mono-infection. 2 out of 3 cytology samples with a high-grade lesion showed an HR-HPV in mono-infection. However, the variable 'poly-infection', even in HR-HPV types, was associated with low-grade lesions in this series. The influence of mono or poly-infection incidence on the development of cytological lesions is a controversial issue [13, 14]. Data from this study suggest that poly-infection is a protective mechanism based on establishing a balance between the infectious virus types. Consequently, hegemony of any of them is not allowed and cytological morphology progresses to higher levels of LSIL.

As the degree of lesion evolves, the HPV number and types involved decrease. From ASC-US to LSIL, any type of HPV (HR or LR) was found; however, from LSIL onwards, higher frequency of high-risk types 16, 31, 51 and 53 were observed, being considered in our environment as the most oncogenic types. Also, the types 56 and 66, considered HR-HPV, prevailed especially in low-grade lesions which should rethink the definition of their oncogenicity. These types could produce high-grade lesions in selected patients due to immunosuppression or other causes as occurs with many other microorganisms considered non-pathogenic. However, high-grade lesions were not produced by these types in the study population.

In mono-infection, 1.2% of HSIL were associated with LR-HPV exclusively (6 and 11), as well as 11.6% of ASC-H (42,54,67 and 70). Although this unexpected event could rethink the classification of these viruses as LR-HPV, these results could be attributed to the limitations of the techniques used, since most of these results (6 of 8 results) derived from technique 2, in which the correlation between sensitivity and specificity is not its main characteristic. The remaining two cases were detected with techniques 1 and 3.

The high detection rate of HPV 16 (32.36% overall) was independent of the extent of the lesion, appearing even in samples with NORMAL cytological diagnosis. Consequently, screening based only on cytology, infra diagnose this type of infections in which lesions progress slowly.

According to the techniques employed, technique 1 performed a sequence PCR, whereby sequencing of several types at the same time was not possible. Thus, all patients presented mono infection when using this technique; employing technique 2, statistically significant relationship between mono-poly infection and the Papanicolaou staining result ($p = 0.126$) was not discovered; and, with technique 3, a statistically significant relationship between mono-poly infected and Papanicolaou staining was found ($p < 0.05$); a poly infected patient presents 2,159 (1,136-3.44) times more risk of belonging to LSIL than NORMAL. Among the techniques used, technique 3 showed the best data due to its technology and the ability to detect different types of coinfectant viruses by analyzing melting curves. Regarding the present data, greater sensitivity and reproducibility of technique 3 versus 2 is shown. Technique 3 provides a good correlation between sensitivity and specificity, allowing HPV typing in all cases (data not shown).

The data and results provided are based on the opportunistic screening of HPV carried out in Department 11 of the Valencian Community and are representative of this geographical area. In addition, and as far as is known, this is the only descriptive and statistical study of opportunistic HPV screening in the Valencian Community.

At this time, the National Health Service of Spain has set the basis to establish a national system of cervical cancer population screening [6]. This system will allow a control of this pathology, the study of its evolution and possible disease eradication with the establishment of primary and secondary prevention measures.

The GARDASIL 9® vaccine immunizes against types: 6, 11, 16, 18, 31, 33, 45, 52, 58. Although data on the vaccinated population are not yet available, this study concludes that HR-HPV 51 and 53, not included in the vaccine, appear with a frequency (8.97% and 10.13% respectively) similar to some contained in this vaccine. This fact suggests a natural selection of these types compared to others, and therefore raises reconsideration of the types to be included in future vaccines.

The detection of HPV leads in the consideration of other adjuvant factors for better infection management; factors

such as genotype, the presence of a poly or mono-infection or oncogenic capacity, together with the extent of the lesion, will influence the monitoring and treatment of the infection.

FUNDING

None to declare.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/094.2019

Use of micafungin as antifungal prophylaxis in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) in Spain (GETH-MIC)

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Article history
Received: 25 November 2019; Accepted: 28 January 2020; Published: 14 February 2020

ABSTRACT

Introduction. The fungal infections remain an important problem in the allogeneic stem cell trasnsplantation (allo-SCT) setting and thus, anti-fungal prophylaxis is commonly used. The antifungal drug should offer activity, at least against *Candida* and *Aspergillus* spp., a good safety profile and low probability interactions. Micafungin could theoretically fulfill these requisites. The aim of the study was to describe the experience with micafungin as primary prophylaxis in patients undergoing allo-SCT in a cohort of Spanish centres, and to evaluate its efficacy and tolerability in this population.

Material and methods. Retrospective multicentre observational study including all consecutive adult patients admitted for allo-SCT in participating centres of the Grupo Español de Trasplante Hematopoyético (GETH), from January 2010 to December 2013, who received micafungin as primary prophylaxis during the neutropenic period.

Results. A total of 240 patients from 13 centres were identified and 159 patients were included for the analysis. Most patients (95.6%) received 50 mg/day of micafungin. During the follow-up, 7 (4.4%) patients developed breakthrough invasive fungal disease, 1 proven and 6 probable; one patient discontinued the drug because of serious drug interactions. Prophylaxis with micafungin was considered effective in 151 (94.9%) patients.

Conclusions. According to our experience, micafungin is an appropriate alternative for antifungal prophylaxis in patients undergoing an allo-HSCT, because its efficacy, its low profile of drug interactions and side-effects.

Key-words: Stem cell transplantation, micafungin, prophylaxis.

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Profilaxis antifúngica con micafungina en pacientes que reciben un trasplante alogénico de progenitores hematopoyéticos (alo-TPH) en España (GETH-MIC)

RESUMEN

Introducción. Las infecciones fúngicas siguen representando un problema en el trasplante alogénico de progenitores hematopoyéticos (alo-TPH) por lo que es habitual el uso de profilaxis antifúngica en estos pacientes. El tratamiento antifúngico debe presentar al menos actividad frente a *Candida* y *Aspergillus* spp, un buen perfil de seguridad y baja probabilidad de infecciones, siendo micafungina una de las opciones que podría cumplir todos estos requisitos. El objetivo del estudio fue describir la experiencia con micafungina como profilaxis primaria en pacientes sometidos a alo-TPH en una cohorte de hospitales españoles, y evaluar su eficacia y seguridad en esta población.

Material y métodos. Estudio retrospectivo multicéntrico observacional consecutivo de todos los pacientes adultos ingresados para alo-TPH en los centros del Grupo Español de Trasplante Hematopoyético (GETH) desde enero de 2010 a diciembre de 2013 y que recibieron micafungina como profilaxis primaria durante el periodo de neutropenia.

Resultados. Se identificaron 240 pacientes de 13 hospitales y 159 fueron incluidos para el análisis. La mayoría (95.6%) de ellos recibieron dosis de 50mg/día de micafungina. Durante el seguimiento, 7 (4.4%) pacientes desarrollaron infecciones de brecha, 1 probada y 6 probables; en un paciente se suspendió el tratamiento por interacciones medicamentosas graves. La profilaxis con micafungina se consideró efectiva en el 94,9% de los pacientes (151 de 159).

Conclusiones. En base a nuestros resultados, consideramos que Micafungina es una buena alternativa como profilaxis antifúngica en pacientes sometidos a alo-TPH, por su eficacia, el bajo riesgo de interacciones y de efectos adversos.

Palabras clave: trasplante de células madre, micafungina, profilaxis.

INTRODUCTION

Invasive fungal infection (IFI) is an important cause of morbidity and mortality in allogeneic haematopoietic stem cell transplant (allo-HSCT) recipients [1, 2]. Although other fungi such as *Zygomycetes*, *Fusarium* spp. and *Scedosporium* spp. are being increasingly reported as important pathogens in HSCT recipients, the most frequent infections remain those related to *Aspergillus* spp. and *Candida* spp. [3].

The incidence of invasive *Candida* spp. and *Aspergillus* spp. infection is between 5% and 4–15%, with mortality rates around 30–40% in invasive candidiasis and up to 40-80% for *Aspergillus* infection [4]. Because early microbiological diagnosis is usually difficult to obtain, therapeutic strategies of prophylaxis or empirical treatment have been developed. The use of the different formulations of amphotericin B [5], voriconazol or posaconazole in this setting have shown utility, but are also associated with some toxicity and potential drug interactions which difficult their use in some patients.

Echinocandins are highly effective antifungal agents against *Candida* and *Aspergillus* spp., [6, 7]. that have demonstrated their efficacy in fungal infection prophylaxis and neutropenic fever treatment [8-10]. Micafungin provides, compared to other echinocandins, better activity against some *Candida* spp. (specially *C. glabrata*) and also *Aspergillus* spp. [7, 11]. The drug has a convenient once-daily dosage regimen and is associated with relatively few drug-drug interactions [12], positioning micafungin as a good alternative in those patients who need concomitant treatments or present moderate hepatic or renal dysfunction. Several studies have exposed their experience with micafungin as prophylaxis during neutropenia in hematologic patients, including randomized controlled trials [13, 16], and recent guidelines focused on antifungal prophylaxis also supported its use in neutropenic patients after HSCT [17].

The aim of this study was to describe the experience with micafungin as primary prophylaxis during the neutropenic phase in patients undergoing allo-HSCT in a cohort of Spanish transplant centres, and to evaluate its efficacy and tolerability in this population.

MATERIAL AND METHODS

Study design. This is a retrospective multicentre observational study including all consecutive adult patients admitted for allo-HSCT in 13 centres pertaining to the Grupo Español de Trasplante Hematopoyético (GETH) and the European Society for Blood and Marrow Transplantation (EBMT), from January 2010 to December 2013, who received micafungin as primary prophylaxis during the transplant. Patients that received less than 5 days of micafungin were excluded from the analysis. Only patients that received micafungin during the peri-transplant period (15 days before or after the infusion day) were included; patients who received prophylaxis with micafungin in the context of graft versus host disease (GVHD) were not considered for the analysis.

Study variables and data collection. Demographic, clinical, laboratory, microbiological and radiologic data, clinical course and mortality were retrospectively recorded from each patient. Patients were followed-up until hospital discharge. All clinical data of patients submitted to HSCT in Spain are routinely included in the EBMT registry database as a part of a continuous observational study. The data included in this study have been obtained from this registry. The data contained are loaned by patients under informed consent signed by them at the time of transplantation. The Ethics Committee of the GETH approved this study.

Definitions and statistical analysis. Possible, probable or proven IFI was considered according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [1]. Failure of prophylaxis was considered in those patients who developed breakthrough IFI during prophylaxis or in the 30 first days after transplantation or the treatment was discontinued because of toxicity or interactions mild or moderate side effects were considerate when no need of treatment discontinuation was needed. Conversely, severe side effects were considered in those cases where micafungin needed to be discontinued.

Continuous variables are expressed as the median and interquartile range (IQR) or mean and standard deviation as appropriate. Statistical analyses were performed using the statistical software package IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY: IBM Corp.

RESULTS

Baseline characteristics. During the study period, data from 240 patients from 13 HSCT units belonging to the GETH group were collected. Eighty-one patients were excluded for different reasons (figure 1). Finally, 159 patients were included for the analysis. Ninety-four (59%) were men. Mean age was 48 (± 13) years. All the demographic characteristics of the patients at baseline are summarized in table 1.

Prophylaxis with micafungin and outcome. The median (range) days of prophylaxis with micafungin were 18 (13-24) days. The main reason to micafungin discontinuation was instauration of fungal empirical therapy in 7 (4.4%) patients. There was only 1 (0.6%) patient who discontinued because of toxicity. The most common dose used by the centres was 50 mg/day, in 152 (95.6%) patients, while (4.4%) received 100 mg/day.

Breakthrough IFI was unfrequent with 1 and 6 proven or probable IFI documented through the follow-up. Data regarding these patients are summarized in table 2. The median days of treatment with micafungin in these patients was 14 (10-25) days at a dose of 50 mg/day. Five of these patients died: two due to multiple organ failure, 1 patient because of sinusoidal obstruction syndrome and one because of staphylococcal septic shock. Six other patients died because of different complications related to the underlying disease and its treatment. None of the deaths was attributed to fungal infection.

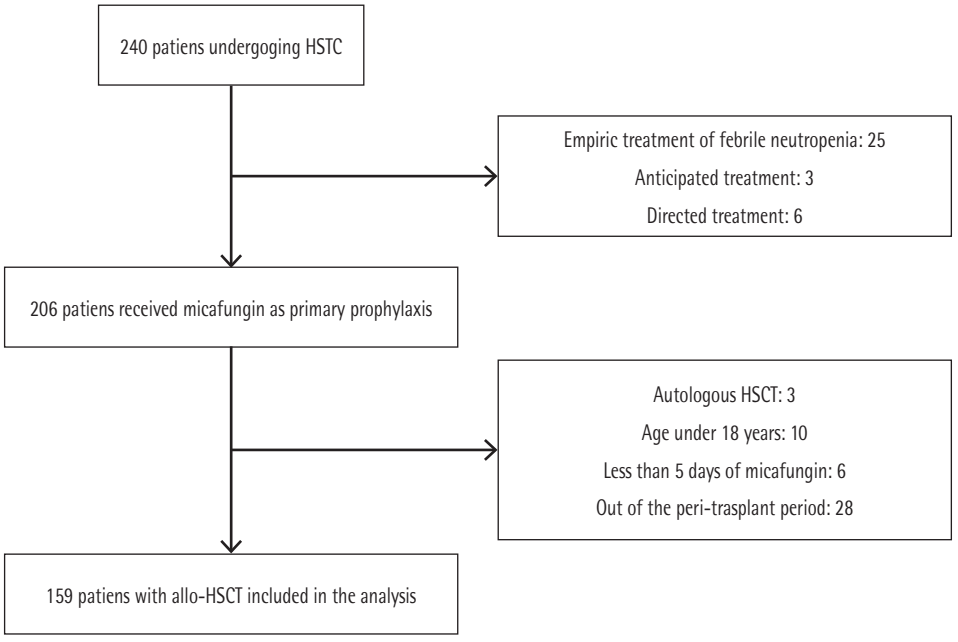

Figure 1 Patient selection flowchart

HSCT: hematopoietic stem cell transplantation

Table 1 Demographic characteristics of all patients (n =159) with allo-HSCT.

Male sex	94 (59.1)
Age, years, mean (SD)	47.8 (±12.7)
Underlying hematologic disease	
Leukaemia	82 (51.6)
Lymphoma	34 (21.4)
Myelodysplastic syndrome	26 (16.4)
Multiple myeloma	8 (5)
Other pathologies	9 (5.7)
Type of allo-HSCT	
Peripheral blood	151 (95)
Bone marrow	6 (3.8)
Umbilical cord	2 (1.3)
Median (IQR) days of neutropenia (< 500 cells x 10^9/L)	16 (12 – 20)
Patients with neutropenia (< 500 cells x 10^9/L) during > 10 days	130 (81.8)

Results are expressed in n (%) unless otherwise stated.
Allo-HSCT: allogeneic hematopoietic stem cell transplantation

Side effects. Only one patient presented serious drug interactions that obliged micafungin cessation consistent in drug-drug interaction with concomitant therapies that were not referred. Two other patients presented mild liver enzyme elevation during treatment but no need of antifungal treatment discontinuation was needed. Overall, prophylaxis with micafungin was considered effective. This is no fungal infection and no requirement to stop de drug because of toxicity/interactions in 151 (94.9%) of the patients.

Prophylaxis efficacy. Besides those patients who developed probable/proven IFI or toxicity, 24 patients changed the antifungal prophylaxis because of suspected IFI, although finally only in seven of them a probable or proven IFI was detected. Therefor micafungin was considered effective in 151 (94.9%) patients.

DISCUSSION

Our study, in different Spanish centres, indicates that the use of micafungin as prophylaxis in the allo-HSCT is well tolerated and effective. The rate of prophylaxis failure (combination of development of IFI or requirement to change the antifungal therapy because of drug-drug interactions) was 5.1%, while it was successful in 151 (94.9%) patients.

Micafungin is a semi-synthetic lipopeptide echinocandins which blocks the synthesis of 1,3-b- D-glucan, a major component of the cell wall of most fungal cells [18]. Its convenient once-daily dosage regimen, the good safety and its low drug-drug interactions profile, [12, 19] have positioned it as a good alternative in patients undergoing allo-HSCT during the neutropenic phase. In 2014 Ziakas *et al.* published a meta-analysis to evaluate the comparative effectiveness of systemic antifungal prophylaxis in HSCT recipients, including data of 4,823 patients from twenty studies considered evaluable. Although

Table 2 Clinical characteristics and putcome of breackthrough IFI.

Case	Underlying disease	Type of HSCT	Micafungin dose (mg/d)	Duration (days)	Days from micafungin initiation to IFI diagnosis	Compatible radiological findings with IFI	Mycological criteria for IFI	EORTC IFI grade	Change to directed antifungal treatment	Outcome	Cause of death	IFI-related death
1	Leukaemia	UC	50	29	10	Yes	Positive GMN	Probable	No	Death	MOF	No
2	Leukaemia	PB	50	12 + 11	N/A	Yes	Positive GMN	Probable	Yes[a]	Death	*S. aureus* bacteremia	No
3	Lymphoma	PB	50	14	13	Yes	Positive GMN	Probable	Yes[b]	Successful	--	--
4	MDS	PB	50	25	17	Yes	Positive GMN	Probable	Yes[c]	Successful	--	--
5	Lymphoma	PB	50	6 + 15	N/A	Yes	Positive GMN	Probable	Yes[d]	Death	MOF	No
6	Lymphoma	PB	50	10	10	Yes	Positive GMN *Aspergillus flavus* in *sputum sample*	Probable	Yes[e]	Death	VOD	No
7	Leukaemia	PB	50	25	24	No	*Fusarium solani* in skin biopsy	Proven	Yes[b]	Successful	--	--

HSCT: Hematopoietic stem cell transplant; IFI: Invasive fungal infection; MDS: myelodysplastic syndrome; UC: umbilical cord; PB: peripheral blood; BM: bone marrow; GMN: galactomannan; MOF: multiple organ failure failure; VOD: veno-occlusive disease; N/A: not applicable.

[a]Case 2 received 12 days of primary prophylaxis with micafungin, substituted for liposomal amphotericin during 11 days, changed again to micafungin at dose of 50mg/d during 11 days, and after changed again to liposomal amphotericin during 20 days and finally to caspofungin for 4 days. The first positive galactomannan was at the end of treatment with caspofungin (24 days after the last dose of micafungin). Treating clinicians considered failure of prophylaxis with micafungin.

[b]Not specified.

[c]To voriconazole.

[d]Case 5 received initially 6 days of primary prophylaxis with micafungin. He developed fever and a lobar infiltrate evident on chest x-ray and directed treatment with liposomal amphotericin was started for 19 days. Prophylaxis with micafungin was restarted at dose of 50mg/d. After 15 days, bilateral nodules and pleural effusion compatible with IFI were evident on the CT scan. Two determinations for GMN were positive and directed treatment with caspofungin was started. Treating clinicians considered failure of prophylaxis with micafungin.

[e]To liposomal amphotericin.

fluconazole continued to be the most widely used agent, micafungin proved to be more effective than fluconazole for the prevention of all mold infections and invasive aspergillosis, reducing the need for empiric antifungal treatment [20]. In the same way Wang et al. analysed data from ten randomized controlled trials involving 2,837 patients with the aim to compare the efficacy and safety between echinocandins and triazoles for the prophylaxis and treatment of fungal infections. The results positioned echinocandins to be as effective and safe as triazoles for the prophylaxis and treatment of patients with fungal infections [21].

Besides efficacy, side-effects with equinocandins, including micafungin, are less common compared to azoles, which conditions better tolerance [15, 21, 22]. All these facts have conducted some guidelines focused on antifungal prophylaxis to recommend the use of micafungin with equal strength to other prophylaxis strategies during the neutropenic phase following allo-HSCT [17, 23]. Nevertheless to date, few observational studies have evaluated the role of micafungin as prophylaxis during the pre-engraftment period of allo-HSCT in day-to-day clinical practice in adult population. Hirata et al. carried out a retrospective study to assess the antifungal prophylactic efficacy, safety, and tolerability of micafungin, 150 mg daily, in a group of patients with haematological malignancies undergoing chemotherapy or HSCT. The strategy led to a significant decrease in IFI with few side effects. However, it must be borne in mind that the results with micafungin were compared to a group of patients who did not receive systemic antifungal prophylaxis [14]. Nachbaur *et. al.* described the results of another retrospective cohort involving one hundred patients with different haematological malignancies at risk for IFI, including patients undergoing allo-HSCT, who received primary antifungal prophylaxis with micafungin at a daily doses of 50 mg during neutropenia. Compared to a historical cohort with posaconzale, micafungin was at least as effective in preventing IFI, with an incidence of proven and probable breakthrough IFI of 3-6% in both groups [24]. Finally, two prospective observational studies have been published in the last years. El-Cheikh *et. al.* carried an observational single-centre trial with 26 patients receiving allo-HSCT from a French hospital who received prophylaxis with micafungin 50 mg/daily, with no *Candida* spp. and/or *Aspergillus* spp. breakthrough infections documented an no drug-related adverse events [25]. More recently, data from a prospective multicentre post-marketing observational surveillance study to assess the safety and efficacy of micafungin in Japanese patients undergoing HSCT. Among 241 patients, breakthrough IFI was documented in 4.4% of the cases. Unlike the previous studies, adverse drug reactions were much more frequent (36%), mainly as hepatobiliary disorders [26].

Our findings, in accordance with other series, show that patients who receive an allo-HSCT can receive micafungin as first-line prophylaxis for IFI, with success rates of approximately 95%. There are two further points in our study that we consider remarkable. First, according to our results, a dose of micafungin 50 mg/daily seems effective in preventing IFI in this high-risk population of patients undergoing allo-HSCT, with a good safety and tolerability profile. These results are consistent with other authors experience [27]. On the other hand, our experience with regard to side effects with micafungin has been quite satisfactory, with only one case of documented serious adverse effect in 159 patients completing a micafungin-based regimen. This factor suggests an advantage of micafungin, which should be taken into account in considering other treatments used previously.

Our study's limitations are those inherent to a retrospective design. Nevertheless, it is, to our knowledge, the only series published to date with micafungin as primary prophylaxis in allo-HSCT recipients in Spain in a very homogeneous group of patients. This posits a useful alternative in day-to-day clinical practice.

In conclusion, according to our experience, micafungin, 50mg/daily, is an appropriate alternative for antifungal prophylaxis in patients undergoing an allo-HSCT, because it's efficacy, its low profile of drug interaction and side effects.

ACKNOWLEDGEMENTS

This study was presented in part as a poster presentation (P169) at the 41st Annual Meeting of the European Society for Blood and Marrow Transplantation (Istambul, March 2015) and as poster presentation (presentation code 725) at the XIX Congreso Nacional de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (Sevilla, March 2015).

FUNDING

This study has been partially funded by Astellas.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi: 10.37201/req/108.2019

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Seroprevalencia de anticuerpos frente al virus del sarampión en Galicia: tendencias durante los últimos diez años en función de la edad y sexo

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Article history
Received: 19 December 2019; Revision Requested: 7 January 2020; Revision Received: 30 January 2020; Accepted: 12 February 2020; Published: 2 March 2020

RESUMEN

Objetivos. En 1998 la Región de Europa de la Organización Mundial de la Salud fijó el objetivo de eliminar el sarampión. En este estudio se analizó la prevalencia de la inmunidad frente al virus del sarampión en la población del área sanitaria de Santiago de Compostela a partir de los datos obtenidos entre 2008-2018.

Pacientes y métodos. Se estudiaron 7.150 pacientes diferentes que se dividieron en grupos según su año de nacimiento: 2010-2017, 2000-2009, 1990-1999, 1980-1989, 1953-1979 y <1953. La determinación en suero de IgG frente al virus del sarampión se realizó mediante un inmunoensayo quimioluminiscente comercializado.

Resultados. Se observó un mínimo (76%) para las tasas de protección frente al virus del sarampión en los nacidos entre 1990-1999. Por grupo de edad se vio que en todos los grupos las mujeres presentaron un porcentaje superior de anticuerpos frente al sarampión. En un modelo de regresión logística con año de nacimiento y sexo se obtuvo una odds ratio para el año de nacimiento (p<0,001) de 1,06 y para el sexo (p=0,0013) de 0,82.

Conclusiones. Se observaron seroprevalencias inferiores a partir de la implantación de la vacuna, un cambio más acusado durante el periodo de implantación y desde el plan de vacunación para el sarampión del año 2000 en Galicia, las tasas de protección frente al virus del sarampión han ido aumentado en nuestra área. Aunque se observó una mayor proporción de mujeres protegidas frente a la de hombres, estas diferencias fueron escasas.

Palabras clave: sarampión; sexo; edad; inmunidad; serología; España; Galicia

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Seroprevalence of antibodies against measles virus in Galicia: trends during the last ten years depending on age and sex

ABSTRACT

Objectives. In 1998, the Europe Region of the World Health Organization set the goal of eliminating measles. In this study, the prevalence of immunity against measles virus in the population of the health area of Santiago de Compostela was analyzed based on data obtained between 2008-2018.

Methods. A total of 7,150 different patients were studied and divided into groups according to their year of birth: 2010-2017, 2000-2009, 1990-1999, 1980-1989, 1953-1979 and <1953. The serum determination of IgG against measles virus was performed using a commercialized chemiluminescent immunoassay.

Results. A minimum (76%) was observed for measles virus protection rates in those born between 1990-1999. By age group it was seen that in all groups the women presented a higher percentage of antibodies against measles. In a logistic regression model with year of birth and sex, an odds ratio of 1.06 (p<0.001) was obtained for the year of birth and of 0.82 (p=0.0013) for sex.

Conclusions. It was observed lower seroprevalences from the implantation of the vaccine and a more pronounced change during the implantation period. From the vaccination plan for measles of the year 2000 in Galicia, the rates of protection against the virus of the measles have been increasing in our area. Although there is a greater proportion of women protected against men, these differences are small.

Key words: measles; sex; age; immunity; serology; Spain; Galicia

INTRODUCCIÓN

El sarampión o primera enfermedad exantemática es una enfermedad febril aguda, muy contagiosa, resultado de la in-

fección por el virus del sarampión, un virus ARN perteneciente al género *Morbillivirus* de la familia Paramyxoviridae [1]. El sarampión puede presentar diversas complicaciones (neumonía, croup, afectación grave del sistema nervioso central (SNC), etc) que son más frecuentes en niños, jóvenes, adultos mayores de 20 años, embarazadas y personas con el sistema inmunitario debilitado. En el tracto respiratorio la neumonía es causa de la mayoría de la mortalidad y morbilidad asociadas al sarampión. La queratoconjuntivitis, otra complicación del sarampión, fue causa frecuente de ceguera antes de la amplia distribución de la vacuna para el sarampión. La infección con sarampión durante el embarazo se asocia con aborto espontáneo, bajo peso al nacer y muerte de la madre. Las complicaciones en el SNC son raras pero muy graves (discapacidad intelectual, sordera, muerte). La mejor manera de prevenir el sarampión y sus complicaciones es mediante la vacunación [1], especialmente teniendo en cuenta que esta enfermedad fue una de las principales causas de mortalidad y morbilidad infantil antes de la introducción de la vacuna en la década de los 60 del siglo pasado. La inmunidad de por vida que sigue al sarampión y a su vacuna se debe a los anticuerpos IgG neutralizantes.

El sarampión posee las siguientes características que hacen factible su control y eliminación de forma eficaz: es una enfermedad viral cuya infección natural confiere inmunidad de por vida; se transmite de persona a persona; no se conocen reservorios diferentes a los humanos; es producida por un solo serotipo con elevada estabilidad antigénica y por último, existe una vacuna eficiente y segura que protege contra la infección y confiere inmunidad.

La primera vacuna antisarampión autorizada en España fue en 1965 pero fue retirada en 1969 por los efectos adversos que provocaba. En 1975 se autorizó una segunda vacuna (vacuna atenuada, cepa Schwartz) que en 1978 el Ministerio de Sanidad la incluyó en el calendario vacunal para niños de 9 meses. La aceptación de esta vacuna fue escasa tanto por los padres como entre el personal sanitario, tal vez por el recuerdo de la vacuna anterior. Así, la cobertura vacunal en 1978 no llegaba al 4% y en 1981 era del 29%. En 1981 se sustituye esta vacuna monovalente para sarampión por la triple vírica sarampión, rubeola, parotiditis (SRP) a los 15 meses y no a los 9. Esta vacuna tuvo gran aceptación. Así la cobertura vacunal llegó al 47% en 1982, al 80% en 1986 y del 90% en 1993 [2]. En 1995 se introdujo una segunda dosis de SRP a los 11 años que alcanzó a los nacidos a partir del año 1984. En 1999 en Galicia se adelantó esta segunda dosis a los tres años para alcanzar los objetivos del Programa de eliminación del sarampión de la Oficina para la Región Europea de la OMS. Con este adelanto se retiró la dosis de SRP a los 11 años de edad, y entre octubre de 1999 y abril de 2000 se desarrolló una campaña de vacunación en la que se ofreció la segunda dosis a los que entonces tenían entre 3 y 11 años de edad. Al final de la campaña se estimó que la cobertura real en estos niños sería próxima al 94%. Finalmente, en enero de 2014 la primera dosis de SRP se adelantó a los 12 meses [3].

En 1998, la Oficina Regional para Europa de la OMS, donde se incluye España, fijó el objetivo de eliminar el sarampión en 2010 [4]. Posteriormente fue ampliado al año 2015 tras la revisión de los progresos realizados por los diferentes países [5, 6].

En España este objetivo de la OMS se estableció en el "Plan de eliminación del sarampión en España" del año 2000. Una de las estrategias para conseguir este objetivo es alcanzar altas coberturas de vacunación sistemática (≥95%) [4].

En Galicia el gobierno autónomo puso en marcha en el año 2000 el "Programa galego de eliminación do sarampelo" [7].

No obstante, a pesar de los esfuerzos realizados se han registrado durante los últimos años diversos brotes de sarampión en España y en otros países europeos [8, 9].

Para evaluar el estado inmunitario frente a las infecciones inmunoprevenibles existen dos aproximaciones, una mediante la revisión de registros de vacunación y otra mediante la realización de estudios de seroprevalencia [10-14].

En este estudio, se analizó la prevalencia de la inmunidad frente al virus del sarampión en la población del área sanitaria de Santiago de Compostela a partir de los datos demográficos y serológicos (IgG Anti- Virus Sarampión) obtenidos en los últimos 10 años (2008-2018).

PACIENTES Y MÉTODOS

Se trata de un estudio observacional descriptivo transversal. A partir del sistema de información del laboratorio del Servicio de Microbiología del Hospital Clínico Universitario de Santiago de Compostela se obtuvieron todos los resultados de la variable anticuerpos de tipo IgG frente al virus del sarampión realizados entre los años 2008 a 2018. Junto a estos resultados también se obtuvieron las variables fecha de nacimiento y el sexo del paciente, así como la fecha de la determinación para calcular la variable edad en el momento de la determinación.

Criterios de selección y población de estudio. Al ser un estudio anonimizado y no disponer del nombre ni del número de historia clínico del paciente, se eliminaron todos los resultados que tenían duplicados la fecha de nacimiento y el sexo. Por otra parte, como la primera dosis de vacuna se recibe a los 12 meses desde de 2014, también se eliminaron los resultados de aquellos pacientes menores de un año en el momento de la determinación. Para el estudio se dividieron los pacientes en los siguientes grupos según el año de nacimiento: 2010-2017, 2000-2009, 1990-1999, 1980-1989, 1953-1979 y <1953. El objetivo de estos grupos fue estudiar la relación entre la proporción de personas con inmunidad para el sarampión y el año de nacimiento. Se dividieron los grupos de edad en intervalos de 10 años desde la implantación de la vacuna triple vírica (1981) para estudiar la evolución del estado vacunal.

Determinación de anticuerpos de IgG. La determinación en suero de anticuerpos de tipo IgG frente al virus del sarampión se realizó mediante un inmunoensayo quimioluminiscente comercializado (LIAISON measles IgG, Diasorin Italy) siguiendo las instrucciones del fabricante.

Análisis estadístico. En el estudio se analizaron las variables independientes edad, sexo, grupo de año de nacimiento y la variable dependiente resultado de anticuerpos IgG para el virus del sarampión. Las variables cuantitativas, se describieron mediante su mediana, mínimo y máximo, percentil 25 y 50, y las cualitativas mediante su distribución de frecuencias. En la comparación de variables cuantitativas se utilizaron el test "t de student", mientras que en la de variables cualitativas, la "chi cuadrado de Pearson". Por último, para el análisis multivariable se utilizó la regresión logística. En todos los casos se utilizó el programa estadístico Stata 13.1.

RESULTADOS

Los resultados que presentamos corresponden a 7.150 pacientes con una mediana de edad de 34 años (máximo de 93 años y mínimo de 1 año con siete días, P25 = 22,5 años, P75 = 49 años), de los cuales 2.468 eran hombres (34,5%) y 4.682 (65,5%) mujeres. En nuestro estudio, no se observaron diferencias significativas para la edad entre hombres y mujeres.

Los resultados de la relación entre la protección frente al virus del sarampión y el año de nacimiento representada por la proporción de anticuerpos IgG anti virus de sarampión y el grupo de año de nacimiento se muestran en la figura 1.

Se observaron diferencias significativas (p<0,001) entre hombres y mujeres en el porcentaje de los que presentaban presencia de anticuerpos protectores frente al virus del sarampión 87% (2.147/2.468) en hombres vs 90,2% (4.225/4.682) en mujeres.

Dentro de cada grupo de edad, la relación entre el sexo con la presencia de anticuerpos frente al virus del sarampión se muestra en la tabla 1.

Con la totalidad de datos se calculó un modelo de regresión logística. La variable dependiente fue el valor negativo o positivo de la presencia de anticuerpos de tipo IgG frente al virus sarampión en suero y como variables independientes el año de nacimiento y el sexo. Se obtuvo un modelo estadísticamente significativo (p<0,001) con una odds ratio para el año de nacimiento (p<0,001) de 1,06 (IC 95% 1,05 a 1,06) y para el sexo (p=0,0013) de 0,82 (IC 95% 0,7 a 0,96). Si consideramos un resultado de anticuerpos de tipo IgG negativo frente al virus del sarampión como factor de riesgo, entonces al aumentar el año de nacimiento (pacientes más jóvenes) este factor de riesgo se multiplica por 1,06, mientras que por otra parte las mujeres tendrían disminuido el factor de riesgo por 0,82.

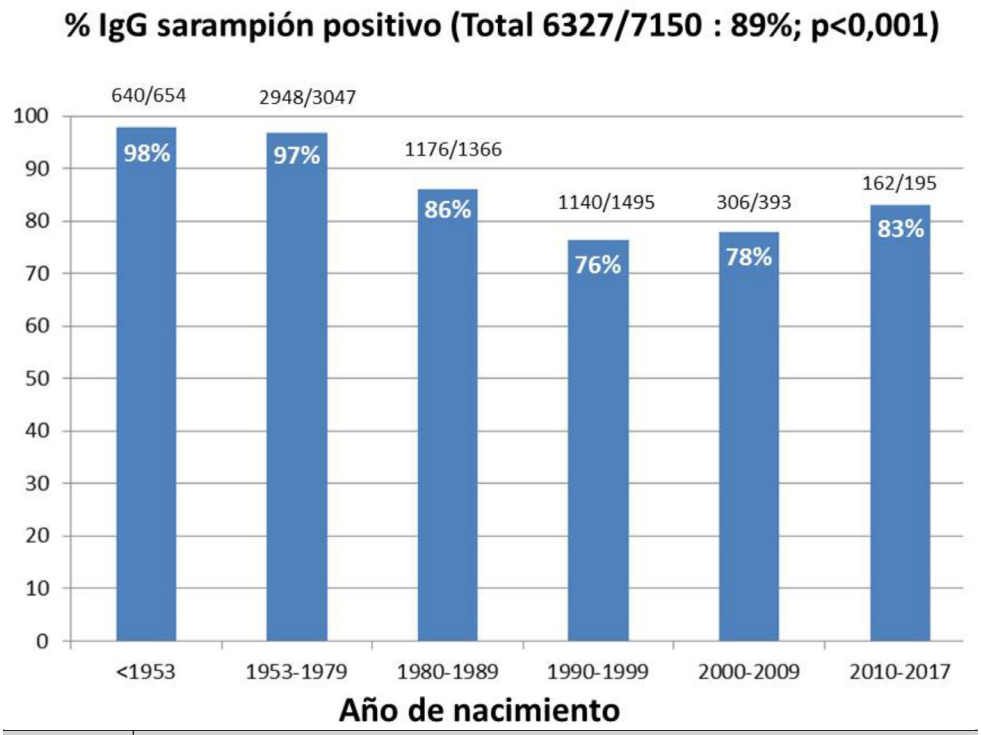

Figura 1 Relación entre la proporción de IgG para sarampión y grupo de año de nacimiento

1990-1999 vs 2010-2017: p=0,033; 1980-1989 vs 1990-1999: p<0,001; 1953-1979 vs 1980-1989: p<0,001.

Tabla 1 **Relación entre el sexo y la presencia de anticuerpos de tipo IgG frente al virus del sarampión dentro de cada grupo de edad**

Grupo edad	Total (%IgG)	Hombres (%IgG)	Mujeres (% IgG)	P
2010-2017	162/195 (83,1)	97/118 (82,2)	65/128 (84,4)	0,687
2000-2009	306/393 (77,9)	151/201 (75,1)	155/192 (80,7)	0,181
1990-1999	1.140/1.495 (76,3)	371/505 (73,5)	769/990 (77,7)	0,07
1980-1989	1.176/1.366 (86,1)	330/398 (82,9)	846/968 (87,4)	0,03
1953-1979	2.948/3.047 (96,8)	925/964 (96)	2.023/2.083 (97,1)	0,092
<1953	640/654 (97,9)	273/282 (96,8)	367/372 (98,7)	0,106
Total	6.372/7.150 (89,1)	2.147/2.468 (87)	4.225/4.682 (90,2)	<0,001

DISCUSIÓN

A pesar de los esfuerzos para erradicar el sarampión, a día de hoy siguen apareciendo casos de esta enfermedad en todo el mundo [4, 15-18]. En Galicia, los dos últimos casos reportados son de 2019, un paciente 37 años en la provincia de Ourense [19] y otro de 39 años de edad en la provincia de Lugo [20].

Los resultados de nuestro estudio señalaron una proporción de IgG positiva para el sarampión de más del 95% sólo en los nacidos antes de 1980. Nuestros resultados coinciden en parte con los obtenidos en la última encuesta de seroprevalencia en Galicia [3] realizado en grupos de edad entre los 18 y los 64 años (nacidos entre 1995 y 1949). En ésta se obtienen resultados de IgG positiva para sarampión superiores al 90% en todos los grupos de edad y por encima del 95% en los nacidos antes de 1983.

En 2015 se publicó la IV encuesta de serovigilancia de la comunidad de Madrid [21]. En esta encuesta los resultados para IgG de sarampión fueron superiores al 95% en todos los grupos de edad desde los 2 a los 60 años.

En el País Vasco, la primera encuesta de seroprevalencia, publicada en 2011 [22], con grupos de edades comprendidos entre los 2 y los 59 años, mostró seroprevalencias del 89% para el grupo 10-14 años (serían los nacidos entre 1999-1995 porque el estudio se realizó en 2009) y del 92% para el grupo 15-19 años (1994-1990). A pesar de que las proporciones en el País Vasco fueron superiores a las de nuestro estudio, en su encuesta también se observó un mínimo en los nacidos entre 1990 y 1999.

A nivel nacional la última encuesta fue en 1996 aunque se está trabajando en una nueva [23]. En la encuesta de 1996, realizada por el Centro Nacional de Epidemiología [24, 25], los grupos de edad se comprendían entre los 2 y los 39 años (nacidos entre 1994 y 1957). Encontraron por debajo del 95% de seroprevalencia para anticuerpos frente al sarampión los grupos de 6-9 años (nacidos en 1990-1987, 90%) y 15-19 años pero con un 94,8%.

En un estudio realizado en Francia el año 2013 con donantes de sangre, se encontró un 10% de los pacientes de entre 18-25 años y un 8,6% entre 26 y 32 años desprotegidos para el sarampión. En este estudio sólo se analizaron estos dos grupos [26].

En Italia, un estudio publicado en el año 2000 con grupos de edad que iban desde los cero a más de 40 años, sólo los grupos de 20 años o más presentaban seroprevalencias superiores al 95% [27].

El European Centre for Disease Prevention and Control (ECDC) publicó en 2018 [28] que sólo unos pocos países en Europa alcanzan el 95% de cobertura vacunal para las dos dosis (Suecia, Islandia, Portugal, Hungría). Según estos mismos datos, España se encontraría con una cobertura vacunal por encima del 95% para la primera dosis y de un 94% para la segunda.

Teniendo en cuenta la implantación de la vacuna para sarampión en nuestro país, se observa que en los nacidos antes de 1981 la inmunidad al sarampión no se debe principalmente a la vacunación sino a la alta incidencia de esta infección en la era prevacunal y en los primeros años de implantación de la vacuna [25]. La protección observada se debería a la transmisión natural del virus.

A partir de 1987 en España se produjo un gran descenso en la incidencia de la infección [25]. En esta situación, los nacidos desde entonces tendrán muy pocas posibilidades de exponerse al virus [3]. Pensamos que la menor proporción de seroprotección en los nacidos entre 1990 y 2000 podría deberse a ser los años iniciales en un cambio en la manera de adquirir la inmunidad: por contacto con el virus en la era prevacunal, con una gran incidencia de la infección, a sólo mediante la vacuna a partir del año 1987. Otro factor a tener en cuenta podría ser el descenso en las vacunaciones por la pérdida de concienciación debido al escaso número de casos.

Desde la implantación de la vacuna, las seroprevalencias que encontramos en nuestro estudio son inferiores a las de los diferentes estudios mencionados en otras regiones de España. Esta infravaloración de nuestros resultados pensamos que es debida a las limitaciones propias de nuestro estudio como podrían ser la falta de aleatoriedad en la selección de los pacientes, el origen desconocido de los mismos, la localidad del estudio, no se excluyeron pacientes con posibles déficits inmunológicos o tratamiento inmunosupresor, o a que se recogieron únicamente datos de pacientes a los que por alguna razón desde el punto de vista clínico se decide hacer el estudio serológico de inmunización frente al sarampión y por tanto puede no representar el conjunto de la población.

Pese a estas limitaciones, en nuestro estudio se observó que la proporción de personas con IgG positivas para el sarampión fue aumentando en las personas más jóvenes a partir del año 2000, cuando comienza el plan de vacunación de la Xunta de Galicia, tras el mínimo alcanzado entre la población nacida entre 1990 y 1999.

Por lo que se refiere a la influencia del sexo, al comparar las tasas de protección frente al virus del sarampión entre hombres y mujeres, se observó que en conjunto las mujeres

parecen tener una cierta "ventaja protectiva" frente a los hombres, en torno a un 3%. Conviene señalar que la diferencia en la respuesta a la vacunación según el sexo ya había sido descrito previamente [29] refiriendo una mejor respuesta de anticuerpos en mujeres al administrar ciertas vacunas como era el caso de la vacuna frente al sarampión. Sin embargo, con respecto al desarrollo de inmunidad, los programas de vacunación en niños asumen que vacunando a todos los niños de la misma manera se asegura alcanzar las concentraciones individuales de anticuerpos protectores necesarios para conseguir la inmunidad de grupo sin importar el sexo. No obstante, también se debe considerar que con independencia de la inmunidad adquirida, la susceptibilidad a algunas enfermedades infecciosas en la infancia difiere entre niños y niñas [30]. Las posibles explicaciones para estas diferencias entre sexos incluirían factores genéticos, ambientales y las hormonas sexuales estradiol y testosterona que tienen una influencia positiva y negativa, respectivamente, en el sistema inmune [31]. En un estudio realizado en Holanda para conocer la respuesta, entre otras vacunas, a la vacuna del sarampión durante la infancia, concluyen que las diferencias entre niños y niñas son escasas y que cuando se observan diferencias, como en el caso del sarampión, las niñas se ven favorecidas [31].

Nuestro estudio tiene las limitaciones, algunas ya citadas anteriormente, inherentes a este tipo de trabajos retrospectivos al no disponer de una muestra que se haya seleccionado para representar de forma fiable a la población en base a criterios como edad, sexo, factores demográficos y socioeconómicos. Estos tipos de estudios requieren un elevado coste, sin embargo nuestra aproximación resulta económica [10].

Como conclusiones, en nuestro trabajo se observó una infravaloración de la seroprevalencia para los grupos de edad incluidos desde la implantación de la vacuna. Esta desviación creemos que se debe a las limitaciones propias de este trabajo junto al cambio producido en la forma de adquirir inmunidad al sarampión desde la implantación de la vacuna. Estos años de cambio coinciden con un descenso en la seroprevalencia que en nuestro estudio es más acusado. Por otro lado, aunque se observa una mayor proporción de mujeres protegidas frente a la de hombres, estas diferencias son escasas, no consistentes y carecemos de resultados como para evaluar si esta pequeña diferencia tiene correspondencia con el curso clínico o el desarrollo de la enfermedad. Por último, con las limitaciones señaladas nuestros resultados apuntan a que desde la instauración del plan de vacunación para el sarampión del año 2000 en nuestra comunidad (Galicia) las tasas de protección frente al virus del sarampión han aumentado en su conjunto para establecer un escudo inmune protector frente a las amenazas que están surgiendo en otros países de nuestro entorno.

FINANCIACIÓN

Los autores declaran no haber recibido financiación para la realización de este trabajo.

CONFLICTOS DE INTERÉS

Los autores declaran no tener conflictos de interés.

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Official journal of the Spanish Society of Chemotherapy

Original breve

Revista Española de Quimioterapia
doi: 10.37201/req/086.2019

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Epidemiología y clínica de las infecciones y colonizaciones causadas por enterobacterias productoras de carbapenemasas en un hospital de tercer nivel

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Article history
Received: 24 October 2019; Revision Requested: 4 December 2019; Revision Received: 29 January 2020; Accepted: 12 February 2020; Published: 9 March 2020

RESUMEN

Objetivos. Describir la epidemiologia de las Enterobacterias portadoras de carbapenemasas (EPC) en un hospital de tercer nivel.

Material y métodos. Estudio observacional retrospectivo, se incluyeron todos los pacientes con muestra positiva para EPC atendidos en hospitalización o en el servicio de Urgencias, entre el 1 Enero de 2014 y el 31 de Diciembre de 2016.

Resultados. Se incluyeron 272 pacientes (316 muestras): 155 (57%) varones. Media de edad de 70,4 años (IC 95% 68,2 -72,7). Media del índice de Charlson 3,6 (IC95% 3,4-3,8). En el 63,2% la adquisición fue nosocomial, en el 35,3% fue asociada a cuidados sanitarios (ACS). Presentaron infección el 55,1%, siendo la más frecuente la infección del tracto urinario (ITU) (58,7%). Las especies más frecuentes fueron *Klebsiella pneumoniae* (62,7%) y *Enterobacter cloacae* (10,1%). Los tipos de carbapenemasa más frecuente fueron OXA-48 (53,8%) y VIM (43%). La adquisición nosocomial se asoció con el género masculino, trasplante, inmunosupresión, ingreso en Unidad de Cuidados Intensivos (UCI) o Servicio Quirúrgico, tratamiento antibiótico previo, *Enterobacter,* VIM, infecciones respiratorias e intraabdominales. La adquisición ACS se asoció con mayor edad y comorbilidad, procedencia de residencia, sondaje vesical, mayor número de procedimientos ambulatorios, ingreso hospitalario previo, *K. pneumoniae* y *E. coli*, OXA–48, coproducción de betalactamasas de espectro extendido, ITU y sepsis.

Conclusiones. Los pacientes que adquieren la EPC en residencias presentan frecuentemente infección. Los pacientes con adquisición nosocomial se colonizan por EPC en la UCI, en relación a procedimientos invasivos y trasplante. Esta población presenta mayor mortalidad por desarrollar infecciones respiratorias por EPC.

Palabras clave: Carbapenemasa, enterobacterias, multirresistencia.

Epidemiology and clinical of infections and colonizations caused by Enterobacterales producing carbapenemases in a tertiary hospital

ABSTRACT

Objective. To describe the epidemiology of Enterobacterales producing carbapenemases (EPC) in a tertiary hospital.

Material and methods. A retrospective observational study, all patients with a positive sample for EPC treated in hospitalization or in the Emergency Department were included, between January 1, 2014 and December 31, 2016.

Results. A total of 272 patients (316 samples) were included: 155 (57%) male. Mean age of 70.4 years (95% CI 68.2 -72.7). Mean Charlson index was 3.6 (95% CI 3.4-3.8). In 63.2% the acquisition was nosocomial, in 35.3% it was health-care associated (HA). 55.1% presented infection, the most frequent infection was urinary tract infection (UTI) (58.7%). The most frequent species were *Klebsiella pneumoniae* (62.7%) and *Enterobacter cloacae* (10.1%). The most frequent types of carbapenemase were OXA-48 (53.8%) and VIM (43%). The nosocomial acquisition was associated with the male gender, transplantation, immunosuppression, admission to the Intensive Care Unit (ICU) or surgical service, prior antibiotic treatment, *Enterobacter*, VIM, respiratory and intra-abdominal infections. The HA acquisition was associated with age and comorbidity, nursery home origin, bladder catheterization, greater number of outpatient procedures, previous hospital admission, *K. pneumoniae* and *E. coli*, OXA-48, coproduction of extended spectrum betalactamases, UTI and sepsis.

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Conclusions. Patients who acquire EPC in nursery homes frequently have an infection. Patients with nosocomial acquisition are colonized by EPC in the ICU, in relation to invasive procedures and transplantation. This population has a higher mortality due to developing respiratory infections by EPC.

Key-words: Carbapenemase, Enterobacterales, multirresistance

INTRODUCCIÓN

La resistencia a antibióticos representa un problema sanitario a nivel global [1]. Entre los microorganismos resistentes, las enterobacterias representan un reto importante por su rápida adquisición y difusión de resistencias. Las especies principales de enterobacterias portadoras de carbapenemasas (EPC) son *Klebsiella pneumoniae, Enterobacter* spp. y *Escherichia coli* [2]. De acuerdo a la clasificación de las betalactamasas, las carbapenemasas pertenecen a la clase A (KPC), clase B o metalobetalactamasas (VIM, IMP y NDM) y clase D (OXA-48). Se ha observado un aumento progresivo de la incidencia de EPC de acuerdo con los datos de Programa de Vigilancia de la Resistencia a Antibióticos del Centro Nacional de Microbiología [2]. En 2012, España se encontraba en situación epidemiológica de brotes ocasionales hospitalarios [3]. Actualmente, se encuentra en situación epidemiológica de distribución inter-regional [4]. En 2013, un estudio en el que se incluían 83 hospitales españoles [5] describió la situación epidemiológica de las colonizaciones e infecciones por EPC en España.

Los objetivos de este estudio, son describir las características epidemiológicas y clinicas de las infecciones y colonizaciones por EPC en un hospital de tercer nivel; y establecer los factores de riesgo tanto de infección frente colonización, así como los factores de adquisición nosocomial frente a la asociada a cuidados sanitarios (ASC).

MATERIAL Y METODOS

Se trata de un estudio observacional retrospectivo, en el que se incluyeron todos los pacientes con muestra positiva para EPC obtenida en la actividad clínica hospitalaria (muestras clínicas o de cribado) atendidos en el Hospital Universitario Puerta de Hierro Majadahonda (HUPHM), entre el 1 Enero de 2014 y el 31 de Diciembre de 2016, identificados por el Servicio de Microbiología. Se incluyeron los pacientes que requirieron ingreso en hospitalización y/o atención en el Servicio de Urgencias y se excluyeron aquellos procedentes de consultas o muestras notificadas de atención primaria o residencias. El HUPHM es un hospital de tercer nivel, referencia del área sanitaria del noroeste de la Comunidad de Madrid. Es además un hospital de referencia nacional en trasplante de órgano sólido y médula ósea.

Los datos se recogieron en una base de datos diseñada específicamente para el estudio tras una revisión exhaustiva de la historia clinica informatizada del centro, de la aplicación del laboratorio de microbiología que abarca toda el area sanitaria y la revisión de la aplicación de atención primaria e informes de alta de toda la comunidad. Se incluyeron como variables los datos demográficos, comorbilidades y el servicio de ingreso en el que se aisló por primera vez la EPC. Tmabién se recogieron datos del contacto previo con sistema sanitario, los factores de riesgo extrínsecos tales como los procedimientos invasivos realizados durante el ingreso en los 3 meses previos a la adquisición de la EPC y los antibióticos recibidos durante al menos 3 días consecutivos dentro de los 3 meses previos a la adquisición.

Se consideró que la adquisición de la EPC había sido nosocomial cuando se había adquirido después de las 48h de ingreso y cuyos síntomas no estaban presentes previamente. Se consideró infección ACS a toda infección presente al ingreso en los pacientes procedentes de residencia, o con ingreso hospitalario en el último año o que habían sido sometidos a procedimientos invasivos diagnósticos o terapéuticos de forma ambulatoria en los 30 días previos al ingreso. Y se consideró adquisición estrictamente comunitaria el resto de casos. Se clasificó a los pacientes en infectados y colonizados, considerando los criterios de las definiciones de los CDC para cada tipo de infección [6]. Se valoró la presencia de sepsis definida como una puntuación ≥ 2 escala SOFA y la presencia de shock séptico. Se recogieron datos de evolución como la recurrencia definida como nuevo episodio de infección por EPC dentro del año tras el alta y la mortalidad por todas las causas durante el ingreso y hasta el mes tras alta.

Se recogieron tanto el género, la especie causante como el tipo de carbapenemasa para cada infección individualizada. Los aislados bacterianos que presentaron resistencia a carbapenems, fueron enviadas al Instituto Carlos III de Majadahonda para la confirmación fenotípica y genotípica mediante la realización de PCRs múltiples e individuales para detectar los genes que codifican los distintos tipos de carbapenemasas. Se estudió la presencia de betalactamasas de espectro extendido (BLEE) en los casos con perfil fenotípico sospechoso. La sensibilidad antibiótica fue estudiada mediante microdilución y los resultados se interpretaron de acuerdo a los puntos de corte del *Clinical and Laboratory Standards Institute* (CLSI) [7].

Para la descripción de las variables cualitativas se emplearon medidas de distribución de frecuencia en número de casos y porcentaje. Para la descripción de las variables cuantitativas se utilizó la media con el intervalo de confianza y la mediana con el rango. Para la asociación de variables se utilizó el Test de Chi al cuadrado o Test de Fisher. La magnitud de la asociación se calculó mediante regresión logística expresando resultado con Odd Ratio y su intervalo de confianza (IC) al 95%. Posteriormente, el análisis multivariante se ajustó por Índice de Charslon, sexo y edad. El análisis estadístico se realizó mediante el programa estadísticico STATA 14.3.

Dado que se trata de un estudio retrospectivo observacional mediante la revisión de historias clínicas, esta investigación no requirió la obtención de consentimiento informado. Los investigadores preservaron en todo momento la confidencialiadad de los datos mediante el tratamiento codificado de los mismos. Este estudio fue aprobado por el Comité Ético de Investigación Clínica del HUPHM (referencia PI - 154/19).

RESULTADOS

Se incluyeron 272 pacientes (316 muestras). 232 pacientes presentaron un único tipo de EPC (85,3%) y 40 presentaron 2 o 3 EPC diferentes. La incidencia global fue de 0,52 casos por 1000 estancias al año. Presentaron infección clínica 150 pacientes (55,1%) y colonización 122 pacientes (49,9%). De los 272 pacientes, 117 (43%) fueron mujeres y 155 (57%) varones. La media de edad fue de 70,4 años (IC 95% 68,2 -72,7). La media del índice de Charlson fue 3,6 (IC 95% 3,4-3,8).

En 172 pacientes (63,2%) la adquisición fue nosocomial, en 96 pacientes (35,3%) fue ACS y en 4 casos estrictamente comunitaria. Con respecto a la adquisición nosocomial, el servicio donde más frecuentemente se adquirió la EPC fue en la Unidad de Cuidados Intensivos (UCI) (46,5%). La estancia hospitalaria media previa a la adquisición fue de 29,7 días (27,9 - 32,2) con una mediana de 18 días. La estancia media en UCI previa a la adquisición fue de 24,4 días (21,9 – 27,0) con una mediana de 14 días. Durante la hospitalización previa a la adquisición se sometieron al menos a un procedimiento invasivo 156 pacientes (89,5%). La media de procedimientos fue 5,6 (IC 5,3 - 5,8). El procedimiento que se realizó más frecuentemente fue el sondaje vesical en 141 pacientes (82%). El 36,4% (99 pacientes) fueron sometidos a intervención quirúrgica correspondiendo 22 de ellas (8%) a trasplante de órgano. Entre los pacientes con adquisición ACS el 71,9% habían tenido algún ingreso en el último año. La cateterización urinaria fue el procedimientos ambulatorio más frecuentemente realizado en el último mes (31,3%). El 67,7% de los pacientes procedían de residencias de ancianos. Con respecto a la exposición previa a antibióticos, 206 pacientes (76,1%) recibieron al menos un antibiótico previo a la adquisición. La media de antibióticos que recibió cada paciente fue 2,1 (IC 95% 2,0 – 2,2).

De las 316 muestras positivas con EPC, 195 se identificaron en muestras clínicas (61,7%) y 121 se identificaron en muestras de exudado rectal. La muestra clínica en la que se identificó más frecuentemente fue en orina (60%). En 29 urocultivos (24,8%) se catalogó bacteriuria asintomática. El género más frecuentemente aislado fue *Klebsiella* con 226 casos (71,5%), seguido de *Enterobacter* con 48 a casos (15,2%). La especie más frecuente fue *K. pneumoniae* con 198 casos (62,7%), seguido de *Enterobacter cloacae* con 32 casos (10,1%), *Klebsiella oxytoca* con 28 casos (8,9%), *E. coli* con 21 casos (6,6%) y otras especies con 37 casos (11,7%). El tipo de carbapenemasa más frecuente en las EPC fue OXA-48 con 170 casos (53,8%), seguido de VIM 136 casos (43%), KPC 9 casos (2,8%) y un único caso de NDM.

La localización más frecuente fue la infección del tracto urinario (ITU) con 95 episodios (58,7%), seguida de infección respiratoria (14,8%), infecciones de piel y partes blandas (IPPB) (11,7%) e infección intraabdominal (10,5%). Se objetivaron 31 bacteriemias (16,6%), 3 asociadas a catéter intravascular, 4 bacteriemias primarias, y el resto secundarias, siendo el foco más frecuente el urinario (48,4%). Con respecto a la gravedad, 40 pacientes (27%) presentaron sepsis y 15 pacientes (10,2%) cumplieron criterios de shock séptico.

En las tablas 1 y 2 se muestran las características epidemiológicas según el lugar de adquisición y los factores de riesgo de infección respectivamente. La adquisición nosocomial se asoció con el género masculino, el trasplante, el tratamiento inmunosupresor, el ingreso en UCI o Servicio Quirúrgico y haber recibido tratamiento antibiótico previamente. La adquisición ACS se asoció con mayor edad, mayor comorbilidad, procedencia de residencia, ser portador de sonda vesical, mayor número de procedimientos ambulatorios en el último mes e ingreso hospitalario previo en el último año. Desde el punto de vista microbiológico, las especies *K. pneumoniae* y *E. coli*, la carbapenemasa tipo OXA-48 y la coproducción de BLEE se asociaron estadísticamente con la adquisición ACS mientras que el género *Enterobacter* y la carbapenemasa tipo VIM se asociaron con la adquisición nosocomial. La infección se asoció con la adquisición ACS, mientras que la colonización se asoció con la adquisición nosocomial. La ITU y la sepsis fueron más frecuentes en los pacientes con adquisición ACS, mientras que las infecciones respiratorias e intraabdominales se asociaron con la adquisición nosocomial. La recurrencia durante el primer año se presentó más frecuentemente en los pacientes con adquisición ACS. Si comparamos aquellos pacientes que fallecieron con los supervivientes, las infecciones respiratorias causaron mayor mortalidad, 27,5% vs. 11,8%, OR 2,8 (IC 95%: 1,2 -7,0), p = 0,024, mientras que las ITU causaron menor mortalidad 45,0% vs 67,3%, OR 0,4 (IC 95%: 0,2- 0,8), p = 0,015.

DISCUSIÓN

Podemos distinguir dos perfiles de poblaciones diferentes que pueden presentar colonización o infección por EPC. Aquellos pacientes de edad avanzada con un Índice de Charlson elevado a costa de comorbilidades asociadas a la edad, que presentan ITU de adquisición ACS, con antecedente de sondaje vesical, causadas por *K.pneumoniae* y *E.coli* portadoras de OXA-48 y BLEE. Esta población se asoció a infección por EPC frente a colonización, presentando con más frecuencia sepsis y recurrencia pero no mayor mortalidad. Y otra población más joven, frecuentemente hombres, con ingreso en UCI o Servicios Quirúrgicos, que habitualmente se colonizaron por EPC tipo VIM y cuyos factores de riesgo de adquisición fueron la antibioterapia previa, trasplante y la realización de procedimientos invasivos. En caso de presentar infección, estos pacientes presentaron más frecuentemente infecciones respiratorias o intraabdominales.

Son escasos los estudios que comparan los pacientes con adquisición ACS frente a los pacientes con adquisición nosocomial. En el estudio de Tang et al. [8] se compara las características de los pacientes según el lugar de adquisición, encontrando mayor edad y mayor proporción de mujeres entre aquellos con adquisición ACS. En el estudio de Palacios–Baena et al. [5], observaron asociación entre la EPC tipo OXA-48 con los pacientes de mayor edad, adquisición ACS (fundamentalmente residencias), ITU, *Klebsiella spp* y BLEE; mientras que los pacientes portadores de EPC del tipo metalobetalactamasas (VIM n=53, IMP n=5) se asociaron a adquisición en UCI y *Enterobacter* spp.

Tabla 1 **Factores de riesgo de adquisición de EPC nosocomial frente ACS en pacientes infectados o colonizados.**

	ACS (n = 96)	Nosocomial	Análisis univariante			Análisis ajustado por edad, sexo e Índice de Charlson		
	n (%)	(n = 172) n (%)	OR	IC 95%	p	OR	IC 95%	p
Edad, media (IC95%)	81,4 (80,0 – 82,8)	64,4 (66,0 - 65,7)	0,93	(0,91-0,95)	< 0,001			-
Sexo (varón)	47 (49,0)	108 (62,8)	1,76	(1,06-2,92)	0,028			-
Comorbilidad								
Insuficiencia cardiaca	41 (42,7)	66 (38,4)	0,84	(0,50-1,39)	0,487			-
Hemiplejia	18 (18,8)	16 (9,3)	0,44	(0,21-0,92)	0,026			-
Demencia	57 (59,4)	27 (15,7)	0,13	(0,07-0,23)	0,001			-
Enf. pulmonar crónica	28 (29,2)	53 (30,8)	1,08	(0,63-1,87)	0,778			-
Diabetes Mellitus	32 (33,3)	48 (27,9)	0,77	(0,45-1,33)	0,352			-
Neoplasia	16 (16,7)	29 (16,9)	1,01	(0,52-1,98)	0,968			-
Trasplante (TOS + MO)	6 (6,3)	34 (19,8)	3,70	(1,49-9,16)	0,003			-
Inmunosupresión	12 (12,5)	41 (23,8)	2,19	(1,09-4,41)	0,025			-
Media Índice de Charlson (IC95%)	4,3 (4,0 – 4,6)	3,3 (3,1 - 3,5)	0,87	(0,79-0,96)	0,004			
Servicio de ingreso								
Médico	88 (91,7)	61 (35,5)	-			-		
Quirúrgico	4 (4,2)	31 (18,0)	11,18	(3,75-33,30)	< 0,001	10,84	(3,46-33,99)	< 0,001
UCI	4 (4,2)	80 (46,5)	28,85	(10,04-82,94)	< 0,001	16,71	(5,44-51,30)	< 0,001
Contacto previo sistema sanitario								
Ingreso año previo	69 (71,9)	84 (48,8)	0,37	(0,22-0,64)	< 0,001	0,50	(0,27-0,93)	0,030
Residencia	65 (67,7)	24 (14,0)	0,08	(0,04-0,14)	< 0,001	0,14	(0,07-0,29)	< 0,001
Sondaje vesical 1 mes previo	30 (31,3)	14 (8,1)	0,20	(0,10-0,39)	< 0,001	0,22	(0,10-0,48)	< 0,001
Media de procedimientos (1 mes previo al ingreso) (IC95%)	0,42 (0,36 - 0,48)	0,15 (0,12 - 1,18)	0,33	(0,20-0,57)	< 0,001	0,26	(0,13-0,53)	< 0,001
Media de antibióticos recibidos en los 3 meses previos (IC95%)	0,94 (0,83 - 1,05)	2,76 (2,60 - 2,92)	2,13	(1,69-2,70)	< 0,001	2,08	(1,59-2,73)	< 0,001
Género y especie								
Klebsiella spp.	82 (85,4)	128 (74,4)	0,50	(0,26-0,96)	0,038	0,82	(0,38-1,76)	0,610
Klebsiella pneumoniae	76 (79,2)	108 (62,8)	0,44	(0,25-0,79)	0,006	0,81	(0,41-1,58)	0,530
Klebsiella oxytoca	6 (6,3)	20 (11,6)	1,97	(0,76-5,10)	0,060	1,17	(0,40-3,42)	0,781
Enterobacter spp.	4, (4,2)	30 (17,4)	4,86	(1,66-14,25)	0,004	2,66	(0,83-8,52)	0,098
Enterobacter cloacae	2 (2,1)	25 (14,5)	7,99	(1,85-34,53)	0,005	5,07	(1,09-23,66)	0,039
Otros *Enterobacter*	2 (2,1)	5 (2,9)	1,41	(0,27-7,40)	0,687	0,47	(0,07-3,12)	0,432
Escherichia coli	8 (8,3)	3 (1,7)	0,20	(0,05-0,75)	0,018	0,12	(0,02-0,73)	0,021
Otros géneros	2 (2,1)	11 (6,4)	3,21	(0,70-14,80)	0,135	2,47	(0,45-13,62)	0,301
Tipo de carbapenemasa								
OXA48	85 (92,4)	60 (35,9)	-			-		
VIM	7 (7,6)	107 (64,1)	21,65	(9,41-49,81)	< 0,001	10,92	(4,44-26,82)	< 0,001
BLEE	48 (50,0)	36 (20,9)	0,26	(0,15-0,46)	< 0,001	0,42	(0,23-0,76)	0,005
Clínica								
Colonización	23 (24,0)	98 (57,0)	-			-		
Infección	73 (76,0)	74 (43,0)	0,24	(0,14-0,42)	< 0,001	0,30	(0,16-0,57)	< 0,001
Tipo de Infección								
ITU	59 (61,5)	30 (17,4)	0,13	(0,07-0,23)	< 0,001	0,16	(0,09-0,31)	< 0,001
Respiratoria	4 (4,2)	20 (11,6)	3,03	(1,00-9,13)	0,040	2,85	(0,86-9,50)	0,088
IPPB	9 (9,4)	10 (5,8)	0,60	(0,23-1,52)	0,276	0,81	(0,29-2,22)	0,677
Intraabdominal	2 (2,1)	15 (8,7)	4,49	(1,00-20,07)	0,033	4,71	(0,96-23,17)	0,057
Bacteriemia	11 (10,3)	20 (11,6)	1,13	(0,51-2,53)	0,943	0,80	(0,31-2,09)	0,654

Tabla 1 **Factores de riesgo de adquisición de EPC nosocomial frente ACS en pacientes infectados o colonizados. (cont.)**

	ACS	Nosocomial	Análisis univariante			Análisis ajustado por edad, sexo e Índice de Charlson		
	(n = 96) n (%)	(n = 172) n (%)	OR	IC 95%	p	OR	IC 95%	p
Gravedad								
Sepsis	30 (41,1)	9 (12,5)	0,20	(0,09-0,47)	< 0,001	0,24	(0,09-0,62)	0,003
Shock séptico	8 (11,0)	7 (9,7)	0,88	(0,30-2,55)	0,807	0,93	(0,27-3,23)	0,910
Evolución								
Recurrencia primer 1 año tras el alta	16 (16,7)	11 (6,4)	0,34	(0,15-0,77)	0,007	0,29	(0,11-0,78)	0,015
Mortalidad (ingreso)	12 (12,5)	38 (22,1)	1,99	(0,98-4,01)	0,056	1,86	(0,84-4,13)	0,126
Mortalidad (hasta 1 mes tras alta)	17 (17,7)	41 (23,8)	1,45	(0,77-2,73)	0,244	1,50	(0,73-3,07)	0,271

Enf. = Enfermedad. TOS = Trasplante órgano sólido. MO = Médula ósea. UCI = Unidad de Cuidados intensivos. BLEE = betalactamasas de espectro ampliado. ITU = Infección del tracto urinario. IPPB = Infección de piel y partes blandas.

En nuestro estudio, se encuentra una mayor proporción de pacientes infectados entre los pacientes con adquisición ACS que en los que presentan adquisición nosocomial como se ha publicado previamente [8-10]. El porcentaje de infección frente colonización puede variar entre el 30 – 60% según el tipo de hospital, así en hospitales de media estancia (HME) y complejidad menor, la infección clínica puede representar el 33,5% a costa de mayor frecuencia de colonización [9, 11], y por el contrario en hospitales de mayor complejidad el porcentaje de infección asciende al 66% [5, 11, 12].

En el estudio de López–Dosil et al. [9], se analizó las infecciones y colonizaciones adquiridas en ambiente nosocomial frente ACS en dos hospitales: un hospital de agudos (HA) frente a un HME, que atienden a pacientes del mismo área que el HUPHM. La colonización fue más frecuente en los pacientes del HME, mientras que los pacientes del HA presentaron más frecuentemente infección. Los pacientes del HA presentaron más frecuentemente infección adquirida ACS, mientras que los pacientes del HME presentaron en la mayoría de los casos adquisición nosocomial. En la discusión, atribuyeron estas diferencias a la procedencia de los pacientes, en el caso del HA un 67,3% provenían de residencias, mientras que en el HME fue un 33,3%. En nuestro estudio, el 37,3% de los pacientes con infección provenían de residencia frente un 27% en los colonizados, estando en el límite de la significación estadística (p = 0,072). Por lo tanto, de acuerdo a nuestro estudio, la infección se asocia con la adquisición ACS (fundamentalmente en residencias) y la colonización con la adquisición nosocomial.

Las formas clínicas de infecciones por EPC más frecuentes fueron las ITU como en otros estudios [5, 9]. La mayor parte de la información clínica y terapéutica se ha obtenido de estudios que incluyen bacteriemias por *K. pneumoniae* sin incluir otras especies de EPC ni otros tipos de infección [13], por lo tanto, pueden no ser representativos de la mayoría de las infecciones por EPC. Las ITU son además, más frecuentemente adquiridas ACS [8-10]. La adquisición ACS se asoció con sepsis y recurrencia de la infección. En los estudios de Palacios–Baena et al. [5] y Tumbarello et al. [14] que incluyeron distintas localizaciones de infección, el 15% de los pacientes presentaron sepsis o shock séptico. Por lo tanto, los pacientes del HUPHM presentaron infecciones con mayor gravedad. Esto puede ser debido a una alta frecuencia de infecciones por EPC en residencias en nuestro medio como también discutió en su artículo López–Dosil et al. [9].

En la práctica clínica existe gran dificultad para diferenciar entre ITU y bacteriuria, sobre todo en pacientes de edad avanzada con enfermedades neurológicas provenientes de residencia. Nuestro estudio revela una importante proporción de estos pacientes con infección, lo que contribuye a una mayor sospecha clínica de infección frente a colonización en estos pacientes. Los estudios de López–González et al. [12] y Qureshi et al. [15] trataron de establecer los factores de riesgo de ITU frente bacteriuria. Son escasos los estudios que tratan de establecer los factores de riesgo de infección frente colonización independientemente del tipo de infección [16].

Gran parte de los pacientes de nuestro estudio que adquirieron colonización por EPC lo hicieron durante el ingreso en UCI, incrementándose además el riesgo de infección cuanto mayor fue la estancia en UCI. Estos pacientes están sometidos con frecuencia a procedimientos invasivos y trasplante de órgano. Borer et al. [16] de forma similar a nuestro trabajo, estableció como factores de riesgo de infección: un Índice de Charlson ≥ 3, la DM, hemiplejia y la enfermedad tumoral. Por otro lado, estableció como factor de desarrollo de infección la exposición a procedimientos invasivos. En nuestro caso, la realización de procesos invasivos se asoció a colonización al ser los pacientes con adquisición nosocomial más frecuentemente sometidos a procedimientos invasivos. En el estudio de Qureshi et al. [15] se estableció como factor de riesgo de ITU frente bacteriuria un mayor Índice de Charlson. Pero al contrario que nuestros resultados, la edad más joven, el sexo masculino y el trasplante de órgano en el estudio de Qureshi et al. [15] se asociaron con riesgo de infección. En este aspecto, nuestro estudio no objetivó mayor riesgo de infección entre los pacientes trasplantados, posiblemente esto se deba a la alta proporción de pacientes trasplantados que se colonizaron tras la cirugía en la UCI y que por tanto se sobreexpresan en el grupo de colonización. En el estudio de Patel et al. [17] observaron que los pacientes trasplantados tienen mayor riesgo de adquisición de EPC, sobre todo en el periodo inmediato

Tabla 2 **Factores de riesgo de infección frente a colonización en pacientes portadores de EPC.**

	Colonización	Infección	Análisis univariante			Análisis ajustado por edad, sexo e Índice de Charlson		
	(n = 122) n %	(n = 150) n %	OR	IC95%	p	OR	IC95%	p
Edad, media (IC95%)	65,63 (63,81 - 67,45)	74,31 (72,93 - 75,67)	1,03	(1,01-1,04)	< 0,001			-
Sexo (varón)	72 (59,0)	83 (55,3)	0,86	(0,53-1,40)	0,542			-
Comorbilidad								
Insuficiencia cardiaca	49 (40,2)	59 (39,3)	0,97	(0,59-1,57)	0,889			-
Hemiplejia	14 (11,5)	21 (14,0)	1,26	(0,61-2,59)	0,536			-
Demencia	30 (24,6)	54 (36,0)	1,72	(1,02-2,93)	0,043			-
Enf. pulmonar crónica	36 (29,5)	45 (30,0)	1,02	(0,61-1,73)	0,930			-
Diabetes Mellitus	28 (23,0)	54 (36,0)	1,89	(1,10-3,23)	0,020			-
Neoplasia	13 (10,7)	32 (21,3)	2,27	(1,13-4,56)	0,018			-
Trasplante (TOS + MO)	18 (14,8)	22 (14,7)	0,99	(0,51-1,95)	0,984			-
Inmunosupresión	21 (17,2)	32 (21,3)	1,30	(0,71-2,40)	0,394			-
Media Índice de Charlson (IC95%)	3,02 (2,81 - 3,24)	4,08 (3,85 - 4,31)	1,18	(1,07-1,31)	0,001			-
Servicio de ingreso								
Médico	55 (45,1)	98 (65,3)	-			-		
Quirúrgico	10 (8,2)	25 (16,7)	1,40	(0,63-3,14)	0,409	1,57	(0,68-3,61)	0,287
UC	57 (46,7)	27 (18,0)	0,27	(0,15-0,47)	< 0,001	0,35	(0,18-0,66)	0,001
Adquisición								
ASC	23 (18,9)	73 (48,7)		(0,00-0,00)				
Nosocomial	98 (80,3)	74 (49,3)	0,24	(0,14-0,42)	< 0,001	0,29	(0,16-0,55)	<0,001
Estancia hospitalaria previa a adquisición	25,08 (22,22 - 27,94)	35,99 (31,19 - 40,78	1,01	(1,00-1,02)	0,049	1,01	(1,00-1,02)	0,025
Estancia UCI previa a adquisición	19,37 (17,05 - 21,71)	32,15 (26,72 - 37,54)	1,02	(1,00-1,03)	0,023	1,02	(1,00-1,03)	0,022
Contacto previo con el sistema sanitario								
Ingreso año previo	63 (51,6)	90 (60,0)	1,40	(0,87-2,28)	0,167	1,03	(0,61-1,74)	0,901
Residencia	33 (27,0)	56 (37,3)	1,61	(0,96-2,70)	0,072	0,95	(0,50-1,80)	0,884
Sondaje vesical 1 mes previo a ingreso	15 (12,3)	29 (19,3)	1,71	(0,87-3,36)	0,120	1,23	(0,60-2,55)	0,571
Media de procedimientos (1 mes previo al ingreso) (IC95%)	0,19 (0,15 - 0,23)	0,29 (0,25 - 0,33)	1,54	(0,92-2,59)	0,100	1,27	(0,74-2,17)	0,391
Media de procedimientos (3 meses previos dentro del ingreso) (IC95%)	4,39 (4,06 - 4,72)	2,83 (2,52 - 3,14)	0,90	(0,84-0,96)	0,001	0,95	(0,88-1,02)	0,151
Media de antibióticos recibidos en los 3 meses previos (IC95%)	2,30 (2,11 - 2,49)	1,89 (1,74 - 2,05)	0,90	(0,80-1,02)	0,099	0,97	(0,85-1,10)	0,608
Género y especie								
Klebsiella spp.	91 (74,6)	122 (81,3)	1,48	(0,83-2,65)	0,181	1,13	(0,61-2,08)	0,699
Klebsiella pneumoniae	78 (63,9)	109 (72,7)	1,50	(0,90-2,51)	0,123	1,15	(0,66-2,00)	0,623
Klebsiella oxytoca	13 (10,7)	13 (8,7)	0,80	(0,35-1,79)	0,580	0,91	(0,38-2,17)	0,823
Enterobacter spp.	18 (14,8)	17 (11,3)	0,74	(0,36-1,50)	0,403	1,05	(0,50-2,23)	0,892
Enterobacter cloacae	13 (10,7)	14 (9,3)	0,86	(0,39-1,91)	0,717	1,18	(0,51-2,72)	0,692
Otros *Enterobacter*	5 (4,1)	3 (2,0)	0,48	(0,11-2,04)	0,318	0,71	(0,16-3,24)	0,663
Escherichia coli	3 (2,5)	8 (5,3)	2,23	(0,58-8,61)	0,243	2,67	(0,63-11,26)	0,182
Otros géneros	10 (8,2)	3 (2,0)	0,23	(0,06-0,85)	0,028	0,24	(0,06-0,94)	0,041
Tipo de carbapenemasa								
OXA48	49 (41,5)	100 (69,0)	-			-		
VIM	69 (56,5)	45 (31,0)	0,32	(0,19-0,53)	< 0,001	0,43	(0,24-0,78)	0,005
BLEE	30 (24,6)	56 (37,3)	1,83	(1,08-3,10)	0,025	1,33	(0,76-2,33)	0,320
Evolución								
Mortalidad (ingreso)	16 (13,1)	34 (22,7)	1,94	(1,01-3,72)	0,045	2,42	(1,21-4,84)	0,012
Mortalidad (hasta 1 mes tras el alta)	18 (14,8)	40 (26,7)	2,10	(1,13-3,90)	0,018	2,44	(1,27-4,68)	0,007

Enf. = Enfermedad. TOS = Trasplante órgano sólido. M.O = Médula ósea. UCI = Unidad de Cuidados intensivos. ASC = Asociado a cuidados sanitarios. BLEE = betalactamasas de espectro ampliado.

postrasplante. Dado que el HUPHM es de referencia en trasplante el conocimiento de la epidemiología y los factores de adquisición de las EPC podría ayudar a reducir la frecuencia de colonización, y el posterior desarrollo de infección. La mortalidad observada es similar a la que se publica en estudios en los que se incluye además de las bacteriemias, otros tipos de infecciones [5]. La mayoría de estudios sobre mortalidad incluyen bacteriemias sin incluir otros tipos de infecciones clínicas [18-20]. Como en otros estudios, encontramos menor mortalidad entre las ITU y mayor mortalidad en las infecciones respiratorias [5]. Por lo tanto, al ser las ITU las infecciones más frecuentes en nuestro estudio, se objetiva menor mortalidad global. Por otro lado, observamos una mayor mortalidad en los pacientes con adquisición nosocomial. Estos pacientes presentaron infecciones respiratorias más frecuentemente.

Entre las principales limitaciones de este trabajo, se encuentra el ser un estudio retrospectivo observacional con las limitaciones que este tipo de estudios conllevan. Sin embargo, se ha realizado una exhaustiva revisión de las historias clínicas y se ha aplicado rigurosamente las definiciones y criterios definidos en el protocolo de recogida de datos. Se trata de un estudio unicéntrico, pero con un tamaño muestral relevante superior a los 245 casos del estudio multicéntrico de Palacios – Baena et al. [5]. Se incluyen además de pacientes con infección, pacientes con colonización, además de los diversos tipos de localizaciones de infección por EPC y distintos tipos de EPC frente otros estudios donde se incluye exclusivamente las bacteriemias por *Klebsiella*. Dados los resultados obtenidos, consistentes con la mayoría de la literatura revisada, los resultados de este trabajo son se suponen extrapolables a otras poblaciones.

Este estudio aporta conocimiento acerca de la epidemiología de las EPC. Los pacientes con EPC de adquisición ACS (fundamentalmente residencias) presentan más frecuentemente infección, estando asociada la ITU y *K.pneumoniae* con OXA-48. Los pacientes con adquisición nosocomial se colonizan por EPC en relación a ingreso en UCI, realización de procedmientos invasivos y trasplante. Esta población en caso de adquirir infección presentan localizaciones que asocian mayor mortalidad.

FINANCIACIÓN

Los autores declaran no haber recibido financión para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Official journal of the Spanish Society of Chemotherapy

Clinical-Pathologic Conference

Revista Española de Quimioterapia
doi:10.37201/req/100.2019

Neck infection after allogenic hematopoietic progenitors transplantation

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Article history
Received: 5 December 2019; Revision Requested: 16 January 2020; Revision Received: 21 January 2020; Accepted: 28 January 2020; Published: 14 February 2020

PRESENTATION OF CASE (DR. JOSEP. MENSA)

A 65-year-old woman, diagnosed with Acute Myeloid Leukemia (AML) in December 2015, electively enters the Hematology Service in August 2016, to receive an allogeneic hematopoietic cell transplantation (ALLO-HCT), of peripheral blood, from an unrelated donor with an HLA 10/10 identity.

The transplant is performed after conditioning with fludarabine and busulfan. She received cyclophosphamide as prophylaxis for graft-versus host disease (GVHD) and subsequently maintained immunosuppression with tacrolimus and mycophenolate. Donor and recipient were seropositive for CMV and the patient received anti-infectious prophylaxis with levofloxacin (500 mg/d), fluconazole (400 mg/d) and acyclovir (800 mg/12h).

In the immediate post-transplant period, she presented grade IV mucositis and required parenteral nutrition and analgesia with opioids.

On the 5th post-transplant day, fever appeared, with no apparent focus, and empirical antibiotic treatment with meropenem was initiated (1 g/ 8 h in 4 h extended perfusion).

Cervical bulk and cardio-respiratory arrest. On the 12th postransplant day, fever persisted and a right submandibular mass appeared. An Ear, Nose & Throat (ENT) consultation suggested a probable sialoadenitis and daptomycin (10 mg/kg/day) was added to the treatment with meropenem. Some blood test data at that time were as follows: PCR 18 mg/dL; Glucose 189 mg/dL; Creatinine 0.76 mg/dL; Glomerular Filtration 83 ml/min; ASAT 73 IU/L; ALAT 34 IU/L; GGT 107 IU/L; Total Bilirubin 0.9 mg/dL; Direct bilirubin 0.4 mg/dL; Alkaline Phosphatase 94 IU/L; LDH 614 IU/L; Na 130 mEq/L; K 3.1 mEq/L; Leukocytes 0.04 x 10^9/L; Hb 9.3 g/dL; Platelet count 38x10^9/L. Ferritin 1,080 ng/mL and sideremia 106 µg/dL. Blood cultures, urine culture and serum Aspergillus Galactomannan Assay (AGA) determinations were negative. Cytomegalovirus (CMV) blood viral load was undetectable in 3 determinations.

In the next 24 hours, the inflammation spreaded through the laterocervical and parotid region and, in the early morning of the 14th post-transplant day, the patient developed a rapidly progressive respiratory difficulty with costal tirage and laryngeal stridor. She was transferred to the emergency operative room where she was admitted with a 3 Glasgow Coma Score and agonic respiration, so advanced Cardio-Pulmonary Resuscitation was initiated. Oro-Tracheal intubation was unsuccessfully attempted under direct vision and with glidescope. Manual ventilation with bag-mask was performed while the ENT surgeon performed an successful emergent tracheostomy, with patient`s recovery of pulse and oxygen saturation. Approximate time without effective ventilation was calculated in about 10 min.

Given the thrombocytopenia and the possibility that the abrupt deterioration was due to a hemorrhagic complication, a continuous perfusion of platelets was maintained during the operation and in the following 24 hours.

ICU admission. The patient was admitted to the Intensive Care Unit (ICU), where on admission macroglossia was observed, making it impossible to assess the situation of the oral cavity. A large indurated mass was palpated in the right latero-cervical and submandibular region. The facies was edematous and there was epistaxis, contained with anterior nasal packing.

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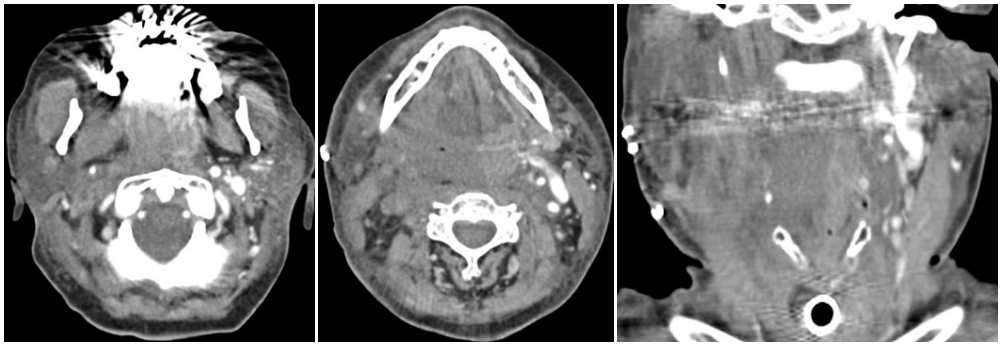

Figure 1 | **Computed tomography scan. Day + 14 after transplantation**

Some analytical data in blood were at that moment as follows: PCR 29 mg/dL, Glucose 158 mg/dL, Cr 1.17 mg/dL, Glomerular Filtrate 48 ml/min, ASAT 121 UI/L, ALAT 43 UI/L, GGT 132 UI/L, Total Bilirubin 2.6 mg/dL, Alkaline Phosphatase 60 UI/L, LDH 826 UI/L, Sodium 136 mEq/L, Potassium 2.6 mEq/L. Leukocytes 0.07 x 10^9/L, hemoglobin 13.7 g/dL, HT 37%, MCV 87.9 fl, platelets 24,000/uL. Hemostasis with Prothrombin Time of 80% and activated Partial Thromboplastin Time (aPTT) of 40 sec, with international normalized ratio (INR) of 1.13. Blood cultures were negative and after post-surgical stabilization, cervical computed tomography (CT) was performed (figure 1).

The CT scan was reported as follows: "Occupation of soft tissue extending from the nasopharynx to the trachea, completely collapsing the airway. Well positioned permeable endotracheal tube. Thickening of the cervical subcutaneous cellular tissue and right predominant platysma muscle. Extensive ill-defined hypodense area with loss of differentiation of the structures of the floor of the mouth affecting the muscles of the sublingual space with hypocaptation of the right submaxillary gland with some gas bubble inside and at the level of vallecules.

The right deep cervical planes are also hypodense with a puffy aspect with loss of differentiation of the parapharyngeal, retropharyngeal, carotid and prevertebral spaces on the right side with extension of the phlegmonous process towards the contralateral parapharyngeal and vascular space.

Internal jugular and right common carotid do not appear opacified and therefore thrombosis cannot be ruled out. Mucosal occupation of maxillary, ethmoidal and sphenoidal paranasal sinuses".

Treatment with meropenem (2 g/ 8 h), daptomycin (10 mg/kg/24h) and clindamycin (600 mg/8 h) was maintained. Bleeding persisted around the tracheostoma and oral cavity. Continuous platelet perfusion was maintained for the next 48 hours until the figure of 80,000 platelets/uL was achieved.

Sedo-analgesia was discontinued for neurological assessment. No response to stimuli and persistence of low level of consciousness was observed. Cranial CT scan was performed, showing no significant findings.

EEG showed signs compatible with severe anoxic encephalopathy.

Forty-eight hours later, right arreactive anisocoria was objective. In a new cranial CT scan, ischemic stroke was observed in the territory of the right posterior inferior cerebellar artery (PICA). In this context, she presented hemodynamic instability requiring vasoactive drugs to maintain Mean Arterial Pressure around 65 mmHg.

Evoked potentials showed absence of bilateral cortical evoked response by stimulating both medium nerves.

After informing the family of the poor life prognosis, it was decided to prioritize the patient's comfort measures and limitation of therapeutic effort. The patient was deceased and a necropsy was performed.

DIFFERENTIAL DIAGNOSIS OF THE PATIENT (DR. CARLOS DUEÑAS)

The circumstances that contribute to the etiology of the infection in this patient are those accumulated by the basic pathology of the recipient, in this case AML, those derived from the invasive procedure (in this case practically null) and those related to immunosuppressive treatment (in this case busulfan, fludarabine, cyclophosphamide, mycophenolate and tacrolimus).

AML is the haematological disease with the highest risk of Invasive Fungal Infections (IFIs) with an incidence that varies between 10 and 25 percent, according to studies by SEIFEM [1]. The risk factors for IFI in AML are classified in 4 categories (table 1)

Looking at it from the point of view of infections in ALLO-HCT there are also a number of factors that according to Wingard et al. [2]. influence its presentation (table 2).

One aspect that should be pointed out, after reviewing the risk factors involved in this patient, is that of iron overload. Several articles underline that in patients with ALLO-HCT, iron overload is a poor prognostic factor for survival [3-5].

In this patient, another aspect of interest is the poten-

Table 1 Risk factors for Invasive Fungal Infections (IFIs) in Acute Myeloid Leukemia (AML)

a) Factors related to the Leukemia
Advanced stage of the disease
Complete remission failure
b) Host-related factor
Comorbidities
Older age
Organ dysfunction
Unfavorable genetic compatibility
c) Treatment related factors
Severity and duration of neutropenia (The duration of neutropenia is 10-14 days after autologous HCT and 15-30 days after Allo-HCT using ablative therapy and 5-7 days if ablative therapy is not used)
Severe mucositis, associated with chemotherapy
d) Factors related to exposure to fungi
Rooms without HEPA filter and previous IFIs

Table 2 Factors influencing the risk of infections in ALLO-HCT.

PATIENT DEPENDENT FACTORS
Advanced age
High comorbidity index for ALLO-HCT
Factors related to underlying disease or previous treatments
(corticoids, cyclophosphamide)
Underlying diseases
Previous HCT transplant
Previous donor or recipient infections
Previous immunity for CMV, VHS, VVZ, VEB
Iron overload
TRANSPLANT DEPENDENT FACTORS
Myeloablative regimens
Donate/receiver HLA compatibility: 10/10
Type of cell transplant performed: umbilical cord or peripheral blood
T-cell deplection
Immunosuppressive Regime: Methotrexate
INMUNOGENETICS
Polymorphisms that increase or decrease the risk of infection
PROLONGED AND SEVERE NEUTROPENIA
MASCC risk index: (13 points in this case, representing a high risk of poor prognosis)
Acute or chronic and prolonged GVHD and its treatment: above all use of high-dose corticosteroids and new immunosuppressants that delay immune reconstitution.
Infection with immunomodulatory virus: CMV
Graft failure
Respiratory complications

tial role of the myeloablative regimen in the evolution of this case. The regimen consisting of busulfan and cyclophosphamide is considered the classical myeloablative treatment and is associated with earlier, longer-lasting toxicities and higher mortality, primarily in older people than treatment with busulfan and fludarabine [6]. Fludarabine is less toxic than cyclophosphamide, maintaining its immunosuppressive efficacy and the combination busulfan-fludarabine is associated with a lower risk of sinusoidal obstruction syndrome characterized by hyper-bilirrubinemia (> 2 mg/dl), painful hepatomegaly and weight gain secondary to ascites. It is also associated with a lower risk of documented infections. The risk of grade 3-4 mucositis or graft rejection is similar with both drugs.

In order to discuss the possible etiology of this patient's disease, it is indicative to review the relationship that the infections have with the post-transplant moment. In the first 30 days after the procedure, the major problems are damage to the phagocytosis and the mucocutaneous barrier as a consequence of the treatment in preparation for the transplant. Prolonged neutropenia is added to this. As a consequence of the above, the main sources of infection will be the oropharyngeal, gastrointestinal and cutaneous flora.

Frequent use of intravenous catheters can serve as a gateway for opportunistic skin colonizing pathogens such as coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, *Enterococcus* spp. and *Candida* spp. In general, the focus of infection is not usually found and antibiotic treatment is usually empirical, covering bacterial infections. Colony stimulating growth factors are often used to decrease the duration of neutropenia and its complications, and in the case of persistent neutropenia is often associated with the appearance of *Aspergillus* spp. Reactivation of Herpes simplex virus (HSV) may also occur [7].

Fever in the neutropenic transplant recipient is common in the pre-graft phase. Fever typically appears from the 3rd to the 5th day after the onset of neutropenia and may be the only symptomatology of the infection. The most frequent in this situation are bacterial infections, but in many cases microbiological documentation is not obtained due to early initiation of broad-spectrum antibiotic treatment. There are only positive blood cultures in 10-25% of the cases. When foci of infection are identified (20-30% of occasions) the most frequent origins are: the lungs, the skin, especially in the insertion zone of the catheter and the perianal area, the genitourinary tract, the oral cavity, and the gastrointestinal tract. In the absence of apparent focus, the standard antibiotic treatment is monotherapy with a broad-spectrum Beta-lactam drug, such as piperacillin/tazobactam, ceftazidime, cefepime or a carbapenem with broad Gram-negative spectrum of activity (BGNs) including *P. aeruginosa*.

In the presence of catheter-related infection, cellulitis,

pneumonia, mucositis or methicillin-resistant *S. aureus* (MRSA) colonization, drugs with MRSA activity such as glycolypopeptides should be added.

Persistence of fever may be due to a delay in response to the initial regimen established, the existence of Gram-positive infection not treated with the initial antibiotic regimen, the presence of infection with BGNs resistant to the treatment established, the presence of untreated fungal infections or causes that are not infectious.

We would like to briefly discuss the value of acute phase reactants in this situation. The specificity of PCR is low for infections. It usually increases within 24 hours of infection and can predict the development of fever and sepsis in neutropenic patients. Procalcitonin (PCT) is more specific for the diagnosis of bacterial infections in febrile neutropenia with a very high NPV for bacteremia [8].

Our patient had mucositis. Mucositis serves as a gateway to microorganisms in the blood, such as bacteremia by *S. viridans* and CoNS. Oral bacterial microbiota usually changes after chemotherapy and increases in colonization by microorganisms such as *Enterococcus faecalis* and *Candida* spp. have been described in ALLO-HCT.

Searching for an etiologic diagnosis of the potential infection, our patient was receiving prophylaxis against viral infection. All recipients with positive CMV serology and all negative recipients with positive CMV donor antibodies, should receive ganciclovir prophylaxis from before transplantation until 100 days later. For HSV, acyclovir prophylaxis should be offered to all patients with positive serology to prevent reactivation in the early phase after grafting and maintain it until mucositis resolves and in the post-graft phase, approximately until 30 days after transplantation [9-11]. The prophylaxis with acyclovir and the repeated negativity of the CMV viral load in this patient, invite me to rule out the participation of both viruses in the case that we are discussing.

Prophylactic treatment of bacterial infection, in these circumstances, with fluoroquinolones, has a role in the prevention of early infection and there are studies indicating that the use of prophylactic antibiotics reduces the number of bacteremias, without reducing the associated mortality [12]. In contrast, the use of fluoroquinolones is associated with the development of quinolone-resistant CoNS infections, *Streptococcus* of the *viridans* group, and *E. coli*. Levofloxacin is preferred over ciprofloxacin because of its better coverage against *S. viridans*. The use of vancomycin is associated with the appearance of vancomycin-intermediate *S. aureus* and vancomycin-resistant *Enterococcus* [13] and the use of fluoroquinolones is associated with the appearance of BGN infections resistant to them [14].

In our opinion, in this case, the bacterial infection, due to intense antibiotic treatment, is either not present or is due to the presence of MDR microorganisms not treatable with the multiple antimicrobials administered.

Regarding fungal infections, this patient received prophylaxis with fluconazole, which decreases the risk of invasive candidiasis during the neutropenic phase, especially in centers where *Candida albicans* is the main cause of infections in the pre-graft phase. The problem is that fluconazole is not active against certain *Candida* species such as *C. krusei* or a significant percentage of *C. glabrata*, nor against filamentous fungi. Prophylactic strategies have led to a decrease in systemic *C. albicans* infections, although esophageal infection often persists in these cases. *C. glabrata* and *C. kefyr* are associated with the presence of oral ulcers in TCH. Filamentous fungi such as *Aspergillus* and any of the mucorales may produce lesions that mimic mucositis [15].

The prevalence of invasive fungal infection demonstrated in autopsies during the last 2 decades in these patients oscillates around 31%, and most of them are a necropsic finding. The most frequently isolated fungi are *Candida* spp. and *Aspergillus* spp. and, less frequently, *Fusarium*, *Scedosporium*, Mucorales and other emerging fungi, probably related to the selection of species produced with the use of the new antifungals. Predisposing factors are: advanced age, type of underlying haematological disease, immunosuppression, administration of broad-spectrum antibiotics, use of central venous catheter (CVC) for long periods of time, administration of parenteral nutrition, malnutrition, alteration of anatomical barriers (skin and mucous membranes), prolonged neutropenia, use of corticoids in rejection, iron overload and presence of genetic polymorphisms TLR-4, dectin 1 and pentraxin. The absence of *Candida* isolates in any sample or time and the prophylaxis with fluconazole invite me to put *Candida* infection in a very secondary place of preference as the cause of this patient's process.

Among the filamentous fungi, *Aspergillus* has a particular importance. Most cases are caused by *Aspergillus fumigatus* and are followed in frequency by *A. flavus*, *A. terreus*, *A. niger* and *A. glaucus*. The incidence can reach 8% in TPH and the most relevant risk factors are: prolonged neutropenia, intensity of immunosuppressive treatment, treatment with corticoids and concomitant viral infections by CMV and respiratory syncytial virus (RSV). Diagnosis of invasive aspergillosis is difficult because there are no characteristic clinical signs, imaging tests are not always conclusive, and laboratory methods have little sensitivity. Biomarkers such as galactomannan antigen have variable sensitivity and specificity in transplant recipients, depending on the type of sample, frequency of the sample, and the type of antigen, sampling and interpretive cut-off points. The greatest experience and usefulness has been demonstrated in granulocytopenic TPH, with positive and negative predictive values of 94.4 and 98%, respectively, preceding the appearance of symptoms in up to 80% of patients. False positive results associated with various biological factors are also known, such as colonization by *Bifidobacterium*, absorption of food galactomannan in patients with chronic GVHD, treatment with old piperacillin-tazobactam or amoxicillin-clavulanic preparations, and cross-reactivity with other fungi such as *Penicillium*, *Alternaria* or *Paecilomyces* [16]. In this patient, *Aspergillus* has

not been isolated at any time, there is no lung infection and galactomannan is reported as repeatedly negative, making the diagnosis of invasive aspergillosis unlikely.

Following with other filamentous fungi, all deep infections caused by *Scedosporium*, *Fusarium* and *Mucoraceae* are very serious and potentially fatal [17, 18]. Good evolution, when occurs, is usually related to a quick diagnosis (by direct microscopic vision of the samples of biopsied lesions), early antifungal treatment (often combining two or more drugs), extensive surgical debridment of the accessible lesions and the recovery of the patient's immune status, especially the neutropenia.

Fusarium incidence has increased due to the increased use of cytostatic treatment and ALLO-HCT and has been associated with soft tissue infections, onychomycosis and keratitis in immunocompromised patients from where it can disseminate. Inhalation of spores is another portal of entry producing sinusitis and pneumonia. Nucci et al. [19]. described a trimodal distribution of fusariosis in allo-HCT: a first peak before neutropenia recovery, a second peak at 62 days of mean transplant and a third peak after one year of transplantation. It usually presents with persistent fever and very pleomorphic skin metastatic lesions (nodules, ulcers) that can evolve towards central necrosis. Galactomannan can be useful for diagnosis and blood cultures are usually positive up to 50% of the time. There are no skin lesions in this patient, the presentation is like a cervical mass in the neck, galactomannan is negative and we are not informed of any positive blood cultures, so we believe that *Fusarium* is very unlikely to cause this picture.

Scedosporium, is another gender to consider in this disease. It can be a filamentous fungui of very aggressive behavior in neutropenic patients. *Scedosporium apiospermum* is angioinvasive and can cause sinopulmonary affection, endophthalmitis and dissemination to the central nervous system (CNS). *Scedosporium prolificans* is a dematiaceous fungus, phylogenetically close to the genus *Petriella* which causes disseminated infections with high mortality due to its special virulence and resistance to almost all available antifungals. Curiously, most of the IFIs by S. prolificans have been described in the Iberian Peninsula, California, United Kingdom and Australia [20, 21]. It is not possible to find information of *Scedosporium* infections with neck soft tissue invasion similar to this patient. It is a cause, however of sinusitis and otomycosis [22-26] which makes it unlikely that this fungus is the causal agent in this case.

The most frequent agents of mucormycosis are fungi of the genera *Rhizopus*, *Mucor* or *Lichtheimia* and risk factors for its appearance include prolonged neutropenia, treatment with corticoids, diabetic ketoacidosis and iron overload, many of which are present in this patient. Mucormycosis in ALLO-HCT usually appears early in the graft or later, as a complication of graft rejection. It is usually manifested as a rhinocerebral or pulmonary disease. In the rhinocerebral form the most frequent presentation is fever, facial pain and headache. It can present extension into contiguous spaces towards the orbit, palate or brain and is the first cause of invasive sinusitis in haematological patients. Its speed to invade tissues and spread through blood vessels (angioinvasion) is one of the causes of the high mortality rate (> 90%).

Mucormycosis can cause tissue invasion in the neck and mimic Ludwig's Angina [27].

Regarding the ferric overload in this disease, iron is an essential element for the growth and virulence of most microorganisms. The states of iron overload increase the risk of IFI by increasing the concentration of free iron, a necessary element for fungal growth and for the development of its mechanisms. Ferric overload and the consequent increase in the concentration of free iron are risk factors for the development of IFI by *Mucoraceae*. During ALLO-TCH pre-conditioning the IST can reach 100% on the second day with the consequent increase in iron not linked to transferrin. *Mucoraceae* are more dependent on the availability of free iron in the medium than *Aspergillus*, as this genus has more efficient mechanisms for its uptake from serum transferrin. Therefore, due to the rapid progression of the disease and the factors above mentioned, it cannot be excluded a mucormycosis as the final cause of death of this disease. The rapid progression of the neck lesion could be attributed to a suppurative septic thrombophlebitis of the jugular. This complication of neck infections is a multisystemic infection with septic emboli and possible thrombotic extension to the CNS [28].

Finally, we consider the parasitic aetiology of this picture unlikely. It is not known the serological status of this patient against *Toxoplasma* but anti-toxoplasma prophylaxis should be evaluated in case of positive serology recipient with acute graft rejection or history of toxoplasmic chorioretinitis, in principle with TMP-SMX [29, 30].

From all of the above, it is believed that this patient does not have a viral or parasitic infection, bacterial infection is unlikely, and we are inclined to think that it is a fungal infection. Of these, mucormycosis seems to be the most compatible with the clinical picture but it is not possible to rule out other filamentous fungi such as *Fusarium, Scedosporium* or dematiaceous fungi.

Infection with multi-resistant Gram-negative bacilli should be contemplated but seems highly unlikely in this disease.

EVOLUTION OF THE PATIENT (DR. JOSEP MENSA)

The summary of the autopsy report reads: Angioinvasive Mycosis disseminated by *Mucor* sp. with involvement of the cervical area with necrotic tumour that exceeds the midline and compresses the upper airway. Subglottic hematoma covered by laryngeal mucosa.

- Extensive cerebral vascular involvement with thrombosis by fungal stuctures and parenchymatous infarction (area of the right posteroinferior cerebellar artery and the territory of the left temporal lobe).

- Extensive fungal invasion of the arteries of the gastric

submucosa with phenomena of ischemic necrosis of the gastric wall in the body and fundus.

- Multiple fungal pulmonary thromboembolism with thrombus in distal LII and proximal LSI arteries. Extensive ischemic-type pulmonary infarcts with fungal overgrowth. Fungal thrombosis of the splenic vein. Extensive coagulative necrosis in the tail of the pancreas, peripancreatic fat and splenic parenchyma. Septic emboli in adrenal fat with secondary fat necrosis.

DISCUSSION OF CASE

Lanternier and colleagues reviewed 101 cases of mucormycosis (60 tested, 41 probable) in France, 50% of them with haematological malignancy. The episodes occurred in an average time after the onset of the disease of 8.8 months. The authors established an estimated incidence of mucormycosis in organ and stem cell transplant recipients of 8% [31].

The involvement of the mouth's floor and the upper part of the neck as a presentation of mucormycosis is uncommon but well described [27, 32]. Surgery is an essential component of the therapeutic strategy because antifungal treatment alone is often ineffective in controlling infection. In a series of 49 patients with rhinocerebral mucormycosis, mortality was 70% in cases treated with antifungals alone versus 14% treated with antifungals and surgery [33].

The antimicrobial treatment of rhinocerebral mucormycosis is the association of amphotericin B with an echinocandin that may be synergistic [34]. *Rhizopus oryzae* expresses the gene that encodes the proteins of the 1,3-b-D-glucan synthase complex, whose activity is inhibited by caspofungin. At a dose of 0.5 mg/kg every 12 h caspofungin improved survival in a model of mucormycosis in diabetic rats [35].

FINAL DIAGNOSIS

Angioinvasive mycosis disseminated by *Mucor* sp. with involvement of the cervical area.

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Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/078.2019

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Detección de *Streptococcus pyogenes* en muestras faringoamigdalares mediante técnica de detección de antígeno

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Article history
Received: 10 October 2019; Revision Requested: 29 October 2019; Revision Received: 21 November 2019; Accepted: 26 November 2019; Published: 24 March 2020

Sr. Editor*: Streptococcus pyogenes* (SGA) es el agente bacteriano más común de faringoamigdalitis agudas (FAA) en niños (30-40% entre los 3-13 años) y susceptible de tratamiento antibiótico. El tratamiento precoz reduce la severidad y duración de los síntomas, disminuye la transmisión y previene las complicaciones supurativas y no supurativas como la fiebre reumática y las infecciones invasivas [1].

El cultivo es el gold-standard para el diagnóstico de SGA pero la obtención de un resultado requiere un mínimo de 18-24 horas. Los test de detección de antígeno (TRDA) aportan rapidez al resultado y aunque no existe un criterio unánime se han incluido en diversas guías de práctica clínica por su utilidad para guiar las decisiones terapéuticas en las FAA [1-3]. Entre los TRDA destacan los inmunocromatográficos que por sus características pueden usarse como "pruebas en el punto de atención al paciente" [4].

El objetivo del estudio fue evaluar en nuestro medio el test Alere™TestPack+Plus with OBC Strep A (Alere-StrepA) para detección de SGA en niños con sospecha de FAA. El cultivo fue el método de referencia.

Desde enero de 2016 a enero de 2018 se estudiaron exudados faríngeos de 815 pacientes (59,7% niños) de 3 a 14 años (media de edad 6,5 años), con un score de más de 2 puntos en la escala de Centor, atendidos en las urgencias de pediatría del Hospital Virgen de las Nieves (Granada). De todos se tomó una muestra para cultivo en medio de transporte de Amies (Transystem™ COPAN Italia) y otra en un hisopo sin medio de transporte para Alere-StrepA (COPAN Italia). Ambas pruebas se realizaron en el laboratorio de Microbiología por el personal técnico o facultativo.

El cultivo se efectuó en agar sangre carnero 5% (Columbia agar 5% sheep blood; DIFCO) en anaerobiosis durante 48 horas. *S. pyogenes* se identificó mediante espectrometría de masas MALDI-TOF (MALDI Biotyper Bruker Daltonik, Germany) y determinación del antígeno A de Lancefield por aglutinacion con látex (Oxoid Streptococcal Grouping Reagents). Alere-StrepA es un método inmunocromatográfico que aporta el resultado en 15 minutos y se hizo siguiendo las recomendaciones del fabricante.

De las 815 muestras, el cultivo fue positivo en 293 (35,95%) y negativo en 522. De las 293 muestras positivas por cultivo Alere-StrepA fue positivo en 235 (80,2%) y negativo en 58. De las 522 muestras con cultivo negativo 7 (1,3%) fueron positivas con Alere-StrepA. Ningún resultado fue indeterminado.

La sensibilidad y especificidad de Alere-StrepA fueron del 80,2% (IC 95%: 75,47- 84,93%) y del 98,7% (IC 95%: 97,57-99,74%) respectivamente y los valores predictivos positivo y negativo del 97,1% (IC 95%: 94,78- 99,42%) y 90,0% (IC 95%: 87,32- 92,43%).

Previos estudios y metaanálisis de TDRA para detección de SGA en faríngeos indican considerable variabilidad en su sensibilidad que oscilaría del 70-90% y menor variabilidad en la especificidad que se sitúa en torno al 95% [4, 5].

Los valores analíticos obtenidos con Alere-StrepA quedarían dentro del rango de valores considerados habituales para este tipo de pruebas. La sensibilidad fue inferior a la de los estudios de Lasseter (95%) [6], Regueras (86,5%) [7] o Penney (81,4 %) [8] y al indicado en el insert del test (97,6%) y superior a la del estudio de Lacroix (75,3%) [8]. En la evaluación de Lasseter, estudio *in vitro*, la sensibilidad aumentó con el inóculo. En el estudio de Regueras también se observó un efecto del espectro clínico en la sensibilidad. La menor sensibilidad en este estudio podría deberse al sesgo de espectro, grupo de población estudiada o a la idoneidad en la toma de muestra.

Respecto a otros test inmunocromatográficos, en las evaluaciones de Flores [9] y Contessoto [10] la sensibilidad fue superior a la de nuestro estudio, pudiendo deberse a caracte-

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rísticas inherentes al test o a las diferentes condiciones de los estudios. Flores describe una sensibilidad inferior en pacientes con una clínica menos grave.

De las características técnicas del test destacamos su fácil uso e interpretación, formato en casette [6] y los controles adicionales, siendo factible usarlo en el punto de atención al paciente.

El estudio tiene limitaciones, los resultados discrepantes no se verifican con un método de referencia y no se determinan las causas de los falsos negativos y positivos pudiendo deberse a causas descritas previamente [3].

Dados los resultados obtenidos, la alta especificidad permite adoptar una decisión terapéutica; pero sobre todo en resultados negativos Alere-StrepA se debe acompañar de cultivo. En estos casos MALDI-TOF permite identificar SGA en 18-24 horas. Además consideramos importante incidir en la mejora continua de factores que influyen en el rendimiento del ensayo (toma de muestra, realización del test,...).

Aunque existen diferencias entre distintas guías de práctica clínica respecto al diagnóstico y tratamiento de la faringitis hemos optado por la realización de un TDRA con cultivo solo de las muestras negativas [1-3] y de algunas positivas para vigilancia de las resistencias de SGA. Este procedimiento permite además detectar faringitis por otros estreptococos beta hemolíticos.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

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Official journal of the Spanish Society of Chemotherapy

Letter to the Editor

Revista Española de Quimioterapia
doi:10.37201/req/079.2019

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Unusual case report of skin infection by *Paenibacillus timonensis*

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Article history
Received: 11 October 2019; Revision Requested: 2 December 2019; Revision Received: 2 December 2019; Accepted: 28 December 2019; Published: 9 March 2020

Sir,

Paenibacillus is a genus of gram-positive bacilli endospore forming aerobic or facultatively anaerobic bacteria, that were originally included in the genus Bacillus [1, 2]. These bacteria are well adapted to the environment, and have been isolated from various sources including water, soil, food and plants, but they are not usually associated with infection. In the last decades, reports of infection in humans has increased through the years, and has been isolated mostly from wound exudates, and others from mitral endocarditis, bone infection and bacteremias [3-6]. Due to its widespread distribution when *Paenibacillus* is isolated, it is important to discriminate infection from contamination; in this context repeated isolation form multiple samples may indicate clinical significance.

Here we report, to our knowledge, the first case of soft tissue and skin infection by *Paenibacillus timonensis*, and we describe the second case of human infection by this species [7].

A 37-year-old man with a medical history of arterial hypertension and neurofibromatosis type 1, also known as von Recklinghausen's disease, was admitted to the emergency room of our university hospital for treatment of a severe pain and swelling in lower left limb due to strong trauma on May 2019. He was operated urgently due to a compartment syndrome in the left knee. After leaving the operating room, he presented serous secretion at one of the points of the scar, sending deep samples for culture to the microbiology laboratory.

After 24 hours of incubation, greyish, translucent and shiny colonies grew in pure culture in aerobic blood agar (BD Columbia Agar 5% Sheepblood®, Becton Dickinson) and chocolate agar in a 5% CO_2 atmosphere (BD Choco Agar, Becton Dickinson). Catalase test was positive and oxidase was negative. Gram strain revealed Gram-positive rod-shaped bacteria. Identification was performed using MALDI-TOF MS (Bruker Biotyper, MA, USA), with the result of *P. timonensis* with best-match score values of 1.96. Considering that a minimum score of 2 for species determination, the identification was confirmed by the partial sequencing (949 bp) of the 16S rRNA gene using universal primers. This isolate shared 99.88% sequence similarity with the reference sequence of *P. timonensis* available in GeneBank (KT719432.1). Susceptibility testing (Minimun Inhibitory Concentration) was carried out by the E-test method in Mueller Hinton 5% Blood Agar (Becton Dickinson) incubated in aerobiosis at 37°C with readings after 24 and 48 hours. As no specific clinical breakpoints have been established for *Paenibacillus*, we used the EUCAST PK/PD (non-species related) clinical breakpoints. *P. timonensis* was susceptible to trimethoprim-sulfamethoxazole (<0.02 mg/L), gentamicin (0.094 mg/L), erythromycin (0.5 mg/L), cefotaxime (0.064 mg/L), rifampicin (0.125 mg/L), vancomycin (0.25 mg/L), and resistant to ampicilin (>32 mg/L).

To rule out the presence of *P. timonensis* as a possible contaminant, we asked for new samples from the wound, that were sent during the next week. *P. timonensis* was isolated in 4 subsequent samples, thus considering it the cause of the infection. According to susceptibility testing results and the literature [8], the patient was treated with vancomycin intravenously (1 g/12h) for 10 days, followed by 3 weeks of trimethoprim/sulfamethoxazole orally.

Antibiotic treatment was accompanied with the use of negative pressure wound therapy with the use of portable device (PICO™), that has been shown to optimize patient outpatient care and promote rapid wound healing [9, 10]. The patient presented a good evolution of the wound and continued to perform local cures every 7 days until full recovery.

In summary, the genus *Paenibacillus* is an unusual cause of surgical wound infection. Within this group, *P. timonensis* had never been described as a pathogen in this clinical sce-

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nario. In our patient, rapid identification of the etiologic agent allowed quick initiation of antimicrobial treatment. We believe that subsequent isolation of *P. timonensis* in the following samples and clinical improvement after antibiotic treatment helped to show its clinical significance and to discriminate from a possible contamination.

FUNDING

None to declare.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Official journal of the Spanish Society of Chemotherapy

Letter to the Editor

Revista Española de Quimioterapia
doi:10.37201/req/081.2019

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Joint infection due to *Elizabethkingia miricola*

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Article history
Received: 14 October 2019; Revision Requested: 5 November 2019; Revision Received: 10 December 2019; Accepted: 7 January 2020; Published: 10 March 2020

Sir,

Elizabethkingia miricola is a non-fermenting Gram-negative rod, non-motile and non-spore-forming which was firstly described in 2003 when it was isolated from condensation water on the space station MIR [1]. Initially named *Chryseobacterium miricola*, it was reclassified along with *Chryseobacterium meningosepticum* into the new genus *Elizabethkingia,* this was due to the phylogenetic analysis, based on the sequencing of the 16S rRNA gene [2]. *E. miricola* has been demonstrated to be pathogenic, with reports of bacteremia, sepsis and pulmonary abscesses [3-5]. To our best knowledge, we report the first case of infection in a native joint due to *E. miricola*.

A 52-years-old immunocompetent woman came to the Emergency Department of our hospital due to non-favorable evolution of a catastrophic right foot caused by a traffic accident. The diagnosis was made by means of a foot x-ray and it was observed that the affected bones were the calcaneus, talus, scaphoid and cuboid. Patient refers poor evolution of skin injuries during a month with pain that has slightly subsided. The clinical history was unremarkable and no underlying diseases were reported. No antibiotic treatment during that month was administered, and only wound cures every two days were performed. Due to the infectious aspect of the right ankle, a surgical procedure with extensive debridement was then carried out; also, five bone biopsies from different sites were taken and sent to the microbiology laboratory for culture. The patient did not have indwelling devices or invasive catheters before infection developed. After processing, the samples were inoculated in blood agar (both aerobic and anaerobic) (BD Columbia Agar 5% Sheepblood®, Becton Dickinson), chocolate agar (BD Choco agar, Becton Dickinson) and thioglycollate broth (BD Fluid Thioglycollate Medium, Becton Dickinson). All media were incubated at 37º C during 5 days.

Direct Gram staining of the samples showed abundant Gram-negative bacilli, and on the first day of incubation growth of grey colonies in pure culture was observed on all plates above mentioned. The oxidase test was positive (Oxidase Reagent Droppers, Becton Dickinson). The microorganism was identified by mass spectrometry (MALDI-TOF MS, Bruker Biotyper, Billerica, MA, USA) as *E. miricola* (Log score 2.3). The MIC of different antibiotics was carried out by the MicroScan device (Beckman Coulter). According to the breakpoints of CLSI for non-fermenting Gram-negative rods, *E. miricola* was susceptible to ciprofloxacin (MIC 0.38 mg/L), levofloxacin (MIC 0.25 mg/L), piperacillin-tazobactam (MIC 16 mg/L) and resistant to amikacin (MIC>256 mg/L), cefepime (MIC>256 mg/L), ceftazidime (MIC>256 mg/L), colistin (MIC>256 mg/L), fosfomycin (MIC>256 mg/L), gentamicin (MIC 32 mg/L), imipenem (MIC>32 mg/L), meropenem (MIC>32 mg/L) and tetracycline (MIC >24 mg/L). MIC to tigecycline was 0.5 mg/L. At this stage, antimicrobial treatment was started with piperacillin-tazobactam 4gr/8h i.v, but after 15 days of treatment the patient had poor renal function without a known cause, and it was decided to change the treatment to oral levofloxacin 500 mg/24h for three weeks. The patient presented good evolution and after going to successive consultations, 4 months later she was definitively discharged.

At this time, the most common microorganisms causing bone infection are staphylococci or Gram-negative bacilli, including *Pseudomonas aeruginosa*. From cultures of intraoperative specimens, *Staphylococcus aureus* is the main causative agent of chronic bacterial osteomyelitis, accounting for about two thirds of isolates, followed by *Pseudomonas* and Enterobacterales [6]. However, other rare microorganisms could be implicated in the etiology of these infections, as in our case, so physicians and microbiologist should be aware about this possibility.

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The first case of human infection due to *E. miricola* was reported in 2008 in an adult with mantle cell carcinoma who underwent stem cell transplantation [3]. After this, *E. miricola* has demonstrated to cause bacteremia, sepsis, and pulmonary and tract urinary infections [3-5, 7]. From these patients, three of them had underlying comorbidities such as cancer, alcoholic pancreatitis and cystic fibrosis. In all cases, the isolate was identified by MALDI-TOF MS. Thus, the recent introduction of mass spectrometry for routine identification in the clinical laboratories may help to identify some rare pathogens and to know the true incidence of infections due to these microorganisms.

Currently, there are no CLSI/EUCAST guidelines for *E. miricola*. This bacterium has been found to be multidrug resistant, similar to *E. meningoseptica* which is known to harbor β-lactamases showing resistance to β-lactams and carbapenems. *E. miricola* isolates have been found to be resistant to many antibiotics. A study showed resistance to imipenem, ceftazidime, cotrimoxazole and variable susceptibility to quinolones [8]. In a recent study, all isolates of *E. miricola* were susceptible to tetracyclines and piperacillin-tazobactam. However, 50% of the isolates were susceptible to levofloxacin and tigecycline [9]. Another study showed that 91% and 77% of *Elizabethkingia* spp isolates were resistant to ciprofloxacin and levofloxacin, respectively [10]. The most prevalent alterations were two single mutations in GyrA, Ser83Ile and Ser83Arg. In our case, however, the isolate was susceptible to both levofloxacin and ciprofloxacin.

In summary, we here presented the first case of bone infection due to *E. miricola*. Until now, the presence of this pathogen is rare as cause of human infections, but the recent introduction of MALDI-TOF MS can help to identify some microorganisms, as in our case, which rarely produces bone infections. On the other hand, susceptibility to these isolates should be performed due to the fact that several species often exhibit extensive antimicrobial resistance.

FUNDING

None to declare.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/082.2019

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Aumento de *Staphylococcus aureus* resistente a meticilina y sensible a ciprofloxacino en infecciones osteoarticulares, de piel y tejidos blandos

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Article history
Received: 14 October 2019; Accepted: 18 November 2019; Published: 11 March 2020

Sr. Editor: *Staphylococcus aureus* es el agente etiológico más frecuente de las infecciones osteoarticulares, de piel y tejidos blandos (OPTB) [1]. La resistencia a antibióticos de *S. aureus* dificulta el tratamiento eficaz de estas infecciones, en especial las causadas por cepas resistentes a oxacilina/meticilina (SARM) [2]. La vigilancia de la resistencia a antibióticos en *S. aureus* es necesaria para adecuar el tratamiento empírico de las infecciones OPTB. En las últimas décadas han emergido y se están extendiendo clones de SARM asociados a la comunidad (SARM-AC) [3]. Estos clones son sensibles a antibióticos no betalactámicos con más frecuencia que los clones de SARM asociados a cuidados sanitarios (SARM-CS). La resistencia a ciprofloxacino es habitual en las cepas de SARM-CS, pero poco frecuente en los clones de SARM-AC [4, 5]. Por ello, la sensibilidad a quinolonas se ha utilizado como marcador fenotípico de las cepas SARM-AC [6, 7]. El objetivo de este estudio fue conocer la frecuencia de cepas SARM y SARM sensible a ciprofloxacino (SARM CIP-S) en infecciones OPTB por *S. aureus* en nuestra área en el periodo 2017/2018.

Se revisó la base de datos del laboratorio de microbiología en busca de pacientes que, en 2017 o 2018, tuvieran un aislamiento de *S. aureus* a partir de muestras osteoarticulares, heridas, abscesos o tejidos blandos. Se recogieron la edad del paciente y el antibiograma de la cepa. En pacientes que, en el mismo año, tuvieron más de una muestra con *S. aureus*, se consideró sólo la primera. Si en la primera muestra había más de una cepa de *S. aureus*, se contabilizaron como cepas distintas las que diferían en el resultado del estudio de sensibilidad a oxacilina (o cefoxitina), ciprofloxacino, eritromicina, gentamicina, rifampicina, tetraciclina, cotrimoxazol o vancomicina. Se revisó la historia clínica de los pacientes con SARM CIP-S para determinar si se trataba de una infección comunitaria o asociada a cuidados sanitarios. Para definir la infección asociada a cuidados sanitarios se usaron los criterios siguientes [8]: a) infección producida en el hospital 48 o más horas tras el ingreso, b) hospitalización, cirugía o diálisis en el año previo al cultivo de SARM, c) paciente institucionalizado en el año previo al cultivo de SARM, d) presencia de un dispositivo médico percutáneo en el momento del cultivo de SARM, o e) cultivo previo positivo para SARM. Si no se cumplía ninguno de los anteriores criterios, se consideró que la infección había sido adquirida en la comunidad. Para comparar proporciones se usó la prueba de chi cuadrado, y se consideró que había una diferencia significativa si $p < 0,05$.

Tabla 1 **Distribución por edad de la frecuencia de resistencia a meticilina en *S. aureus* y de la frecuencia de sensibilidad a ciprofloxacino en SARM en los años 2017 y 2018.**

	2017	2018	p[a]
Oxacilina-R en *S. aureus*[b]			
Todos los pacientes	153/623 (25)	194/632 (31)	0,01
Edad <65 años	35/264 (13)	53/255 (21)	0,02
Edad ≥65 años	118/357 (33)	141/377 (37)	>0,05
Ciprofloxacino-S en SARM[c]			
Todos los pacientes	10/153 (6,5)	32/186 (17)	0,003
Edad <65 años	6/35 (17)	24/51 (47)	0,004
Edad ≥65 años	4/118 (3,4)	8/135 (5,9)	>0,05

[a]Chi cuadrado.

[b]Resultados expresados como: nº de cepas resistentes a meticilina/nº cepas testadas (%).

[c]Resultados expresados como: nº de cepas de SARM sensibles a ciprofloxacino/nº de cepas de SARM testadas (%).

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Tabla 2 **Infecciones comunitarias por SARM sensible a ciprofloxacino (CIP-S).**

	Nº pacientes (nº ingresos hospitalarios)	Nº de casos 2017/ nº casos 2018
Síndrome		
Artritis séptica	2 (2)	0/2
Absceso	4 (2)	0/4
Celulitis	3 (3)	0/3
Infección de úlcera	3 (0)	2/1
Infección pie diabético	1 (1)	0/1
Impétigo ampolloso	1 (0)	0/1
Edad (años)		
<65	11 (8)	0/11
≥65	3 (0)	2/1

En 2017 hubo 603 pacientes de los que se aislaron 623 cepas de *S. aureus* a partir de muestras OPTB y en 2018, 612 pacientes y 632 cepas. La frecuencia de resistencia a meticilina fue mayor en 2018 que en 2017, particularmente en pacientes menores de 65 años de edad (tabla 1). La frecuencia de sensibilidad a ciprofloxacino en SARM aumentó entre 2017 y 2018, de forma significativa en pacientes menores de 65 años (tabla 1). Considerando la totalidad del periodo, 30 (71%) de las 42 cepas SARM CIP-S se aislaron de pacientes menores de 65 años. En 2017, las cepas SARM CIP-S fueron 10 (1,6%) de las 623 cepas de *S. aureus* aisladas, y esta proporción subió a 32 (5,2%) de las 614 cepas testadas en 2018 (p = 0,0005). La proporción de cepas SARM resistentes a ciprofloxacino sobre el total de cepas de *S. aureus* testadas no varió significativamente: fueron 143 (23%) de 623 en 2017 frente a 154 (25%) de 614 en 2018 (p = 0,38). Entre los 38 pacientes con SARM CIP-S que tenían una historia clínica completa, 14 (37%) habían adquirido la infección en la comunidad. De estas 14 infecciones comunitarias, ocho pacientes (todos ellos menores de 65 años) requirieron ingreso hospitalario, entre ellos dos varones de 60 años con artritis séptica de articulación nativa (tabla 2). En 2018, 12 (2%) de los 612 pacientes tuvieron una infección comunitaria por SARM CIP-S, frente a 2 (0,3%) de 603 en 2017 (p = 0,008; tabla 2).

El aumento de la frecuencia de SARM CIP-S y de infecciones comunitarias por SARM CIP-S que observamos en nuestro estudio sugiere un aumento de la circulación de clones de SARM-AC, que en España son generalmente sensibles a ciprofloxacino [5]. Las infecciones comunitarias por SARM CIP-S afectaron sobre todo a pacientes menores de 65 años de edad y fueron a menudo graves. Los casos de artritis séptica comunitaria por SARM CIP-S son de especial interés, ya que en España la artritis séptica por SARM se ha descrito principalmente asociada a cuidados sanitarios [9, 10]. El aumento de la frecuencia de SARM como causa de artritis séptica de adquisición comunitaria obligaría a incluir un antibiótico activo frente a SARM, como vancomicina o linezolid, en el tratamiento empírico de esta infección.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

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Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/083.2019

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Síndrome hemofagocítico por *Leishmania* en paciente con síndrome poliglandular

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Article history
Received: 16 October 2019; Revision Requested: 25 November 2019; Revision Received: 27 November 2019; Accepted: 10 December 2019; Published: 11 March 2020

Sr. Editor: El síndrome hemofagocítico (SHF), también llamado linfohistiocitosis hemofagocítica, es una patología considerada dentro del grupo de las histiocitosis. Causado por una alteración en la regulación de los macrófagos, origina una producción descontrolada de citoquinas por linfocitos T e histiocitos activados, que provoca finalmente una activación persistente del sistema inmune [1].

Dependiendo de su clasificación encontramos el SHF primario o familiar y el SHF secundario o reactivo a diferentes enfermedades, entre ellas las infecciones y las enfermedades autoinmunes [2].

El diagnóstico definitivo se basa en los hallazgos clínicos (fiebre persistente, erupción cutánea, artralgia y esplenomegalia), analíticos (citopenia, hipofibrinogenemia, hipertrigliceridemia), histológicos (hemofagocitosis) y moleculares establecidos por el por el Study Group of the Histiocyte Society y posteriormente modificados en 2004 [3].

Ha sido relacionado de forma excepcional como complicación de la leishmaniasis visceral, también conocida como kala-azar o fiebre negra, una enfermedad parasitaria endémica en ciertas zonas geográficas como el área mediterránea. Esta asociación origina particularidades en el caso clínico: características clínico-patológicas comunes, complejidad en el diagnóstico de la parasitosis y distinto enfoque terapéutico.

Presentamos el caso de una paciente de 24 años que debutó con fiebre de 20 días de evolución, inicialmente intermitente y en la en la última semana persistente, mal estar general, aftas orales dolorosas, artromialgias y pérdida de peso, sin foco infeccioso aparente.

Antecedentes personales de hipogonadismo hipergonadotropo secundario a síndrome de Turner parcial en mosaico, enfermedad celiaca y síndrome pluriglandular autoinmune tipo 2, con debut diabético en el primer año de vida, hipotiroidismo primario a los 18 años y enfermedad de Addison un año más tarde.

En la exploración presentaba fiebre, afectación del estado general, palidez mucocutánea y esplenomegalia palpable. No se apreciaron lesiones cutáneas ni adenopatías. La auscultación cardiorrespiratoria resultó anodina.

El estudio inicial de laboratorio puso de manifiesto la presencia de anemia, trombopenia y leucopenia así como elevación de transaminasas, LDH y PCR. La pancitopenia se agravó durante la evolución del cuadro clínico, con posterior recuperación gradual de la misma (figura1).

Se completó el estudio con pruebas de laboratorio, de imagen y aspirado de médula ósea, destacando una hipergammaglobulinemia policlonal, hipofibrinogenemia, aumento de triglicéridos y ferritina y serología frente a *Leishmania donovani* positiva (Ig G:1/1280, Ig M:1/100).

El TAC toracoabdominal mostró hepatoesplenomegalia homogénea y un ligero derrame pleural.

El aspirado de médula ósea reveló una médula hiperregenerativa, con numerosos fenómenos hemofagocíticos, aunque sin visualización de parásitos. La detección de DNA de *Leishmania* mediante PCR en el aspirado resultó positiva.

Se diagnosticó Leishmaniasis visceral con síndrome hemofagocítico secundario.

Se inició tratamiento con anfotericina liposomal intravenosa, que produjo una reacción infusional grave, por lo se indicó glucantime intramuscular, con buena tolerancia y mejoría clínica. En el control realizado tras completar el tratamiento, la paciente estaba asintomática, con recuperación completa de la pancitopenia, PCR frente a *L. donovani* en sangre periférica negativa y normalización de cifras de triglicéridos, ferritina y fibrinógeno. Finalmente, se realizó el estudio genético de lin-

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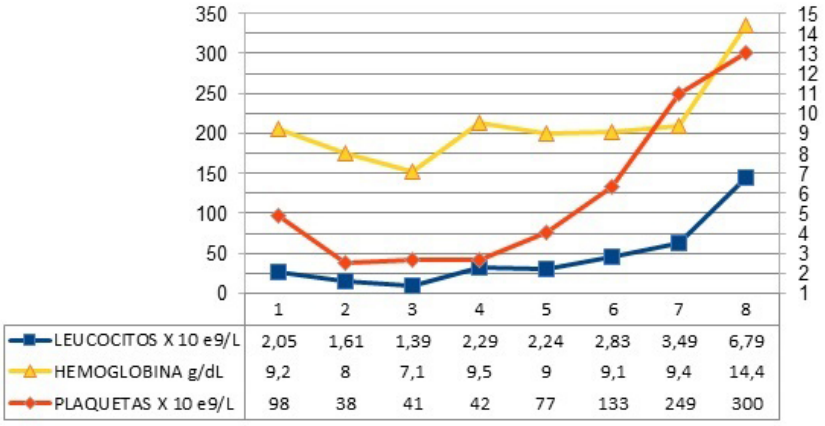

Figura 1 | **Evolución de las cifras de plaquetas, leucocitos y concentración de hemoglobina**

fohistiocitosis hemofagocítica familiar mediante secuenciación masiva, que descartó una causa primaria del SHF.

Se piensa que en la etiología del SHF, está implicada una inflamación multisistémica que amenaza la vida, causada por múltiples moléculas de interleuquinas, factor de necrosis tumoral alfa, interferón gamma, factores inflamatorios producidos por células tisulares y linfocitos T que originan un efecto tóxico en el organismo. Como causa secundaria del SHF, entre los trastornos autoinmunes, la causa principal es el lupus eritematoso sistémico, seguido de enfermedad de Still en adultos, artritis reumatoide, síndrome de Sjögren y esclerosis sistémica [4].

Encontrar un determinante infeccioso en un paciente diagnosticado de SHF no debe obviar el estudio genético en busca de una predisposición que viene dada en el SHF primario. En esta paciente, finalmente se descartó la causa primaria mediante estudio genético [2].

Es el primer caso descrito en la literatura que vincula el síndrome de activación de macrófagos con la enfermedad celíaca y síndrome pluriglandular autoinmune tipo 2 [5].

Este caso excepcional demuestra la dificultad en el diagnóstico y manejo de una paciente con importante base autoinmune y síndrome hemofagocítico secundario a leishmaniasis visceral. Pretendemos una mayor conciencia de este posible diagnóstico entre los médicos que tratan a pacientes con clínica similar.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

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Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/087.2019

Dalbavancina combinada con linezolid en infección protésica de cadera

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Article history
Received: 5 November 2019; Revision Requested: 25 November 2019; Revision Received: 1 December 2019; Accepted: 16 December 2019; Published: 11 March 2020

Sr. Editor: Dalbavancina, al igual que ha ocurrido con linezolid, se utiliza cada vez más en la infección osteoarticular aunque actualmente no tenga indicación para ello [1-5]. Son pocos los casos publicados de infección protésica articular tratados de forma prolongada (más allá de las dos dosis semanales autorizadas en infecciones de piel y partes blandas) con dalbavancina [3], y hasta la fecha no se ha publicado ningún caso de tratamiento combinado con linezolid para el tratamiento de rescate de la infección protésica articular que ha fracasado tras desbridamiento y antibioterapia convencional. Se describe a continuación un caso de infección protésica aguda tratada de forma prolongada con dalbavancina combinada con linezolid.

Mujer de 44 años, sometida a implante de prótesis de cadera por coxalgia secundaria a displasia. Dos semanas después desarrolló signos de infección protésica aguda tratándose con desbridamiento y recambio de la cabeza femoral cerámica. En las tres muestras para cultivo obtenidas se aisló *Staphylococcus epidermidis* sensible a vancomicina (CMI 2 mg/L), daptomicina (CMI ≤1 mg/L), linezolid (CMI ≤2 mg/L), ceftarolina (CMI ≤0,5 mg/L), trimetoprin/sulfametoxazol (CMI ≤2/38 mg/L), rifampicina (CMI ≤0,5 mg/L), y resistente a oxacilina (CMI >2 mg/L), levofloxacino (CMI >4 mg/L) y clindamicina (CMI ≤0,25 mg/L pero con test de resistencia inducible positivo). La proteína C reactiva era de 98 mg/L. Se inició tratamiento con vancomicina (15 mg/kg/8 horas iv). A los cinco días del desbridamiento quirúrgico, comenzó con exudación purulenta por la herida quirúrgica, objetivándose por ecografía colecciones líquidas que llegaban a contactar con hueso, con persistencia de cultivos positivos en dos muestras obtenidas por punción, pese a comprobar niveles valle de vancomicina apropiados (17 mg/L). Se realizó un segundo desbridamiento quirúrgico a los 15 días del previo, con cultivos intraoperatorios positivos en las tres muestras que se tomaron. La antibioterapia se modificó a linezolid (600 mg iv cada 12 horas). A las dos semanas del segundo desbridamiento comenzó de nuevo con exudación por la herida por lo que se puso fecha para nuevo desbridamiento con recambio protésico en dos tiempos, añadiéndose al tratamiento dalbavancina (dosis de carga de 1000 mg). Tras ello, desapareció a los pocos días la exudación por la herida, decidiéndose posponer el recambio protésico pendiente de evolución, y procediéndose al alta con tratamiento combinado con linezolid oral y dalbavancina iv semanal durante 8 semanas. A las dos semanas tras el alta se realizó punción aspirado bajo la herida, obteniéndose escaso líquido serohemático y cuyo cultivo fue negativo en las dos muestras obtenidas. A las 6 semanas se suspendió linezolid por anemización progresiva, y ante la buena evolución de la herida quirúrgica se decidió suspender también dalbavancina tras 7 dosis semanales de 500 mg. En la última revisión (a las 16 semanas tras el último desbridamiento) la paciente se encontraba sin dolor, con muy buen aspecto de la herida quirúrgica, y con normalización de la proteína C reactiva (4,6 mg/L).

Durante la artroplastia, la infección es una de las complicaciones más graves, ya que puede generar reintervenciones quirúrgicas, uso prolongado de antibióticos y recambios de material protésico. La incidencia de infecciones protésicas de cadera en nuestro medio es de aproximadamente el 1%, siendo los estafilococos coagulasa negativa y *Staphylococcus aureus* los agentes etiológicos predominantes (30-40% y 12-23%, respectivamente) [6, 7].

Cuando la infección protésica aguda se maneja con desbridamiento y retención del implante, se aconseja mantener la antibioterapia durante 6-8 semanas si la evolución es satisfactoria, y pueden emplearse pautas de consolidación consideradas óptimas (levofloxacino más rifampicina para la infección estafilocócica o ciprofloxacino para bacilos gramnegativos) [8, 9]. Fuera de este escenario la duración apropiada del tratamiento es incierta, al igual que la actitud tras el fracaso del desbridamiento combinado con antibioterapia [8].

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Dalbavancina y linezolid son agentes antibacterianos con gran actividad sobre microorganismos grampositivos. Dalbavancina es un antibiótico con larga vida media (372 horas) que permite su administración semanal [1,2]. Ambos fármacos han mostrado buenos perfiles de penetración en hueso así como en líquido sinovial [5]. Un estudio *in vitro* ha demostrado la sinergia entre ambos fármacos para el tratamiento de cepas de *S. aureus* resistente a meticilina [10]. Basados en estos datos, y ante el fracaso a los tratamientos realizados, decidimos el empleo de esta combinación, la cual evitó los problemas derivados de haber realizado un recambio protésico, aunque no se haya descrito hasta la fecha ningún caso similar en la literatura.

En conclusión, dalbavancina junto con linezolid podría mostrarse como alternativa de rescate en tratamiento prolongado de infecciones rebeldes de material protésico por bacterias grampositivas que no responden a los antibióticos autorizados, permitiendo además acortar la estancia hospitalaria.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

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Official journal of the Spanish Society of Chemotherapy

Carta el Director

Revista Española de Quimioterapia
doi:10.37201/req/089.2019

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Análisis de los tipos y subtipos gripales en función de la edad en las últimas cuatro temporadas epidémicas

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Article history
Received: 11 November 2019; Revision Requested: 4 December 2019; Revision Received: 9December 2019; Accepted: 16 December 2019; Published: 10 March 2020

Sr. Editor: La gripe es una infección vírica que se presenta de forma estacional o epidémica y en ocasiones pandémica. Aunque afecta a la población general, los menores de 4 años parecen ser la población con mayor prevalencia [1]. Los diferentes tipos (gripe A y B) y subtipos gripales (H1N1 y H3N2) se distribuyen de forma no homogénea entre los grupos etarios presentando implicaciones clínicas y epidemiológicas [1].

Para conocer la situación epidemiológica actual, se ha estudiado la distribución de los casos de gripe entre la población infantil durante las últimas cuatro temporadas (2015-2019). Se ha dividido a la población, siguiendo los criterios epidemiológicos, entre los comprendidos entre los 0-4 años y los 5-15 años [2].

A cada paciente con sospecha de gripe o cuadro gripal se le tomó un aspirado nasofaríngeo o frotis faríngeo para el estudio de los virus respiratorios. La detección se realizó mediante una RT-PCR comercial en tiempo real (Allplex RV; Seegen, Corea del Sur) que permite detectar 16 virus distintos y tipar y subtipar los virus gripales tipo A. Los linajes de la gripe B fueron establecidos mediante una RT-PCR específica (Centro Nacional de Microbiología, Majadahonda, Madrid).

Globalmente y de forma acumulada, en este estudio se han detectado 1064 casos de gripe, correspondiendo 678 (63.7%) al grupo de 0-4 años y 386 (36.3%) al grupo de 5-15 años. Del mismo modo los subtipos gripales detectados han sido A (H1N1)pdm09 360 casos (33.8%), A (H3N2) 323 casos (30.3%) y gripe B 381 casos (35.8%) (tabla 1).

El número total de casos de gripe ha variado en cada temporada epidémica, siendo 118 en la temporada 2016-17 (sin circulación de gripe B) hasta 370 casos en la 2015-16 (con circulación preferente de gripe B). Por grupos de edad el subtipo A (H1N1)pdm09 ha predominado en el grupo de 0-4 años (42%), el subtipo A (H3N2) en el grupo de 5-15 años (36.1%) y la gripe B en este mismo grupo etario (44.5%) (figura 1). De los 360 casos de gripe A (H1N1)pdm09 el 79.1% se presentaron en el grupo de 0-4 años, al igual que la gripe A (H3N2) (56.9%) y la gripe B (54.8%).

Tabla 1 **Distribución de los tipos y subtipos gripales en función de la edad en las últimas temporadas epidémicas.**

Edad	A (H1) n (%)	A (H3) n (%)	IB n (%)	Total
0-4 años	285 (42,1)	184 (27,1)	209 (30,8)	678
5-15 años	75 (19,4)	139 (36,1)	172 (44,5)	386
	360	323	381	1.064

A (H1): gripe A (H1N1)pdm09; gripe A (H3): A (H3N2); IB: gripe B

Se han observado importantes variaciones en función de la temporada gripal y las cepas circulantes en ellas. Así en la 2015-16 la gripe B (linaje Victoria) predominó en ambos grupos etarios, representando el 62.4% de todos los casos de gripe infantil; mientras que en la 2016-17 fue el subtipo A (H3N2) predominó en ambos grupos de edad (91.5%). En la temporada 2017-18 hubo un predominio mixto entre la gripe A (H1N1) pdm09 (46.6%) y la gripe B (linaje Yamagata) (39.6%). Finalmente en la última temporada 2018-19, al no circular la gripe B, se ha observado de nuevo un predominio del subtipo A (H3N2) (74.8%).

En este estudio hemos analizado cuatro temporadas gripales, dos con ausencia de gripe B y dos con presencia de la misma. En la primera circuló mayoritariamente el linaje Victoria, que parece afectar de forma preferente a la población infantil y en la segunda el linaje Yamagata, que afecta de forma prioritaria a la población de mayor edad y con patologías crónicas [2,3].

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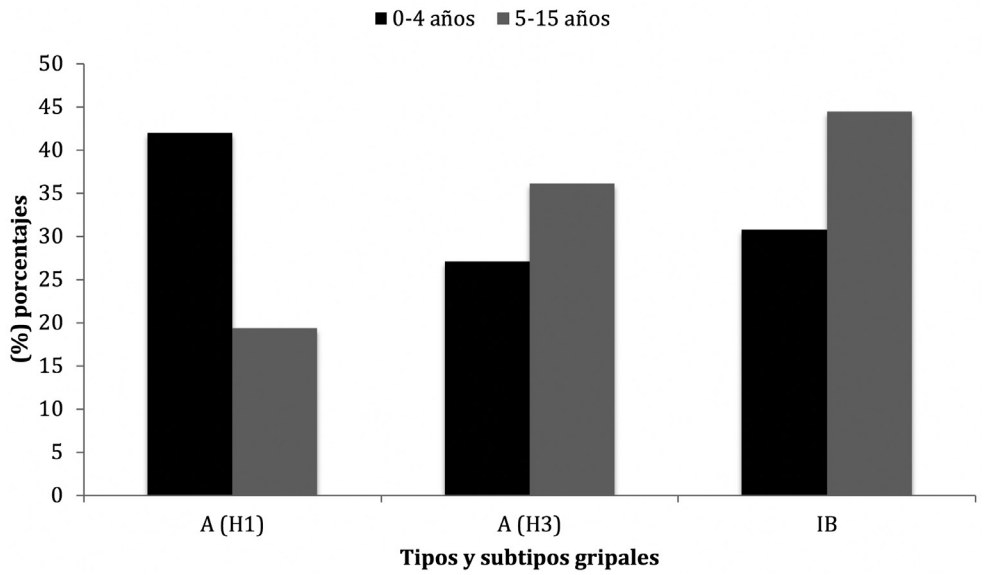

Figura 1 | **Distribución global de los casos de gripe por grupos etarios y tipos y subtipos gripales detectados en las últimas temporadas epidémicas.**

Coincidiendo con la mayoría de estudios, la gripe infantil afecta en cada temporada de forma preferente al grupo etario de 0-4 años, que alcanza las tasas epidemiológicas más elevadas [1, 3, 4]. Este grupo es el mas afectado por la gripe, tasas mas elevadas de ingreso hospitalario y morbimortalidad, probablemente debido a su no vacunación y a la inmadurez del sistema inmunológico para hacer frente cada temporada a una nueva cepa gripal [5]. Desde el punto e vista de salud pública este es el grupo prioritario ya que es el responsable de la introducción, mantenimiento y transmisión de la gripe en el ámbito comunitario [1, 3, 5].

En cuanto al predominio de los tipos y subtipos gripales, globalmente no se ha observado una diferencia entre ellos, aunque si por grupos de edad, ya que se confirma la mayor incidencia del subtipo A (H1N1)pdm09 en el grupo de 0-4 años, dato ya observado en la introducción de esta cepa en la pandemia de 2009 (6). No existen apenas publicaciones que relacionen el subtipo gripal con la edad y sólo en el estudio de Glezen et al. [1] se comunica que de 204 niños con infección por gripe A, 178 (87.2%) pertenecían al subtipo A (H3N2) y 26 (12.8%) al subtipo A (H1N1), aunque estos datos son anteriores a la pandemia de 2009.

La vacunación frente a la gripe durante la gestación y a partir de los seis meses comportaría un importante impacto de salud pública en las epidemias estacionales comunitarias de gripe.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

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Official journal of the Spanish Society of Chemotherapy

Consensus document

Revista Española de Quimioterapia
doi:10.37201/req/2065.2020

Recommendations for the diagnosis and treatment of *Clostridioides difficile* infection: An official clinical practice guideline of the Spanish Society of Chemotherapy (SEQ), Spanish Society of Internal Medicine (SEMI) and the working group of Postoperative Infection of the Spanish Society of Anesthesia and Reanimation (SEDAR)

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Article history
Received: 2 January 2020; Acepted: 26 January 2020; Published: 20 February 2020

ABSTRACT

This document gathers the opinion of a multidisciplinary forum of experts on different aspects of the diagnosis and treatment of *Clostridioides difficile* infection (CDI) in Spain. It has been structured around a series of questions that the attendees considered relevant and in which a consensus opinion was reached. The main messages were as follows:

CDI should be suspected in patients older than 2 years of age in the presence of diarrhea, paralytic ileus and unexplained leukocytosis, even in the absence of classical risk factors. With a few exceptions, a single stool sample is sufficient for diagnosis, which can be sent to the laboratory with or without transportation media for enteropathogenic bacteria. In the absence of diarrhoea, rectal swabs may be valid. The microbiology laboratory should include *C. difficile* among the pathogens routinely searched in patients with diarrhoea.

Laboratory tests in different order and sequence schemes include GDH detection, presence of toxins, molecular tests and toxigenic culture. Immediate determination of sensitivity to drugs such as vancomycin, metronidazole or fidaxomycin is not required. The evolution of toxin persistence is not a suitable test for follow up. Laboratory diagnosis of CDI should be rapid and results reported and interpreted to clinicians immediately.

In addition to the basic support of all diarrheic episodes, CDI treatment requires the suppression of antiperistaltic

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agents, proton pump inhibitors and antibiotics, where possible. Oral vancomycin and fidaxomycin are the antibacterials of choice in treatment, intravenous metronidazole being restricted for patients in whom the presence of the above drugs in the intestinal lumen cannot be assured. Fecal material transplantation is the treatment of choice for patients with multiple recurrences but uncertainties persist regarding its standardization and safety. Bezlotoxumab is a monoclonal antibody to *C. difficile* toxin B that should be administered to patients at high risk of recurrence. Surgery is becoming less and less necessary and prevention with vaccines is under research. Probiotics have so far not been shown to be therapeutically or preventively effective. The therapeutic strategy should be based, rather than on the number of episodes, on the severity of the episodes and on their potential to recur. Some data point to the efficacy of oral vancomycin prophylaxis in patients who reccur CDI when systemic antibiotics are required again.

Key-words: *Clostridiodes difficile*, *Clostridium difficile*, Diarrhoea associated to *C. difficile*, Vancomycin, Metronidazole, Fidaxomicin, Fecal Material Transplantation (FMT), Bezlotoxumab, Vaccines, Probiotics, Monoclonal antibodies.

Recomendaciones para el diagnóstico y tratamiento de la infección por *Clostridioides difficile*. Guía de práctica clínica de la Sociedad Española de Quimioterapia, Sociedad Española de Medicina Interna y grupo de trabajo de Infección Postoperatoria de la Sociedad Española de Anestesia y Reanimación

RESUMEN

El presente documento recoge la opinión de un foro multidisciplinar de expertos sobre distintos aspectos del diangóstico y tratamiento de la infección por *Clostridioides difficile* (CDI) en España. Se ha estructurado alrededor de una serie de preguntas que los asistentes consideraron pertinentes y en las que se llegó a una opinón de consenso. Los principales mensajes fueron los siguientes:

CDI debe sospecharse en pacientes mayores de 2 años de edad ante la presencia de diarrea, ileo paralítico y leucocitosis inexplicada, aún en ausencia de los factores de riesgo clásicos. Salvo excepciones, es suficiente con una sola muestra de heces para su diagnóstico que pueden ser enviadas al laboratorio con o sin medio de transporte para bacterias enteropatógenas. En ausencia de diarrea, pueden ser válidos los isopados rectales. El laboratorio de microbiología debe incluir a *C. difficile* entre los patógenos buscados de rutina en pacientes con diarrea.

Las pruebas de laboratorio en diferentes esquemas de orden y secuencia incluyen la detección de GDH, la presencia de toxinas, las pruebas moleculares y el cultivo toxigénico. No se precisa la determinación inmediata de sensibilidad frente a fármacos como vancomicina, metronidazol o fidaxomicina. La evolución de la persistencia de toxina no es un test adecuado para el seguimiento del proceso. El diagnóstico de laboratorio de CDI debe ser rápido y los resultados informados e interpretados a los clínicos con carácter inmediato.

Además del soporte básico de toda diarrea, el tratamiento de CDI requiere la supresión de los agentes antiperistálticos, de los inhibidores de la bomba de protones y de los antibióticos, cuando sea posible. Vancomicina oral y fidaxomicina son los antibacterianos de elección en el tratamiento, restringiéndose metronidazol intravenoso para enfermos en los que no se pueda asegurar la presencia en la luz intestinal de los fármacos anteriores. El trasplante de materia fecal es el tratamiento de elección para pacientes con múltiples recurrencias pero persisten incertidumbres sobre su estandarización y seguridad. Bezlotoxumab es un anticuerpo monoclonal frente a la toxina B de *C. difficile* que debe administrarse a pacientes con alto riesgo de recurrencias. La cirugía es un procedimiento cada vez menos necesario y la prevención mediante vacunas se encuentra en fase de investigación. Los probióticos no han demostrado, hasta el momento, eficacia terapéutica ni preventiva. La estrategia terapéutica debe basarse, más que en el número de episodios, en la gravedad de los mismos y en la potencialidad de recurrir. Algunos datos apuntan a la eficacia de la profilaxis con vancomicina oral en pacientes que recurren cuando vuelven a precisar antibióticos sistémicos.

Palabras clave: *Clostridiodes difficile*, *Clostridium difficile*, diarrea asociada a *C. difficile*, vancomicina, metronidazol, fidaxomicina, Trasplante de materia fecal, bezlotoxumab, vacunas, probióticos, anticuerpos monoclonales

INTRODUCTION

Clostridiodes difficile (CD) is the leading cause of infectious diarrhea in adults in contact with the health-care setting [1, 2], but also an increasing proportion of *C. difficile* infections (CDI) are either community-acquired or of community onset [3-7]. In Spain, the estimated incidence of CDI acquired in relationship with HealthCare Facilities is 6,5 episodes per 10,000 patient-days of admission and 22.3 episodes per 100.000 inhabitants [8], but many episodes remain undetected. The underdiagnosis was evaluated in three different Nationwide studies in Spain. The results across these studies showed a decrease in missed diagnoses from 76% to 50% between 2008 and 2013 [8-11]. The underdiagnosis, in Spain, is due to the lack of clinical suspicion or to the use of insensitive diagnostic tests. In the European EUCLID study, that followed the methodogy of the Spanish Studies, the mean number of CDI episodes was of 7 episodes of CDI per 10,000 patient-bed days and it was estimated that 23% of the cases were missed [12]. Most cases described in Spain have mild or moderate severity, are health care-associated, and have a recurrence frequency ranging from 12% to 18% [6, 11, 13].

Recurrent CDI (rCDI) represents an incremental morbidity and cost for patients and institutions. It falls not just on the length-of stay, which has the highest weight, but also on re-hospitalisation, serious complications, laboratory tests and medications [14, 15]. In addition to the economic burden, an important factor that needs to be taken into account in pa-

tients suffering from rCDI is the impact on the quality of life (QoL) of this disease [16, 17].

Accurate diagnosis of CDI is suboptimal and laboratory methods to diagnose the disease can be misleading due to the development in the last years of multiple tests with different sensitivities, specificities and targets (bacterium, toxins, cell membrane enzyme, toxin genes) [8, 10, 18, 19].

Finally, current recommendations for treatment vary according to the country issued and the clinical definition, and have been linked mainly to the number of episodes of the patient and the severity of CDI. However, treatment of patients with CDI recurrences and those with severe complicated forms is not so clear and is based on limited clinical evidence, and new treatments or strategies are needed [2, 20, 21]. Several recently completed prospective, randomized, double blind clinical trials have showed that fidaxomicin can be as effective as vancomycin in achieving clinical cure and superior in preventing recurrence [22, 23]. New published evidence has demonstrated that patients receiving bezlotoxumab, a fully human monoclonal antibody especific against the toxin B of CD, plus antibiotic treatment against CDI had a 40% of relative reduction of rCDI at 12 weeks [24].

However, the administration of antimicrobial agents to treat rCDI has been put into perspective with another therapeutic and preventive alternative: Fecal Material Transplantation (FMT) [25-27]. FMT is effective in reducing the incidence of multiple recurrences, however, the technique is cumbersome, not available in the majority of institutions and raises concerns related to the best way, the best dose and the potential transmission of currently unknown microorganisms [28-37]. Also, in the near future, there might be the possibility of prevention with vaccination [38, 39] or with antibiotics.

Aware of the problem of CDI, a panel of experts were convened to develop an opinion document with recommendations on the diagnosis and treatment of CDI, based on the best available evidence for achieving the greatest clinical efficacy adapted to the situation in Spain. The present document is structured in several questions, agreed among the participants, about controversial issues in the diagnosis and treatment of CD in our country. Every answer has a review of the evidence supporting or refuting the issues raised. Finally, the recommendations based on this review are issued.

QUESTION 1. When should CDI be suspected in patients older than 2 years?

Traditional risk factors for acquision of CDI are antimicrobial treatment within the previous 6-8 weeks, advanced age and prolonged hospital stay [6, 40-48]. However, recent studies have shown that a significant proportion of CDI episodes affect patients without any of these risk factors, thus outlining the need for a greater awareness of this disease. In 2008, a study performed in more than 100 Spanish hospitals showed that most patients unsuspected to have CDI did not have traditional risk factors for CDI such as advanced age or hospitalization [10]. A two-point prevalence study performed in Spain and other European countries in January and July 2013 showed similar results [8, 12]. CDI is also relatively common in nonhospitalized patients both with prior contact with the health care system or without [6]. When compared with patients with hospital-acquired CDI (HA-CDI), those with community-acquired CDI (CA-CDI) were younger, more likely to be female, had lower comorbidity scores, and were less likely to have severe infection or have been exposed to antibiotics [6, 49]. One North-American population-based study performed in the period 2004-2007 also showed a high incidence rate for CA-CDI and almost a third of these episodes were from patients that had not received antimicrobials in the six months prior to the diagnosis, and 17% did not have any traditional risk factors for CDI [50]. Another study by Naggie et al, showed that 40% of the patients with CA-CDI had not received antimicrobials prior to diagnosis [51]. In a recent study published by Spanish authors, the use of rifaximin in cirrhotic patients was associated with breakthrough CDI [52]. This evidence supports that common risk factors for CDI may not be present in all patients, therefore each probable case has to be evaluated in an individualized manner and in the presence of a diarrheic episode all patients should be suspected of CDI until proven otherwise.

Some people become carriers of CD or develop a mild, self-limited diarrhea while others develop severe colitis and may have multiple relapses of the disease [53, 54]. Patients with CD intestinal disease usually have mild or severe diarrhea and abdominal pain, low-grade to high-grade fever, and leukocytosis; they may have hypovolemia, shock, and hypoalbuminemia. Some patients develop fulminant colitis associated with a colonic ileus, in which case the patient may not have diarrhea. Physicians rarely suspect CD disease in the absence of diarrhea. A colonoscopy to look for pseudomembranes and to obtain a stool sample is not necessary to make or confirm the diagnosis in the majority of the episodes and may even be associated with adverse events. A computed tomography scan may be diagnostic of CD colitis, provided physicians think of this disease in the absence of diarrhea. Patients with acute toxic megacolon may have abdominal pain, fever, leukocytosis [55], and hypoalbuminemia, but they may not have diarrhea. Many clinicians would suspect ischemic colitis, rather than CD colitis. This form of CD disease has a very high mortality rate and a poor response to vancomycin and metronidazole [56, 57].

One of the most uncertain points of CDI diagnosis is the clinical significance of the detection of a toxigenic CD strain in a diarrheic patient aged less than 2 years. The low number of toxin receptors in the intestinal lumen in patients aged less than 2 years and the high frequency of viral pathogens producing diarrheic episodes indicate the doubtful role of CD in the diarrheical diasease in this age group. In a study performed in a Ma-

drid hospital, all diarrheic stool samples received from children younger than 2 years old were screened for CD. Positive (cases) and negative (controls) children were compared and also cases receiving or not specific anti CDI treatment. No differences in clinical behaviour were detected and all the patients, including CD cases, independently of the administration of metronidazole, were cured of the diarrheic episode [58]. A group of experts recently found a lack of evidence of an unmet need to treat CDI infections in infants under 2 years of age [59].

In conclusion, data in the literature supports that CDI should be suspected in diarrhoeic episodes in patients of any age except for those younger than 2 years even in patients with or without traditional risk factors for CDI. Some patients may not have diarrhea, such is the case of colonic ileus or "unexplained" leukocytosis, making the clinical diagnosis more difficult and sometimes resulting in fulminant colitis

QUESTION 2. Which is the optimal number of stool specimens that should be sent to the Microbiology laboratory for the diagnosis of CDI?

The positivity rate of subsequent specimens from patients with a first negative sample is very low and, therefore, testing a second specimen from a negative patient is more likely to be a false-positive [60-62].

International guidelines of three of the most important societies, the Infectious Diseases Society of America (IDSA), the Society for Healthcare Epidemiology of America (SHEA), and the American Society for Microbiology (ASM) recommend to test only one stool specimen per patient for the diagnosis of CDI [20, 63]. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) does not recommend repeated sample submission during the same episode in a endemic situation although it may be useful in a epidemic situation [64].

In conclusion, data in the literature supports that the best cost-effective number of stool specimens needed for the diagnosis of CDI is one stool specimen and, only exceptionally, two or more stool samples.

QUESTION 3. Which transportation media can be used to send specimens to the laboratory for CDI diagnosis?

Although CD is an anaerobic pathogen, the ability of this microorganism to form spores allows the transportation of specimens for the diagnosis of CDI in aerobiated containers without any media to maintain viability of the microorganism [65-68]. Guidelines performed by IDSA and SHEA societies recommend transporting stool specimens in clean, watertight containers, without transport medium to diagnose CDI [63]. However, in a great number of laboratories, stool samples with a clinical request for aerobic enteropathogens like *Salmonella* spp., *Shigella* spp., or *Campylobacter* spp. are sent to the Microbiology laboratory preserved in transport media. One of the most common media used is Cary-Blair medium, that prevents overgrowth of most *Enterobacteriaceae* and is effective in the preservation for long periods of common enteropathogens. Additionally, this medium does not affect the performance of four different diagnostic methods used to diagnose CDI (glutamate dehydrogenase immnunoassay, toxins A and B immunoassay, cell culture cytotoxicity assay and real-time PCR targeting the toxin B gene) [69-71] Samples transported in sporicidal medium like formaldehyde for parasites must be rejected. Samples must be sent as soon as possible to the microbiology laboratory. In general, it is recommended to preserve samples at 2-8ºC the first 48-72 hours or frozen at -60 to -80ºC if samples will not be processed within the following 72 hours [19].

In conclusion, data in the literature supports that both, samples without any transport medium and samples with transport medium for aerobic enteropathogens, such as Cary-Blair, are suitable for the diagnosis of CDI.

QUESTION 4. Should stool specimens without a *C. difficile* request be processed for *C. difficile* diagnosis?

As previously recommended, clinicians should suspect CDI in any patient suffering diarrhea with or without traditional risk factors for this disease, including outpatients. However, clinicians are not always aware of the presence of common or uncommon risk factors for this illness and, even if it is the case, clinicians sometimes do not remember to include the request for CDI diagnosis in samples sent to the Microbiology laboratory. Clinical misdiagnosis can occur even in patients with traditional risk factors like hospitalized patients. Even in the best scenario for CDI recognition, as is the case of nosocomial diarrhea, the clinical suspicion of CDI is far from optimal, where about 30% of nosocomial CDI episodes are missed due to a lack of processing specimens for CDI [9, 55].

In conclusion, it seems clear that the microbiology laboratory has an important role in improving the diagnosis of CDI. Since the degree of suspicion of physicians may be insufficient, the microbiologist should consider, with the available information, to perform diagnostic techniques for *C. difficile* in samples of unformed faeces regardless of what was requested

QUESTION 5. Should specimens other than diarrheic stool specimens be processed for CDI?

There is a general consensus in all international guidelines that watery or loose stools are the only specimens that should be collected to diagnose CDI in diarrheic patients with suspicion of this disease, in day to day clinical practice [63, 64]. However, CD can produce infections in which patients do not develop diarrhea, like ileus, toxic megacolon, pseudomembranous colitis without diarrhea or abdominal distension. In these cases, it may not be possible to obtain an unformed stool specimen for a CDI diagnosis. In these situations, diagnostic procedures recommended by international guidelines are not applicable. English guidelines performed by the Advisory Com-

mittee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) recommend using in these situations procedures such as colonoscopy, white cell count, serum creatinine or abdominal computerized tomography scanning [72]. On the other hand, guidelines performed by the SHEA and IDSA societies recommend rectal specimens obtained by means of cotton swabs for the etiologic diagnosis of CDI [63, 64]. Guidelines from the ASM do not recommend rectal specimens in these situations and only suggest using formed stool specimens, when present, and after consensus with clinicians [73].

Culture of rectal specimens are as sensitive as stool culture for the diagnosis of CDI [74], and the sensitivity, specificity, positive predictive value, and negative predictive value of testing perirectal swabs versus stool specimens using PCR are similar [75]. The culture of colon biopsies obtained by colonoscopy has been an acceptable procedure for diagnosis of CDI for a long time, however, diagnosis based on stool specimens, which are less invasive and cheaper, could be a better option for CDI diagnosis [69]. To clarify this question, a comparison between diagnostic methods in both colon biopsies and stool specimens was evaluated in a retrospective study in Spain. The study showed that sensitivity of colon biopsies to diagnose CDI (21.3%) was significantly lower than that obtained using simultaneous stool specimens (94.7%) [76].

In conclusion, data from the literature shows that rectal specimens are useful for CDI diagnosis in patients in which stool specimens cannot be obtained. Furthermore, stool specimens are more sensitive than colonic biopsies for the diagnosis of CDI in patients with colitis.

QUESTION 6. What combination of laboratory tests is most cost effective and therefore recommended for an optimal diagnosis of CDI?

Currently, there is no diagnostic test for CDI that alone can be sufficiently cost-effective to be used in the rapid diagnosis of the disease. As a consequence, diagnostic algorithms have been designed to take advantage of the benefits of each diagnostic test [77-79]. Rapid detection of toxins A and/or B can be performed with enzyme immunoassays (EIAs). At first, laboratories used EIAs that detected only toxin A but, with the dissemination and higher frequency of strains toxin A-/toxin B+ producing CDI, these were replaced by EIAs capable of detecting both toxins [80-83]. Some comparative studies have shown that EIAs have sensitivity values of 40-60% when compared with toxigenic cultures [84-90]. On the other hand, the specificity of most of these tests is higher than 90%. The American Society for Microbiology, ASM, considers these tests as techniques with low sensitivity and strongly recommends that these tests are not used as stand-alone tests [73]. In Europe, an analysis performed by the ESCMID committee concerning 13 commercial EIAs that detect toxins A and/or B also showed the deficiency of sensitivity of these tests and concluded that CDI diagnosis should be performed with more sensitive tests [64].

Detection of the enzyme glutamate dehydrogenase (GDH), a protein produced in large quantity by most of the CD strains (toxigenic and non-toxigenic), is another rapid test that allows the detection of CD. The sensitivity of this test is higher than toxin detection alone, between 85%-95%, however the specificity and positive predictive value are relatively low since it can detect strains that produce toxins as well as non-producing strains [91, 92]. Due to the relative low specificity of tests based in GDH detection alone, main societies do not recommend the latter as single tests for rapid diagnosis of CDI [63]. Currently, there are commercialized diagnostic tests that include detection of both GDH antigen and toxins A and/or B, with the main advantage of offering results simultaneously.

In recent years, the CDI diagnostic conundrum has been dramatically transformed by the development of commercial nucleic acid amplification tests (NAATs). NAATs are molecular assays that mostly utilize real-time PCR or loop-mediated isothermal amplification to directly detect the *tcdA* or *tcdB* genes encoding toxin A or B, respectively, from stool specimens [93-103]. Due to the rapid uptake of NAATs there are now a great number of commercial products in the market, most of them FDA approved, like the BD MAX system (Becton Dickinson), Xpert® *C. difficile* (Cepheid), Prodesse® ProGastroTM CD (Gen-Probe) and Illumigene® *C. difficile* (Meridian), with an average of turnaround time between 45 min – 3 hours. The majority target the *tcdB* gene, and some can detect one or both genes of the binary toxin, even hipervirulent ribotype 027 strains that have mutations or deletions of the repressor gene *tcdC* [104, 105]. Sensitivity values of most of these techniques are very high with values greater than 90% and specificities greater than 98% when compared with toxigenic culture, however, the positive predictive value of NAATs for CDI can be low to moderate (80%-95%), depending upon disease prevalence and the limit of detection of the assay [21]. The other problem of NAATs is their high cost when used as stand-alone tests. This limitation precludes them as a systematic alternative for diagnosis of CDI in most laboratories [104].

The cytotoxin assay has been traditionally considered the gold standard for CDI diagnosis. This technique uses tissue cultures to detect CD toxins from diluted stool specimens. This assay is highly specific because it uses specific antibodies for neutralization. However, numerous studies have shown that the cytotoxin neutralization assay is only 65 to 80% sensitive to detect toxigenic CD isolates in comparison to toxigenic culture, which is performed by isolating CD on selective media and demonstrating cytotoxin production by the cultured organism [106-108]. A rising group of experts considers that a negative cytotoxin assay with a positive toxigenic culture indicates a low concentration of free toxin in the stools, not able to be detected by the cytotoxic assay alone [79, 109]. For this reason, the ASM recommends using toxigenic culture or NAATs as a confirmatory test of the rapid algorithms [21].

Due to the limitations of each of the individual tests for a rapid and correct diagnose of CDI, several multi-step algorithms have been proposed (figures 1 and 2). These algorithms have as screening test the detection of GDH by EIA due to its high sensitivity to detect CDI [110-113]. As most specimens are negative, the GDH screening step substantially reduces the

number of specimens that require evaluation with more specific methods. Since both toxigenic and nontoxigenic CD strains express GDH, a positive GDH EIA requires confirmation with a sensitive assay for detection of toxin A or B or their genes. Overall performance including turnaround time of a GDH-based algorithm depends on the secondary tests used to follow up a positive GDH result. GDH detection followed by a NAAT is considered a two-step algorithm and has approximately a 90% sensitivity, and specificity higher than 99% [19]. A three-step algorithm detects toxins A and/or B between the GDH detection and NAATs, reducing almost by 50% the number of molecular tests needed [19]. However, in the recent update of CDI guidelines published recently by IDSA and SHEA the recommendation is to use a stool toxin test as part of a multistep algorithm (i.e., GDH plus toxin; GDH plus toxin arbitrated by NAATs; or NAATs plus toxin) [21]. These procedures have been evaluated by several authors and have a sensitivity of 85-90% and specificity greater than 99% [77, 85, 95, 114-117].

Another traditional and less costly method for the diagnosis of CDI is the toxigenic culture; it has increased sensitivity over cytotoxicity and it is based on the detection of toxin production in the microorganism after isolation in culture. A downside to this technique would be the 48 hours to obtain bacterial growth. Published literature shows a controversy in the diagnosis of CDI in patients in which the results of the cytotoxic assays differ from the toxigenic culture. In a study by Reigadas et al, the authors observed that CDI episodes positive by cytotoxicity assay were more severe than those positive only by toxigenic culture. However, in their study, there were a significant proportion of CDI cases (31.9%) that would have been missed if only cytotoxicity had been considered, including 10% of severe CDI cases and one patient with pseudomembranous colitis. Additionally, in the same study, 45% of the CDI cases had a negative toxin portion EIA, which exemplifies the need for further testing samples with a positive GDH portion and a negative toxin EIA portion test, by PCR or toxigenic culture [118].

Some situations call for a change in the diagnostic algorithm. This is the case of CDI due to ribotype 027 strains that are usually more severe, with a higher transmission and recurrence rate than CDI caused by other ribotypes. In case of suspicion of an outbreak due to this ribotype, it is recommended to perform a rapid molecular test that specifically detects this ribotype [19, 119].

In conclusion, data reported in the literature shows that detection of GDH by EIA as a screening test followed by a rapid confirmatory technique as a NAAT alone or together with the detection of toxins by EIA is the most cost-effective procedure for the rapid diagnosis of CDI (figure 1 and 2). Toxigenic culture is a slow but sensitive and low-cost method for detecting CDI in patients with negative EIA or cytotoxicity.

QUESTION 7. When and how to perform antimicrobial susceptibility tests to *C. difficile* isolates?

For a long time, the susceptibility testing of the traditional antimicrobials metronidazole and vancomycin was not even recommended because the universal activity of these drugs was not questioned. However, different *in vitro* susceptibility studies performed during the last years have showed the existence of toxigenic isolates of CD resistant to these drugs.

In 1997, Barbut and colleagues found one resistant strain showing an MIC to metronidazole of 16 mg/L by the agar dilution method [120]. During the period 1993-2000, Peláez and colleagues [121] detected 26 isolates resistant to metronidazole from 415 isolates tested (6.3% of resistance, MICs: ≥32 mg/L, agar dilution method) in Spain. A posterior analysis performed

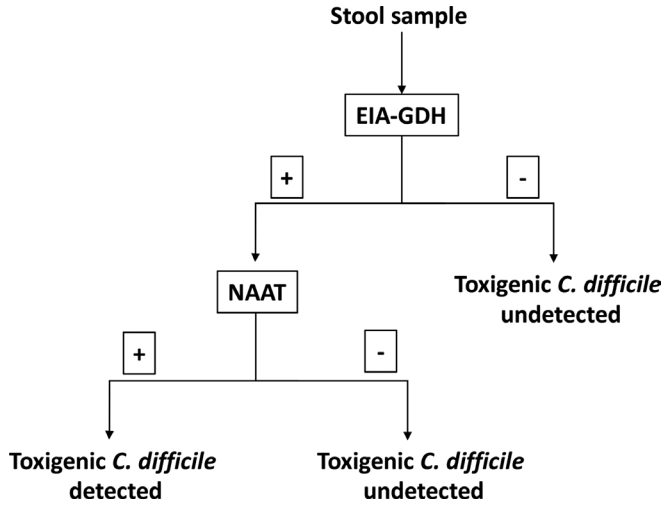

Figure 1 A rapid, cost-effective algorithm for the diagnoses CDI (two steps)

EIA-GDH: Detection of glutamate dehydrogenase by enzyme immunoassay
NAAT: nucleic acid amplification test

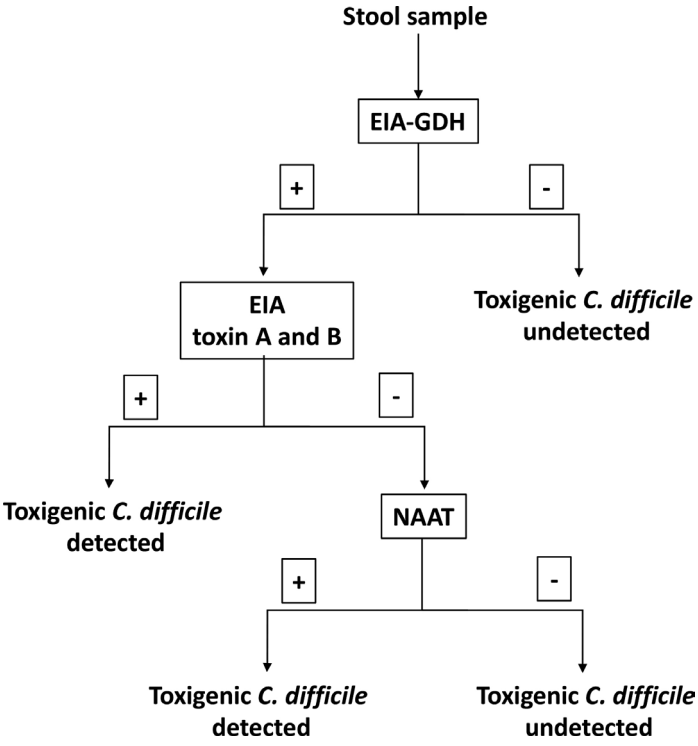

Figure 2 | **A rapid, cost-effective algorithm for the diagnoses CDI (three steps)**

EIA-GDH: Detection of glutamate dehydrogenase by enzyme immunoassay
EIA toxin A and B: Detection of toxin A and B by enzyme immunoassay
NAAT: nucleic acid amplification test

by Peláez et al. showed that resistance to metronidazole was heterogeneous and that it can be lost in strains after prolonged periods of storage due to freezing and thawing [122]. In an Israelite study performed, authors described a 2% of resistance to metronidazole (1/49 isolates, MIC: ≥32 mg/L, E-test method) [123]. A similar resistance rate was found in toxigenic strains isolated during 2004 to 2006 in Ontario, Canada (19/1,080 isolates, MICs: ≥32 mg/L, E-test method) [124]. Recently, Huang and colleagues reported a 23.1% of resistance to metronidazole in primary fresh toxigenic *C. difficile* strains isolated from 2008 to 2009 in China (18/78 isolates, MICs ≥32 mg/L, E-test method) [125]. As occurred in the Spanish study, the Canadian and Chinese isolates had such an heterogeneous resistance that most of the resistant isolates turned into sensitive to metronidazole after serial passages [122, 124]. Although not as frequent, isolates of CD with intermediate resistance to vancomycin (MIC>2 mg/L) have been reported [126-129]. On the other hand, fidaxomicin has shown a good activity against CD with most isolates having MICs lower than 1 mg/L being the highest MIC ever reported, to our knowledge, of 2 mg/L [130-137].

Concentration in colonic mucosa of metronidazole and its metabolite hydroxymetronidazole is considered bactericidal in patients with acute disease receiving oral or intravenous metronidazole, but as the diarrhea improves neither substance is detectable in the faeces of diarrhea caused by CD (mean concentration of 9.3 µg/g in watery stools and of 1.2 µg/g in formed stools)[138, 139]

This finding has led to the EUCAST committee to decrease the metronidazole breakpoint from 16 mg/L to 4 mg/L [140]. Conversely, fecal levels of vancomycin and fidaxomicin in the colon lumen are greater than metronidazole with concentrations of 64-760 µg/g on day 2 and 152-880 µg/g on day 3 post-treatment for vancomycin and as high as 3,000 mg/L for fidaxomicin [120] .

Although Clinical and Laboratory Standards Institute [126] guidelines do not recommend routine susceptibility testing for CD isolates, because correlation of MICs with clinical failures has not been established, they advocate in performing an annual surveillance testing to detect emerging resistance. The surveillance should be done by the hospital laboratory if expertise is available or, if not, by a reference laboratory. If possible, the guidelines recommend to test isolates collected over several months and stored until a total of 50-100 strains are available for later batch testing using preferably an agar dilution method [126].

In conclusion, data in the literature suggests that sensitivity testing should be performed annually to detect the emergence of resistance or in specific situations and in reference laboratories, but not on a routine daily basis

QUESTION 8. Is it necessary to follow-up patients with CDI with laboratory tests?

Generally, in non-complicated CDI cases, the therapeutic response for CDI involves the resolution of fever (if present) on the first day and of diarrhea before the fourth or fifth day [131]. This clinical resolution of the disease may not be accompanied by a microbiological clearance of CD toxins, as CD can survive in the lumen of cured patients during several weeks or months [132, 133]. In a study performed in healthy patients with previous recurrent CDI, authors found that persistence of spores of CD by the end of antibiotic therapy occurred in 56% of patients receiving metronidazole and 43% receiving vancomycin [134]. A similar observational study showed that nearly 20% of patients successfully treated for CDI had detectable spores in stool specimens at the time of the resolution of the diarrhea and it increased to 56% one to four weeks later [141]. The lack of correlation demonstrated in these studies between clearance of colonic CD and resolution of CDI has led to international guidelines to recommend not to use culture or toxin detection to follow-up the evolution of patients with CDI [20, 52]. In order to reduce false positives, some experts suggest that microbiological laboratories reject stool samples from patients treated for CDI with a microbiological diagnosis in the previous seven days [19], as well as from asymptomatic patients (unless suspicion of ileum or toxic megacolon) [21].

In conclusion, data in the literature shows that detection of toxigenic *C. difficile* from stool specimens is not a good method to follow-up the evolution of patients with CDI and should not be performed routinely.

QUESTION 9. When and how to report clinicians the results of laboratory tests for CDI?

Early recognition of an episode of CDI is a critical step to optimize the treatment of CD and to control the transmission to other patients, and must be based in three mainstays: a correct suspicion of the illness by clinicians, an accurate and rapid laboratory diagnosis of CDI, and a rapid and effective transmission of information of these results to the attending physician, infection prevention officer, and nursing staff [20, 52]. The Centers for Disease Control and Prevention (CDC) recommends to work with microbiology laboratories to ensure rapid reporting of test results for CDI, including weekends and holidays, and to ensure that there is a process for providing results to the patient care area so that isolation precautions can be initiated promptly (Center for Diseases Control and Prevention, Guidelines for preventing transmission of MDROs, 2006) [135]. Due to the fact that CD is able to produce spores that persist in the environment for many months and are resistant to cleaning and disinfectant measures, this pathogen is highly transmissible [132, 133]. Transmission of CD to the patient via transient hand carriage on healthcare workers´ hands is thought to be the most likely common way of transmission [136]. Some prominent authors and scientific societies such as the SHEA, the Association for Professionals in Infection Control and Epidemiology, the CDC, the Healthcare Infection Control Practices Advisory Committee, and the Infectious Diseases Society of America [137, 138, 142-146] recommend several points to the health care facilities referring to the quarantine of CDI patients, use of antiseptic procedures such as the utilization of disposable gloves, mask and gown and hand-washing with soap and water, cleaning of patient-care equipment (such as thermometers, stethoscopes, etc.) before it is used with another patient, to enhance environmental cleaning with diluted bleach from all patient contact surface areas, to restrict the use of antimicrobials implicated as risk factors for CDI, to provide an easy laboratory access for prompt and active surveillance toxin B detecting at the earliest indication of a case of CDI and to use rapid and accurate tests to diagnose CDI in the laboratory.

Another important issue is that rapid and accurate laboratory recognition of a CDI episode is a key step to optimize the treatment of patients with CDI. Rapid report of a positive result can facilitate a prompt treatment that avoids the risk that an initial mild CDI episode may progress to severe colitis and toxic megacolon [147]. Delayed diagnosis can increase the time of patient exposition to inappropriate drugs as antiperistaltic or narcotics that can complicate CDI [148]. Similarly, fast information of a negative result favors the withdrawal of antimicrobials in patients with empiric treatment for CDI [52].

In conclusion, data from the literature suggests that rapid laboratory recognition of CDI is crucial for the control and management of this illness. Preliminary phone information of results obtained from the rapid diagnostic tests to the appropriate health care workers is recommended. Ideally, this information should be accompanied by test interpretation and treatment advice

THERAPEUTIC OPTIONS FOR CDI

QUESTION 10. What is the basic support approach for the treatment of patients with CDI?

The basic support approach for the treatment of patients with CDI include: 1) a standard supportive care for patients who are hemodynamically unstable, consisting of rapid fluids and electrolyte intravenous replacement. 2) avoidance of the following precipitating factors: a) agents such as narcotics and loperamide that inhibit intestinal peristalsis, retain intestinal toxins, and increase the risk of toxic megacolon [149-151]; b) concomitant broad-spectrum antibiotics for other concurrent infections [an early switch to reduced-spectrum antibiotics should be performed if complete suspension of the treatment is not possible)]; and c) anti-ulcer medication, especially proton pump inhibitors (PPI)[152, 153].

In conclusion, the basic support approach for patients with CDI includes: fluids and electrolyte replacement, and removal of intestinal peristalsis inhibitors, anti-ulcer medication, and concomitant antibiotics, when feasible.

QUESTION 11. What are the antibiotics of choice for CDI treatment?

The choice of initial antibiotic therapy for CDI depends on the severity of disease, the possibility of oral therapy, and the potential risk for recurrence. Initially, the first prospective, randomized studies in which patients were not stratified by disease severity demonstrated that both oral metronidazole and oral vancomycin were equally effective, over 90%, in the first episode and first recurrence [127, 128]. However, when patients were stratified based on the severity of the infection, vancomycin had a clinical response significantly better than metronidazole in severely ill subjects (97% versus 76%, P = 0.02) [130]. More recent studies have demonstrated that vancomycin provides superior cure rates compared with metronidazole, with reduced side effects, even in mild cases [154, 155].

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Guidelines in 2014 recommended metronidazole as first-line treatment for non-severe CDI and vancomycin as the first choice for severe CDI [20]. Nevertheless, results from a meta-analysis of large multicenter randomized controlled trials (RCT) showed that metronidazole is inferior to vancomycin in the treatment of CDI (non-severe and severe combined, with severe CDI defined as a white blood cell count ≥10,000/mm3, ten or more bowel movements per day, and severe abdominal pain)[154].

Fidaxomicin is a macrocyclic antibiotic approved in the USA and in Europe for the treatment of CDI [156]. Two completed prospective, randomized, double-blind, clinical trials showed that the rates of clinical cure after treatment with oral fidaxomicin (200 mg twice daily for 10 days) were non-inferior to those after treatment with oral vancomycin (125 mg four times daily for 10 days); at the same time, a significant reduction in the rates of recurrence with an increase in the rate of sustained responses was also observed [22, 23]. Oral fidaxomicin is well tolerated, with a safety profile comparable to that of oral vancomycin. There are no differences in the incidence of death or serious adverse events between the two drugs. A downside is that the cost of fidaxomicin is much higher [23, 157]. However, in a recent Spanish study, using a cost–utility analysis model, it has been observed that fidaxomicin is more cost-effective than vancomycin for treatment of CDI in patients with cancer, renal impairment, and/or with concomitant antibiotic treatment [158]. Subsequently, two other studies in patients with cancer or concomitant antibiotic treatment have demonstrated a significant superiority of fidaxomicin over vancomycin [157, 159].

Since the publication of the ESCMID guidance document in which fidaxomicin was reserved for patients with relapsing CDI, a published meta-analysis and indirect treatment comparison suggested that fidaxomicin may be considered as first-line therapy for CDI in patients with a high risk of recurrence [160, 161]. In this regard, recently published IDSA guidelines recommend vancomycin or fidaxomicin for best treatment of initial CDI. The dosage recommended is: vancomycin 125 mg orally 4 times per day or fidaxomicin 200 mg twice daily for 10 days (strong recommendation, high quality of evidence)[21].

Severe CDI cases may be treated with either oral vancomycin or fidaxomicin. A recent prospective, multicenter study demonstrated that courses with either antibiotic resulted in similar treatment outcomes for patients afflicted with severe CDI [21, 162]. When oral treatment is not possible, intravenous metronidazole should be used.

In conclusion, oral vancomycin is the recommended drug for an initial CDI episode; oral fidaxomicin should be considered in initial CDI episodes with a high risk of recurrence. In view of the evidence described above, the opinion of this group of experts is that the use of metronidazole should be restricted to situations in which vancomycin or fidaxomicin are contraindicated or an oral administration is not possible. Combination therapy (i.e. vancomycin and metronidazole) is not recommended in patients with severe CDI, with the exception of severe cases complicated with ileus

QUESTION 12. What additional antibiotics are being studied for the treatment of CDI?

Additional antibiotic options to the ones cited above exist, such as rifaximin [163-165], nitazoxanide [166-168], fusidic acid [169], tigecycline [170, 171], and teicoplanin [128, 172]; however, we do not recommended any of those for routine CDI treatment. Their use in the treatment of recurrences might be overshadowed by the efficacy of the currently available strategies described above. Moreover, there are reports of resistance development to rifaximin [52, 173] and fusidic acid [169], which further discounts the use of these antibiotics as treatment options for CDI in any episode.

Novel antibiotics include cadazolid, a new oxazolidinone. Cadazolid is an inhibitor of CD protein synthesis, causing more suppression of toxin production and spore formation than vancomycin and metronidazole. In pre-clinical studies, cadazolid showed a potent bactericidal in vitro activity against CD (MIC90 of 0.25 mg/L) and a low propensity for resistance development [174-176]. The results for the IMPACT I and IMPACT II phase 3, randomized clinical trials were recently published [177]. While safe and well tolerated, cadazolid failed to achieve the primary end-point of non-inferiority vs. vancomycin for clinical cure [177]. As a result, to the best of our knowledge, efforts to commercialize cadazolid for CDI have been halted.

Ridinilazole is another novel antibiotic currently undergoing clinical trials for the treatment of CDI. The precise mechanism of action of this antibiotic is not clear, however, it appears to impair cell division [178]. With limited activity against Gram-positive and Gram-negative intestinal aneaerobes and a low MIC (MIC90 of 0.125 mg/L) for CD, ridinilazole appears to be a promising candidate for treating CDI. A phase 2, randomized clinical trial comparing ridinilazole with vancomycin for the treatment of CDI demonstrated non-inferiority and a statistically significant superitory at the 10% level. Moreover, the antibiotic was well tolerated with an adverse profile similar to that of vancomycin [179]. Phase 3 clinical trials comparing ridinilazole and vancomycin are ongoing [180].

In conclusion, data from the literature suggests that rifaximin, nitazoxamide, fusidic acid, tygecicline, and teicoplanin are currently not considered as therapy options for CDI, especially since new treatments and strategies for rCDI reduce the risk of recurrence. Cadazolid has failed to achieve the primary end-point of non-inferiority vs. vancomycin for clinical cure in a recent published clinical trial. Ridinilazole is a promising antibiotic with phase 3 clinical trials recruiting patients at present to demonstrate non-inferiority over vancomycin for the treatment of CDI.

QUESTION 13. What are the contributions of bezlotoxumab to the treatment of CDI?

Bezlotoxumab is a recombinant human IgG1/kappa isotype monoclonal antibody approved globally in 2017 for use as an adjunctive treatment in patients at risk for rCDI [181]. Bezlotoxumab binds to regions of the combined repetitive oligopeptide domains of toxin B that partially overlap with putative receptor binding pockets. This monoclonal antibody blocks the action of *C. difficile* toxin B and potentially averts the damage and inflammation that can lead to the symptoms associated with CDI [182].

In two global, phase III trials (MODIFY I and MODIFY II), bezlotoxumab demonstrated significant reductions in CDI recurrence compared with placebo (17% vs 28% in MODIFY I and 16% vs 26% in MODIFY II; $P < .001$) in adults receiving antibiotic treatment for primary CDI or rCDI [183]. In a secondary analysis, bezlotoxumab demonstrated better efficacy results in reducing CDI recurrence in a group of patients at high risk for CDI recurrence (patients with previous CDI episodes, severe CDI, older age (≥65 years old), and infection with hypervirulent strains]. Bezlotoxumab also reduced rCDI, FMT, and CDI-associated 30 day re-admissions in participants with risk factors for rCDI. As a result, the Spanish therapeutic positioning report (IPT) [184] recommends the use of bezlotoxumab as adjuvant therapy for CDI treatment in patients at high risk of recurrence, including patients ≥65 years old with a previous CDI episode in the last six months, immunosuppressed patients, patients with CDI caused by hypervirulent strains (such as 027 and 244), patients with severe CDI (Zar ≥2), and patients that exhibit a high probability of recurrence as evaluated by externally validated predictor models.

In conclusion, the monoclonal antibody bezlotoxumab is the first approved treatment for the prevention of CDI recurrence. It has demonstrated a 40% reduction of CDI recurrence when compared to placebo. Its efficacy is higher in sub-groups of patients at greater risk for recurrence

QUESTION 14. How should bezlotoxumab be used in clinical practice at present?

The efficacy of bezlotoxumab has already been discussed above. A single injection significantly decreases the incidence of recurrences and that difference has been maintained in subgroups of special populations such as immunosuppressed, transplanted, elderly patients, patients in renal failure and other subgroups. The patients to be selected are obviously those in whom a high risk of recurrence is predictable. In this sense, the best-known elements of risk associated to the host are advanced age (≥65 years), the need to maintain antibiotic treatment for baseline infection, deficiencies in humoral immunity response, serious underlying diseases and the need to continue taking proton pump inhibitors, among others [185]. As the microorganism is concern, it seems clear that strains with high toxin production, as is the case with many of those grouped as 027, are associated with an increased risk of recurrence [186-189]. Despite all these data, risk scores for predicting recurrences, based on the association of clinical signs or symptoms, have not functioned adequately on most studies [190-196] and only in some works are they attributed a certain orientative value [197, 198]. Some authors have used also toxin production, through what we might consider a surrogate marker, that would be the amplification cycle of PCR curves. Early amplifications before cycle 24 would be associated with worse evolution and very late cycles (amplification cycles beyond 28) would be associated with colonization [6, 199-203].

Interestingly, these scoring systems show us that certain patients in the first episode of CDI have a higher risk of recurrence than other patients in the second episode [185].

Data derived from the Modify I and II studies analyzed by Gerding et al [185] suggest that 75.6% of hospitalized patients meet one or more risk factors for recurrence who would, therefore, be natural candidates to receive bezlotoxumab. In this study, the risks of recurrence are proportional to the risk factors of each patient. With one risk factor the recurrence rate was 31% but with 3 or more risk factors, recurrences reached 46%. In this most-at-risk population, the reduction in recurrent episodes after receiving bezlotoxumab was 53%. However, not all risk factors are equally predictive [193, 194, 198, 204] and none of these models or scores seem to have been widely accepted.

Unfortunately, the latest clinical practice guidelines issued recently by the Infectious Disease Society of America (IDSA), although they include 53 therapeutic recommendations, do not provide recommendations or guidance on the use of bezlotoxumab in clinical practice [21].

This working group, thinking of the need for a progressive introduction of this drug in the medical practice of our country and considering economic factors, proposes a score-guidance to decide the use of bezlotoxumab, based on a points-based score, in which risk factors and patient conditions do not receive the same weight. We believe that age >65 years, immunodeficiency, a severe or persistent disease and an amplification cycle of the PCR <24 should be scored with one point each. Diseases or situations such as episodes of CDI in the previous year, malignant underlying diseases, inflammatory bowel disease and liver cirrhosis, should be scored with two points each. Finally, patients with hypertoxigenic or very virulent strains and diseases in which a FMT is indicated and cannot

be performed or in which a previous FMT has failed, should receive 3 points with each of these conditions. In our opinion, patients who accumulate 3 or more points are clear candidates to receive bezlotoxumab, but this score has not been validated. Patients with lower scores, in our opinion, should be considered individually. Whenever possible, concomitant antibiotics and acid-suppressing medications, specifically histamine blockers and PPIs, should be removed [205]. The table 1 summarizes this simple, bedside score system, applied to patients with CDI that could help select patients at most risk for CDI recurrence.

In conclusion, we believe that in our environment, bezlotoxumab should be administered to patients with an episode of CDI that are at high risk of recurrence. At present, after the recent introduction of the drug in the market, and for economic reasons, it is prudent to select patients with high risk of recurrence and for this we offer a risk score recommendation to select the more clear candidates.

QUESTION 15. Are there additional immunotherapy-based options to address CDI?

Immunotherapy consists on using passive immunization with antibody-based products against *C. difficile* surface proteins to complement the deficient immune response of the host [206]. Targeted antigens are usually toxins A and B (TcdA and TcdB) and the main objective of immunotherapy is usually the prevention of recurrences [206]. As described above, bezlotoxumab is the only antibody-based product approved for clinical use to prevent CDI recurrences to date [181].

Another form of immunotherapy, albeit with no proven efficacy to date, is the use of intravenous immunoglobulins (IVIG). IVIG have been used to treat the recurrence of CDI with various success rates. Thus far, randomized studies showing a clear benefit are lacking [182]. A prospective analysis with a small number of patients compared the outcomes between use or no use of IVIG. There were no statistical differences in clinical outcomes as measured by all-cause mortality, colectomies, and length of stay [207]. In intravenous formulations for antibody-based products, the latter must be transferred from the systemic circulation to the intestinal lumen. To eliminate this hurdle, oral formulations of IG have also been explored in hamsters [208].

In conclusion, intravenous immunoglobulins are currently not recommended as adjunctive therapy for CDI since there are no conclusive data that demonstrate their efficacy in the prevention of recurrences.

QUESTION 16. What is the current role of Fecal Microbiota Transplantation (FMT)?

Experience with FMT in refractory or recurrent cases of CDI has accumulated over the years. FMT restores gut microbiota diversity through implantation of donor stools into the gastrointestinal tract of patients with CDI. This treatment has shown good clinical response in adults with refractory or recurrent CDI with few reports of adverse events [209-214].

The FMT technique requires a careful selection of donors to avoid the transmission of any of the known enteric pathogens and potentially of other diseases, that usually leads to the rejection of practically nine out of 10 donor candidates [215]. The reasons for rejection are multiple and include, for example, people who have had tattoos or acupuncture in the last six months or who have travelled to tropical countries in the last half year and those with a body mass index greater than 25 [211]. Today, there is a tendency to rely on repeated donations from a few very well-controlled donors who can supply efficient banks.

Fecal processing is cumbersome and unpleasant, and each donation provides material for approximately two to five transplants. At first, FMTs were performed with fresh material, administered either topically by colonoscopy or by nasoduodenal catheterization, using a minimum of 30 grams of fecal matter. Subsequently, the major milestones to facilitate the process have been to demonstrate that frozen faeces from healthy donors maintain their properties and efficacy and that encapsulated material, either fresh or lyophilized, administered orally in capsules, is as effective as the colonic delivery [26, 216-219].

Liofilization allows preparation and storage for multiple transplants that can be performed almost immediately. This establishes the possibility of creating banks for FMT that permit procedures to be performed quickly after indication. At

Table 1 **Prediction score for recurrent *C. difficile* Infection**

+1 point:	+2 points:	+3 points:
>65 years	Previous CDI (previous year)	FMT failure
Immunosuppressed	IBD	Indication for FMT but not possible
Severe CDI	Malignancy	Hypervirulent strains
Antibiotics for other infections	Other high-risk medical conditions	Recurrent episode
Toxin B Ct <24		
Persistent diarrhea >5 days		

CDI: *Clostridioides difficile* infection, IBD: Inflammatory Bowel Disease, FMT: Fecal Material Transplantation.

present, the administration of four capsules of lyophilized material in a single dose is sufficient [34, 217, 220].

Some commercial companies have made available preparations of fecal material or even preparations of intestinal bacterial pools with satisfactory results [221].

In a recent systematic review [222] that included 37 studies (seven randomised controlled trials and 30 case series), FMT was more effective than vancomycin and the overall case resolution was 92%. In cases of initial failure, consecutive courses of FMT resulted in an incremental effect. Recently, however, a tappering cycle of vancomycin was shown, in a comparative study, to be as effective as an FMT [223], however, the authors selected a suboptimal FMT delivery.

There is a general concern regarding long term safety in patients receiving FMT, particularly in relation to metabolic or immune-based disorders [21]. In an open-label, randomized, controlled trial, and in a systematic review that included 273 patients from 11 studies involving more than 10 analyzed cases each, the short-term safety and acceptability of the technique by the patients was high [224, 225]. The transmission of potential pathogens or resistant microorganisms through faeces has been a cause for concern from the outset, but this risk has been minimal to date [226, 227]. Also of concern is the risk of bacterial translocation with distant infections such as bacteremia in immunodeficient patients or in those with increased enteric barrier permeability. It is recommended to avoid FMT in patients with anaphylactic reactions due to food allergies and to be cautious in patients with decompensated cirrhosis or deeply immunocompromised.

Therefore, in the opinion of this working group, the indications for FMT would be focused on patients with proven recurrences and potentially in cases with poor response to treatment, particularly in patients with severe manifestations and who have failed in tappering treatments with vancomycin or fidaxomycin, as long as the procedure is available, the patient accepts it and none of the exclusion criteria for the procedure are met. At the present time, the indication of FMT for first episodes of CDI has yet to be considered an investigational procedure.

In conclusion, FMT is unquestionably one of the most effective ways to avoid the recurrence of CDI and should be offered to patients with multiple recurrences, particularly to those that failed a tappered cycle of oral vancomycin or fidaxomicin. Uncertainties remain about the standardization of the procedure and particularly about its long-term safety. It is also necessary to study the best combination of FMT with other available therapeutic procedures

QUESTION 17. In which situations should surgical intervention be considered?

Patients with severe complicated or fulminant CDI that do not respond to medical treatment in the first 24-48 hours should be evaluated by a surgeon. A classic review showed data supporting total colectomy with end ileostomy as the primary surgical treatment for patients with severe CDI [228]. However, total colectomy is associated with poor outcomes, signficiant morbidity, and a high mortality rate ranging from 35% to 80% [229]. An alternative to a total colectomy procedure, diverting loop ileostomy, combined with colonic lavage, is a less aggressive alternative [229]. Briefly, this technique consists on performing a diverting loop ileostomy and using mechanical lavage to remove bacteria and toxins from the intestinal lumen, followed by a direct instillation of vancomycin into the lumen to further eliminate the remaining CD. This technique has a significantly lower mortality rate when compared with total colectomy and preservation of the colon was achieved in 39 of 42 patients (93%) [229, 230]. In a retrospective multicenter study including data from ten centers of patients who presented with CDI requiring surgery , when comparing colectomy and loop ileostomy, adjusted mortality was significantly lower in the loop ileostomy group [231]. In a more recent meta-analysis, however, it did not appear that diverting loop ileostomy was clearly assoociated with a decrease in mortality but resulted in increased rates of colonic preservation, restoration of intestinal continuity, and laparoscopic surgery[232].

In conclusion, current therapeutical options have reduced the need to resort to surgical invervention, relegating this option to fulminant, non-responding cases. Patients with CDI that do not respond to treatment in the first 24-48hrs should be evaluated by a surgeon; loop ileostomy and colonic lavage should be considered in severe complicated or fulminant CDI without response to medical treatment. Total colectomy should be avoided if at all possible.

QUESTION 18. What is the situation of the vaccines in the near future?

Vaccine candidates based on altered CD toxins A and B are currently under clinical trial study for the prevention of CDI [233, 234]. Early trials suggest that some of these candidates have an acceptable safety and tolerability profile. However, while select candidates have demonstrated substantial immune response in subjects, a definite dose-response relationship has not been established yet and as a result, the ideal dose remains unknown [190]. There is also some concern related to the short durability of the antibody response with some of these candidates, as this would potentially require the administration of additional doses or boosters to provide patients with a long-term clinical benefit [190, 235, 236]. The target population for vaccine administration requires careful consideration, high-risk groups such as subjects with compromised immunity and the elderly might benefit more, however, data on these populations is lacking [190]. The cost of a vaccination regime must also be considered, particularly if targeted to a high-risk population; as high prices might preclude implementation of this strategy in large groups for the prevention of CDI. Results from ongoing trials will be needed to determine whether vaccines constitute a long-term, cost-effective solution to prevent CDI.

In conclusion, vaccine candidates constitute a promi-

sing solution to prevent CDI, however, substantial clinical trials are necessary to establish the real benefit associated to their use.

QUESTION 19. Can probiotics be used to prevent recurrences?

There is limited clinical evidence related to the use of probiotics in the treatment of CDI. In a meta-analysis, *Saccharomyces boulardii* showed promise for the prevention of CDI recurrences [237]. Another meta-analysis suggested that primary intervention of CDI with specific probiotic agents may be achievable [238]. However, a Cochrane review did not find sufficient evidence for the recommendation of probiotics as adjuvant therapy for CDI [239]. Furthermore, a multicenter, randomized, double-blind, placebo-controlled, trial performed to assess the role of *Lactobacilli* and *Bifidobacteria* in the prevention of antibiotic-associated diarrhea and CDI in older inpatients failed to demonstrate a beneficial effect with probiotics [240]. Even, a recent study shows that the use of probiotics was associated with a higher risk of recurrence [241].

In conclusion, probiotics cannot be recommended for widespread use for the prevention or adjuvant therapy of CDI.

QUESTION 20. How should a patient with a first episode of CDI be managed?

As we previously concluded in question 11, recent literature and guidelines have implicitly agreed that metronidazole should be dismissed as an alternative for the treatment of CDI, even in non-severe cases [21, 162]. In fact, the current approach for treating a first episode of CDI should not be based on the severity of the first episode but instead should be based on the presence or not of risk factors for CDI recurrence (figure 3). With the recent evidence, it seems reasonable and cost-effective to treat a first episode with a low risk for recurrence with vancomycin orally (125 mg four times daily for 10 days) [154, 155]. In a few exceptions, i.e. if the patient has no ability for oral intake, should IV metronidazole be considered.

On the contrary, a first episode with a high risk for recurrence deserves a treatment that has proven to reduce CDI recurrences (i.e. fidaxomicin or bezlotoxumab). Therefore, in our opinion, the current debate consists on trying to identify the patient that, in the presence of a first episode, has a high risk for recurrence and could benefit from new treatment strategies to reduce future recurrences. As we mentioned before, scores for risk factors are needed, and we proposed in question 14 an example of a scoring system for determinig the use of bezlotoxumab in patients at high risk for recurrence (table 1).

Given the demonstrated efficacy of bezlotoxumab in the prevention of recurrent CDI episodes, a regime of oral vancomycin (125 mg four times daily for 10 days) with bezlotoxumab (one dose of 10 mg/kg IV) as adjuvant therapy should be administered for a first episode with a high recurrence risk [183]. A regime of fidaxomicin is also an option in patients with a high risk for recurrence (200 mg twice daily for 10 days), however, it remains a price-sensitive option [160].

Recently, Rubio-Terrés et al. performed a cost-effectiveness analysis in Spain, comparing extended-pulsed fidaxomicin versus vancomycin in patients 60 years and older with CDI. According to their economic model , and the assumptions based on the Spanish National Health Ssystem fidaxomicin is cost-effective compared with vancomycin for the first-line treatment of CDI in patients aged 60 years and older [242].

In MODIFY studies it could not be confirmed that bezlotoxumab reduced the risk of recurrence in patients treated with fidaxomicin, probably because of the small number of

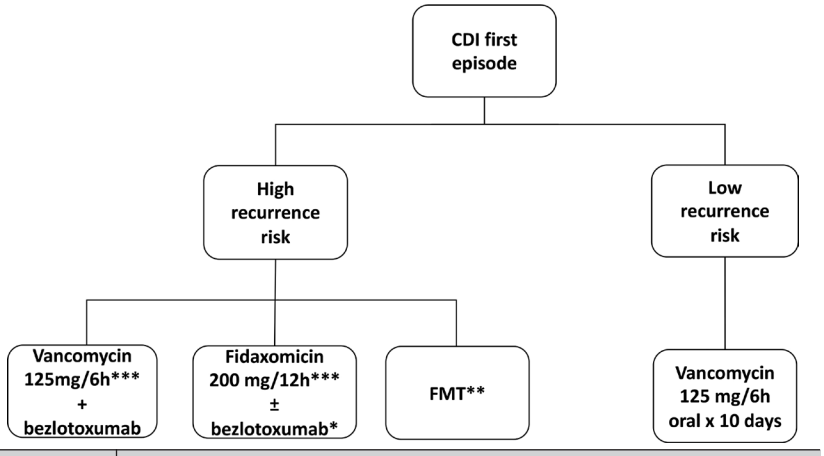

Figure 3 Treatment of first CDI episodes

*Price-sensitive option in some hospitals

**Remains a research alternative for a first episode.

***In patients at high risk of recurrences antibiotics should be administered in a prolonged tappering way.

patients treated with this drug. Addition of bezlotoxumab to fidaxomicin is another treatment option [183], but it would still be affected by the same cost issues faced by the antibiotic alone and more studies are needed to evaluate the efficacy of reducing rCDI with this combination. The use of FMT, in the first episode with high-risk of recurrence, as we mentioned before, is yet only a research alternative. Purportedly, in a setting without economical restrictions, patients at higher risk for recurrence would benefit from fidaxomicin, the best antibiotic option to treat CDI, plus bezlotoxumab, a monoclonal antibody with antitoxin activity.

In conslusion, patients with a first CDI episode and a low risk for recurrence will benefit from oral vancomycin alone. In the case of the presence of high risk factors for recurrence, patients with a first CDI episode will benefit from adding bezlotoxumab or using fidaxomicin.

QUESTION 21. How should a patient with a CDI recurrence be treated?

Ideally, rCDI is a condition to prevent, more than a condition to treat. From a clinical and epidemiological point of view, a recurrence is conventially defined as a CDI episode that re-occurs within eight weeks after complete resolution of the initial or previous episode, confirmed by toxin detection in a stool sample. This definition has been accepted because it is clinically practical and easy to apply; however, patients with previous episodes not considered recurrences because of the time between one episode and the next, should still be managed as patients at high risk for recurrences [194]. The risk of recurrence is variable, ranging between 15% and 25% after the initial episode, reaching rates of up to 60% after a third episode [11, 243, 244].

Treatment options for patients that have recurred are the same as for patients with a first episode with a high risk for recurrence (i.e. fidaxomicin, bezlotoxumab and/or FMT). Recurrence is the highest indicator that a patient needs to be managed focused in preventing recurrences. In these cases, there are different alternatives for treatment that could be applied; i.e. extending or prolonging CDI antibiotic treatment, enhancing the immune system against toxins with bezlotoxumab or microbiota restoration with FMT.

Extending the suppression of CD has been evaluated with strategies that have demonstrated to reduce CDI recurrences. That is the case of vancomycin tapering or fidaxomicin extended treatment. With vancomycin tapering, these regimens typically include a 10- to 14-day course of oral vancomycin at a dose of 125 mg four times per day, followed by a tapering dose over two weeks, followed by "pulsed" dosing with 125 mg once every two or three days for two to eight weeks [223, 245] The other alternative, is a regime of extended-pulsed fidaxomicin: 200 mg oral tablets, twice daily on days 1–5, then once daily on alternate days on days 7–25. This strategy has been compared with standard vancomycin, demonstrating superiority of fidaxomicin regarding sustained cure of CDI and lower rates of recurrence [246].

A controlled clinical trial comparing fidaxomicin extended versus a regimen of vancomycin taper would further support these strategies.

Adding bezlotoxumab to the antimicrobial CDI treatment of patients who already have had a recurrence has proven to reduce CDI recurrences [183]. Additionally, it seems reasonable to considerer giving another dose of bezlotoxumab in a patient with a recurrence in which the monoclonal antibody may have been metabolized since the first episode and the cause of CDI persists (i.e. continuous use of antibiotics). However, there is a need for studies that evaluate the efficacy of repeating a bezlotoxumab dose in a patient who has received the monoclonal antibody for a previous episode. This repetition is not approved.

FMT has also demonstrated efficacy in the treatment of recurrences and should be considered in centers where the procedure is standardized [21]. It is currently uncertain how FMT should be linked to other treatment options; i.e. previous preparation with vancomycin or fidaxomicin before the FMT. These are gaps that must be addressed and evaluated in future studies.

Another situation are the patients with multiple recurrences. These patients enter in a loop of recurrences, receiving numerous treatment options for CDI that are not able to stop the recurrence cycle. The main cause of this situation is the persistence of one or more risk factors for CDI recurrence, i.e. continuous antibiotic use for other infections, persistent immunosuppressive therapy, etc. [247]. In these patients the best treatment options that have proven to reduce CDI recurrences should be used. The management of these patients consist of an art between using the current options for treating rCDI and the experience of the physician. The three available ammunitions (fidaxomicin, bezlotoxumab and FMT) must be used in these scenarios. In our opinion, these patients would benefit from the best antibiotic against CD recurrence (fidaxomicin), immunity against CD toxin B (bezlotoxumab) and restoration of the gut microbiota (FMT).

In conclusion treatment options for patients that have recurred are the same as for patients with a first episode with a high risk for recurrence. However, a different management can be applied (i.e vancomycin taper regime or extended pulse of fidaxomicin). In these patients, in our opinion, FMT is the treatment of choice but the association of bezlotoxumab for immunity against toxin B must be considered. Whenever possible, risk factors for CDI recurrence should be halted in order to prevent future recurrences.

QUESTION 22. In patients that have recurrent episodes of CDI induced by new courses of systemic antibiotics, is oral vancomycin prophylaxis effective?

One of the first studies addressing this matter was the retrospective study performed by Carignan et al. in 2016 [248] in which they studied 551 CDI episodes and observed that oral vancomycin prophylaxis decreased the risk of further recurren-

ce in patients who had a former rCDI episode (AHR, 0.47; 95% CI, 0.32–0.69; P <0.0001)[248]. This reduction was not observed for primary CDI episodes.

More recently, in 2019, two new studies have been published. Knight et al. evaluated retrospectively the long-term efficacy of oral vancomycin prophylaxis in preventing CDI recurrence in subjects who require subsequent antibiotic exposure. They observed that CDI recurrence within 12 months was significantly lower in subjects receiving oral vancomycin prophylaxis compared to those who did not receive it (6.3% vs 28.8%; odds ratio (OR): 0.16; 95% confidence interval (CI): 0.04-0.77; P =0 .011)[249]. Zhang et al. presented a small series of patients in which they observed that prolonged vancomycin prophylaxis at a dose of 125 mg orally daily was an effective and well-tolerated option for secondary prevention of rCDI [250].

Several recent communications have been made at the Infectious Diseases Week meeting, held in Washington (ID Week) in October 2019, providing more evidence on this matter. Two retrospective studies [251, 252] including 72 and 264 patients, respectively, evaluated the use of prophylaxis with oral vancomycin, 125 mg twice daily in patients with a history of CDI and observed that the incidence of CDI was significantly lower in the group receiving oral prophylaxis compared to the control group.

A randomized, prospective study was presented by Johnson et al., in which 100 patients were enrolled 1:1 to either oral vancomycin (dosed at 125 mg once daily while receiving systemic antibiotics and continued for 5 days post completion of systemic antibiotics), or no prophylaxis. No cases of rCDI were diagnosed in the prophylaxis group compared to 6 (12%) in the no prophylaxis group (p = 0.03) [253]. As can be noted, there is still limited data regarding this issue, and more randomized controlled trials are needed. However, the existing literature holds promise for the use of oral vancomycin prophylaxis when a subsequent antimicrobial therapy is planned in a high-risk of rCDI patient.

In conclusion, antibiotic prophylaxis cannot be recommended for widespread use for the prevention of rCDI, however in selected cases that fulfill a high risk profile for rCDI and are scheduled to receive systemic antimicrobials, it can be indicated.

SUMMARY

1. CDI should be suspected in all diarrheic episodes of patients of any age, with or without traditional risk factors for CDI. except for those younger than 2 years.

2. One stool specimen is the best cost-effective number needed for the diagnosis of CDI.

3. Both, samples without any transport medium and samples with transport medium for aerobic enteropathogens, as Cary-Blair, are suitable for the diagnosis of CDI.

4. Microbiologists in the laboratory can have an important role in the improvement of the CDI diagnosis by processing unformed stool specimens from patients older than 2 years, independently of the request by the clinicians.

5. Rectal specimens are useful for CDI diagnosis in patients whose stool specimens cannot be obtained. Stool specimens are more sensitive than colonic biopsies for the diagnosis of CDI.

6. Detection of GDH by EIA as screening test, followed by a rapid confirmatory technique as a NAAT alone or together with a toxin A and B EIA, and the use of toxigenic culture, is the optimal diagnostic combination of laboratory tests to diagnose CDI.

7. Tests of antibiotic susceptibility should be performed annually to detect the emergence of resistance in reference laboratories or in specific situations but not in a daily regular basis.

8. Detection of toxigenic *C. difficile* from stool specimens is not adequate as a follow-up method for the evolution of patients with CDI.

9. Rapid laboratory work-up and reporting of tests for toxigenic *C. difficile* is crucial for the control and management of this illness.

10. The basic support approach for patients with CDI includes: fluids and electrolyte replacement, and removal of intestinal peristalsis inhibitors, antacid medication, and concomitant antibiotics, when feasible.

11. Oral vancomycin is the recommended drug for an initial CDI episode; oral fidaxomicin or bezlotoxumab, should be considered in initial CDI episodes with a high risk of recurrence. Metronidazole should be restricted to situations in which vancomycin or fidaxomicin are contraindicated or an oral administration is not possible.

12. Rifaximin, nitazoxamide, fusidic acid, tygecicline, and teicoplanin are currently not considered as therapy options for CDI. Ridinilazole is a promising antibiotic with phase 3 clinical trials set to start in 2019 to demonstrate non-inferiority over vancomycin for the treatment of CDI.

13. Bezlotoxumab is the first approved treatment for the prevention of CDI recurrence, with a demonstrated higher efficacy in all sub-groups of patients at greater risk for recurrence.

14. Bezlotoxumab should be administered to patients with episodes of CDI that are at high risk of recurrence. We suggest that, despite limitations, risk scores should be used to optimize candidate selection.

15. Given the lack of conclusive data, intravenous immunoglobulins are currently not recommended as adjunctive therapy.

16. FMT should be offered to patients with multiple recurrences, particularly to those who failed a tapered cycle of oral vancomycin or fidaxomicin. Uncertainties remain about the standardization of the procedure and particularly about its long-term safety.

17. Surgery should be considered in those patients with CDI that do not respond to treatment in the first 24-48hrs;

loop ileostomy and colonic lavage should be considered in severe complicated or fulminant CDI without response to medical treatment.

18. Vaccines constitute a promising solution to prevent CDI, however, substantial clinical trials are necessary to establish the real benefit associated to their use.

19. Probiotics cannot be recommended for the prevention or adjuvant therapy of CDI.

20. Patients with a first CDI episode and a low risk for recurrence will benefit from a 10 day course of oral vancomycin. In the case of the presence of high risk factors for recurrence, patients with a first CDI episode should be treated with bezlotoxumab and/fidaxomicin.

21. Recurrences, always imply a high risk of new recurrences and should be treated as such. FMT in these circumstances is probably the treatment of choice. Other options are a vancomycin or fidaxomicin tapering regimes with bezlotoxumab as adjuvant therapy. Whenever possible, risk factors for CDI recurrence should be halted in order to prevent future recurrences.

22. In patients with a history of CDI recurrence in coincidence with the re-introductrion of systemic antibiotics prevention with oral vancomycin (125 mg per day, during the days of use of systemic treatment and for up to 5 days after systemic antibiotics are completed) may be considered.

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