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Scientific letter

Is fosfomycin a good alternative drug for gonorrhoea treatment in our setting?☆

¿Es la fosfomicina una buena alternativa terapéutica para la gonorrea en nuestro medio?

On 27 February 2017, the WHO published the list of the 10 microorganisms resistant to priority antibiotics in the search for therapeutic solutions.[1] *Neisseria gonorrhoeae* resistant to cephalosporins and quinolones is one of them. Although the levels of resistance of *N. gonorrhoeae* to ceftriaxone are still very low in Europe,[2] in the article by Fuertes de Vega et al. 9.1% of gonococcal strains isolated from a tertiary hospital in Barcelona were resistant to cefotaxime, and 17.3% were resistant/intermediate to azithromycin.[3] The increasing resistance to available antibiotics, along with the delay in the development and marketing of new active antibiotics, have aroused interest in the reintroduction of "old" antibiotics such as fosfomycin.

We have studied the sensitivity to fosfomycin of isolated gonococcal strains in patients from five regions of the province of Barcelona: Anoia, Alt Penedès, Garraf, Baix Llobregat and Barcelonès. Strains isolated from urethral (96), vaginal (3), endocervical (2), balano-preputial (1) and semen (1) smears from January 2017 to March 2018 have been prospectively included. Gram staining demonstrated the presence of Gram-negative diplococci in 100/101 samples with staining performed. For the gonococcal isolate, samples were seeded in chocolate agar and Thayer-Martin agar (bioMérieux). The identification of *N. gonorrhoeae* was done using MALDI-TOF-MS. For the sensitivity study using gradient strips (E-test), the CG II agar medium (Becton Dickinson) was used, adjusting the inoculum to a turbidity equivalent to 0.5 of the McFarland scale in physiological serum. The plates incubated at 35 °C, in an atmosphere with 5–7% CO_2 for 20–24 h before reading. The results were interpreted in accordance with the cut-off value of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), Version 8.1, 2018-05-15, for enterobacteria. No isolate showed resistance to ceftriaxone, but the rest of the antibiotics tested did (Table 1). The molecular detection of *Chlamydia trachomatis* and *N. gonorrhoeae* was performed in 48 patients with positive culture, being positive for gonococcus in all cases, highlighting an excellent sensitivity of the molecular method compared to the culture. Coinfection with *C. trachomatis* was detected in 11/48 cases (22.9%). The high percentage of coinfections with chlamydia confirms the need to add the second antibiotic to the treatment with fosfomycin in the case of suspected gonococcal urethritis.

Table 1
Antibiotic sensitivity in *N. gonorrhoeae*.

	% (no.) of sensitive strains	% (no.) of intermediate strains	% (no.) of resistant strains
Penicillin	11.6 (12)	67 (69)	21.4 (22)
Ceftriaxone	100 (103)	–	–
Ciprofloxacin	35 (36)	–	65 (67)
Tetracycline	62.1 (64)	15.6 (16)	22.3 (23)
Fosfomycin	94.2[a] (97)		5.8[b] (6)

[a] MIC ≤ 32 mg/l (EUCAST cut-off value for enterobacteria).
[b] MIC >32 mg/l.

Over time *N. gonorrhoeae* has been acquiring resistance to the main antibiotics used.[4] Currently, the recommended treatment is the combination of ceftriaxone with azithromycin.[5] The emergence of strains resistant to third-generation cephalosporins, or even to the combination of two drugs, has raised alerts about encountering cases of intractable gonorrhoea. The fosfomycin used in the past as an alternative to treatment[6] allows better bioavailability of the drug with its current formulations. In their study, Yuan et al. demonstrated non-inferiority between the fosfomycin trometamol regimen 3 g daily on days 1, 3 and 5 compared to ceftriaxone plus azithromycin.[7] Most of the strains in our study presented an MIC ≤ 32 μg/ml for fosfomycin, sensitivity cut-off value proposed for other microorganisms by EUCAST. If we consider the sensitivity according to the cut-off values of the Clinical and Laboratory Standards Institute (sensitive ≤ 64 mg/l, intermediate = 128 mg/l and resistant ≥ 256 mg/l), only one strain was resistant and one presented intermediate sensitivity. Despite its effectiveness in monotherapy, fosfomycin is more likely to be used in combination therapy. Barbee et al. studied the combination of ceftriaxone with fosfomycin, and although there is no synergy, a lower fractional inhibitory concentration index (0.96) has been observed compared to other combinations studied.[8] Another study also showed no synergy of the combinations of fosfomycin with azithromycin or ceftriaxone.[9] Further studies are needed to ensure adequate concentrations of fosfomycin at the site of infection. Recently, Wijma et al. have shown significant variability in the pharmacokinetics of fosfomycin among healthy volunteers, which could explain therapeutic failures of uncomplicated UTI treatment with the recommended regimen of 3 g of oral fosfomycin in a single dose.[10] Even so, the available data position fosfomycin as a promising therapeutic alternative for uncomplicated cases of genital location.

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Mixed infection of four diarrhoeagenic *Escherichia coli* pathotypes in a case of travellers' diarrhoea: Characterisation of the isolates by whole-genome sequencing☆

Infección mixta por cuatro patotipos diarreagénicos de Escherichia coli *en un caso de diarrea del viajero: caracterización de los aislados obtenidos mediante secuenciación del genoma completo*

Diarrhoea is the most common health problem among travellers from developed countries who visit developing countries and/or tropical and semi-tropical regions, and this diarrhoea is known for this reason as travellers' diarrhoea (TD).[1] More than 60% of cases of TD are caused by bacterial agents, of which diarrhoeagenic *Escherichia coli* (DEC) plays a leading role.[2] Specifically, enterotoxigenic *E. coli* (ETEC) and enteroaggregative *E. coli* (EAEC) are currently considered the most common causes of TD, and it is estimated that together both pathotypes cause about half of the cases of TD in Africa and Latin America and more than a third of the cases of TD in southeast Asia.[1] While their relative importance as TD agents is less, other pathotypes such as verotoxigenic *E. coli* (VTEC), enteropathogenic *E. coli* (EPEC), both typical (tEPEC) and atypical (aEPEC), and enteroinvasive *E. coli* (EIEC) should also be considered as a diagnostic option in these types of infections.[3–5]

In November 2015, a stool sample from a two-year-old girl with a history of travel to Cuba was sent to the Microbiology Department of the Hospital Universitario Río Hortega [Río Hortega University Hospital]. Three days after returning from the trip, the girl had experienced a decrease in consistency and increase in frequency of her bowel movements, without any other associated symptoms. The physical examination was normal and no additional diagnostic tests were required, apart from the stool culture. The condition persisted for 15 days and resolved spontaneously, with administration of probiotics as the only therapeutic measure. In the stool culture the presence of norovirus, adenovirus, astrovirus and rotavirus was ruled out by immunochromatography (CerTest Biotec), and the most common bacterial enteropathogens—*Salmonella*, *Shigella*, *Yersinia*, *Hafnia*, *Aeromonas*, *Plesiomonas* and *Campylobacter*—were ruled out by conventional microbiological methods. Taking into account the history of recent travel, the sample was sent to the Spanish National Microbiology Centre for the diagnosis of DEC infection. The different pathotypes were detected by PCR for the genes encoding the verotoxins of VTEC *(vtx1, vtx2)*, the intimin of EPEC and VTEC *(eae)*, the virulence plasmid of EAEC *(aatA)*, the enterotoxins of ETEC *(eltA, estA)* and the invasion proteins of EIEC *(ipaH)*, as well as the BFP adhesin *(bfpA)*, that differentiates between tEPEC and aEPEC, from a stool culture of the sample in MacConkey agar (Becton-Dickinson), and the detected pathotypes were isolated.[6] The isolates obtained were sequenced on the NextSeq 500 platform (Illumina). For the extraction of genomic DNA, the QIAamp® DNA Mini Kit (QIAGEN) was used and "paired-end" libraries were generated using the Nextera XT DNA Sample Preparation Kit (Illumina). From the readings obtained, the serotype, sequence type, virulence gene profile and resistance gene profile of each isolate were determined with the SerotypeFinder, MLST, VirulenceFinder and ResFinder tools, respectively, available on the Center for Genomic Epidemiology server (https://cge.cbs.dtu.dk//services). Additionally, the antibiotic sensitivity of the isolates was studied using the disc diffusion method, according to EUCAST and CLSI criteria. The antibiotic panel used included ampicillin, cefotaxime, ceftazidime, amoxicillin-clavulanic acid, ertapenem, meropenem, ciprofloxacin, pefloxacin, gentamicin, chloramphenicol, trimethoprim, nalidixic acid, tetracycline, streptomycin, kanamycin and sulfamethoxazole.

The sample was positive simultaneously for VTEC, EAEC, ETEC and aEPEC pathotypes, and the isolation of the four present DEC strains was obtained, the complete characterisation of which is shown in Table 1. Although mixed infections by DEC strains of different pathotypes, as well as coinfections of DEC strains with other bacterial as well as viral or parasitic enteropathogens, are not uncommon, especially in cases of TD,[5,7,8] as far as we know, this is the first case of DEC infection in which the presence of four different pathotypes has been demonstrated. However, despite their frequent presence in children with diarrhoea, the clinical implication of some of these pathotypes, such as EAEC or aEPEC, is not clearly established, and has also been described in asymptomatic children. However, ETEC is a pathogenic group with a clear clinical implication, considered as a leading cause of TD in adults in developed countries and the most significant cause of childhood diarrhoea in developing countries.[9] In this sense, the production of the ST-Ia heat-stable enterotoxin by the ETEC O8:H8

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