New Microbiologica, 41, 3, 235-237, 2018, ISSN 1121-7138

SHORT COMMUNICATION

Susceptibility of clinical isolates of *Campylobacter jejuni* and *Campylobacter coli* to colistin

Antonio Sorlózano-Puerto[1], José Antonio Carrillo-Ávila[1], Miguel Gutiérrez-Soto[2], José María Navarro-Marí[3], José Gutiérrez-Fernández[1,3]

[1]*Departamento de Microbiología, Facultad de Medicina, Universidad de Granada, Instituto de Investigación Biosanitaria de Granada, Spain;*
[2]*Centro de Salud "Polígono Guadalquivir", Córdoba, Spain;*
[3]*Laboratorio de Microbiología, Hospital Virgen de las Nieves, Complejo Hospitalario Universitario de Granada, Instituto de Investigación Biosanitaria de Granada, Spain*

SUMMARY

Campylobacter spp. are one of the most frequent causes of bacterial diarrhea worldwide. Although severe diarrhea is not highly prevalent, the risk of a fatal outcome is increased when infection is caused by strains resistant to macrolides, fluoroquinolones, and/or tetracyclines. It is therefore necessary to test the susceptibility of these bacteria to other antibiotics such as colistin, which may serve as an alternative therapeutic option in these situations. The E-test was used to investigate the activity of erythromycin and colistin against 30 clinical isolates of *Campylobacter* spp. The MIC values obtained (range: 0.38-8 mg/liter) were sufficiently low, given the elevated concentrations that colistin sulfate can reach in the intestinal lumen, for this antibiotic to be considered useful to treat severe diarrhea caused by *Campylobacter* spp. resistant to first-line antibiotics.

Received December 12, 2017 *Accepted* February 15, 2018

Colistin is a polypeptide antibiotic from the group of polymyxins (polymixin E) synthesized by *Bacillus polymyxa* subspecies *colistinus*. Two forms of colistin are commercially available: colistin sulfate for oral or topical utilization, and colistimethate sodium for parenteral administration or nebulization (Falagas and Kasiakou, 2005). This old antibiotic has been given renewed life as an option for: treatment by parenteral injection or nebulization of multidrug-resistant gram-negative bacilli such as *Acinetobacter* spp., *Stenotrophomonas* spp., *Pseudomonas* spp. and carbapenem-resistant *Enterobacteriaceae* (Biswas *et al.,* 2012); the topical treatment of bacterial skin infections (Falagas and Kasiakou, 2005); and intestinal and oropharyngeal decontamination to prevent endogenous infection or ventilator-associated pneumonia (Giamarellou and Poulakou, 2009). Oral treatment with colistin is also indicated to treat enterocolitis from Gram-negative bacteria and diarrhea from pathogenic strains of *Escherichia coli* in children and breastfed infants, because colistin sulfate is poorly absorbed by the gastrointestinal tract and can reach elevated concentrations in the intestinal lumen (Li *et al.,* 2005).

Campylobacter spp. are the main cause of bacterial diarrhea in our setting, ahead of the genus *Salmonella*. They are responsible for 44.0% of cases, a percentage that has substantially increased over recent years (Sánchez-Capilla *et al.,* 2015), as reported in most industrialized countries (Allos, 2001). Although the disease is often mild and self-limiting, it is frequently severe or fatal in immunocompromised patients (Magaz Martínez *et al.,* 2016) or when the infection is caused by bacteria resistant to fluoroquinolones, macrolides or tetracyclines, the principal therapeutic options against these bacteria (Ghosh *et al.,* 2013). It is therefore necessary to test the *in vitro* susceptibility of clinical isolates of *Campylobacter* spp. to different antibiotics that might offer an effective alternative to first-line drugs when these are not active (Sorlózano-Puerto *et al.,* 2017). Colistin may be one such option for the treatment of severe enterocolitis caused by multi-resistant *C. jejuni* or *C. coli*.

However, there has been little research on the activity of colistin against *Campylobacter* spp., and the results have been highly varied (Feizabadi *et al.,* 2007; Komba *et al.,* 2015), making it difficult to establish its true therapeutic potential in these situations. In addition, because breakpoints have been established by the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) for susceptibility to macrolides, fluoroquinolones, and tetracyclines but not to polymyxins, most laboratories do not determine the susceptibility of *Campylobacter* spp. to colistin, among other antibiotics.

A prospective study was performed in the Microbiology Department of the Granada University Hospital Complex (Southern Spain) to investigate the susceptibility to erythromycin and colistin of 24 *C. jejuni* and 6 *C. coli* isolated in fecal samples from patients with acute

Key words:
Colistin, *Campylobacter jejuni*, *Campylobacter coli*, Diarrhea.

Corresponding author:
José Gutiérrez-Fernández
E-mail: josegf@go.ugr.es

Table 1 - *Erythromycin and colistin MICs (in mg/liter) for each isolate of Campylobacter spp. The clinical category according to CLSI (2016) and EUCAST (2017) breakpoints is given in parentheses (S = susceptible, I = intermediate, R = resistant).*

Microorganism	*Erythromycin MIC*	*Colistin MIC*
C. jejuni	0.5 (S)	1.5
C. jejuni	0.25 (S)	2
C. jejuni	0.5 (S)	4
C. jejuni	25 (I/R)*	3
C. jejuni	1 (S)	6
C. jejuni	1.5 (S)	8
C. jejuni	0.75 (S)	2
C. jejuni	0.75 (S)	3
C. jejuni	0.75 (S)	1
C. jejuni	1.5 (S)	6
C. jejuni	1.5 (S)	3
C. jejuni	0.5 (S)	3
C. jejuni	1.5 (S)	4
C. jejuni	0.75 (S)	3
C. jejuni	0.5 (S)	2
C. jejuni	0.5 (S)	2
C. jejuni	0.38 (S)	3
C. jejuni	1.5 (S)	3
C. jejuni	0.38 (S)	0.38
C. jejuni	4 (S)	4
C. jejuni	>256 (R)	3
C. jejuni	0.38 (S)	3
C. jejuni	1.5 (S)	2
C. jejuni	2 (S)	3
C. coli	3 (S)	2
C. coli	>256 (R)	1.5
C. coli	>256 (R)	1
C. coli	>256 (R)	1
C. coli	>256 (R)	1
C. coli	>256 (R)	4

*Intermediate by CLSI and resistant by EUCAST.

diarrhea during June and July 2016. E-test strips containing erythromycin and colistin were purchased from Liofilchem (Roseto degli Abruzzi, Italy). The E-test has demonstrated comparable results to those obtained with standard methods approved by CLSI and EUCAST (Ge *et al.,* 2013). It was carried out using Mueller-Hinton agar plates with 5% sheep blood (Becton Dickinson, Sparks, USA) that were inoculated with 0.5 McFarland inoculum suspensions. After application of the E-test, plates were incubated at 42°C in microaerophilic atmosphere (Campygen®, Oxoid, Basingstoke, UK), and MIC values were read after 24 h.

The study protocol was carried out in accordance with the Declaration of Helsinki. This was a non-interventional study with no additional investigation to routine procedures. Biological material was only used for standard enteric infections diagnostics following physicians' prescriptions. No additional sampling or modification of the routine sampling protocol was performed. Data analyses were carried out using an anonymous database. So, approval was considered unnecessary according to national guidelines (Law on Data Protection-Organic Law 15/1999 of 13 December on the protection of data of a personal nature, https://www.boe.es/buscar/doc.php?id=BOE-A-1999-23750).

The results are summarized in *Table 1*, interpreting susceptibility or resistance to erythromycin according to the clinical breakpoints published by CLSI and EUCAST (CLSI 2016; EUCAST 2016).

From the mid 1990s levels of quinolones resistance in Spain have been high (Ruiz et al., 1998) and this situation is also described in different geographic areas, especially in Asia (Bodhidatta *et al.,* 2002; Pham *et al.,* 2016) and reflected in international travelers (Mason *et al.,* 2017; Ruiz *et al.,* 2007). Although susceptibility to macrolides has remained more stable, high levels of erythromycin resistance have also been described (Post *et al.,* 2017; Sáenz *et al.,* 2000). As stated in some of these studies, tetracycline resistance levels, despite being high, are usually lower than those of quinolones (Carev *et al.,* 2017). In 2014, resistance rates of 87.2% were found for ciprofloxacin, 3.5% for erythromycin and 89.5 % for tetracycline in 86 *C. jejuni* strains isolated from stool cultures in our center, while rates of 100%, 21.4%, and 92.9%, respectively, were recorded in 14 *C. coli* isolates (unpublished data). It is therefore evident that fluoroquinolones and tetracyclines are not adequate therapeutic options in our setting, although susceptibility to macrolides persists, especially among *C. jejuni* isolates, as shown in *Table 1*. In the case of colistin, although no breakpoints have been established, we believe that the MIC values obtained in the present study (range: 0.38-8 mg/liter) were sufficiently low for this antibiotic to be considered active against isolates of *Campylobacter* spp. given the high concentrations of colistin sulfate that can be reached in the intestinal lumen. Accordingly, colistin represents an alternative to fluoroquinolones and tetracyclines for the treatment of severe acute diarrhea produced by these bacteria.

Conflicts of Interest

None of the authors have any conflicts of interest.

References

Allos B.M. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin Infect Dis*. **32**, 1201-6.

Biswas S., Brunel J.M., Dubus J.C., Reynaud-Gaubert M., Rolain J.M. (2012). Colistin: an update on the antibiotic of the 21st century. *Expert Rev Anti Infect Ther*. **10**, 917-34.

Bodhidatta L., Vithayasai N., Eimpokalarp B., Pitarangsi C., Serichantalergs O., et al. (2002). Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone-resistant *Campylobacter*. *Southeast Asian J Trop Med Public Health*. **33**, 752-7.

Carev M., Kovačić A., Novak A., Tonkić M., Jeroncić A. (2017). *Campylobacter jejuni* strains coresistant to tetracycline and ciprofloxacin in patients with gastroenteritis in Croatia. *Infect Dis (Lond)*. **49**, 268-76.

Clinical and Laboratory Standards Institute (CLSI). (2016). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. CLSI document M45, 3rd ed. Wayne, PA.

European Committee on Antibiotic Susceptibility Testing (EUCAST). (2017). Breakpoint tables for interpretation of MICs and zone diam-

eters. Version 7.1., Available at http://www.eucast.org. Accessed: Nov 10, 2017.
Falagas M.E., Kasiakou S.K. (2005). Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis*. **40**, 1333-41.
Feizabadi M.M., Dolatabadi S., Zali M.R. (2007). Isolation and drug-resistant patterns of *Campylobacter* strains cultured from diarrheic children in Teheran. *Jpn J Infect Dis*. **60**, 217-219.
Ge B., Wang F., Sjölund-Karlsson M., McDermott P.F. (2013). Antimicrobial resistance in campylobacter: susceptibility testing methods and resistance trends. *J Microbiol Methods*. **95**, 57-67.
Ghosh R., Uppal B., Aggarwal P., Chakravarti A., Jha A.K. (2013). Increasing antimicrobial resistance of *Campylobacter jejuni* isolated from paediatric diarrhea cases in a tertiary care hospital of New Delhi, India. *J Clin Diagn Res*. **7**, 247-9.
Giamarellou H., Poulakou G. (2009). Multidrug-resistant gram-negative infections: what are the treatment options? *Drugs*. **69**, 1879-901.
Komba E.V., Mdegela R.H., Msoffe P.L., Nielsen L.N., Ingmer H. (2015). Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses Public Health*. **62**, 557-68.
Li J., Nation R.L., Milne R.W., Turnidge J.D., Coulthard K. (2005). Evaluation of colistin as an agent against multi-resistant gram-negative bacteria. *Int J Antimicrob Agents*. **25**, 11-25.
Magaz Martínez M., Garrido Botella A., Pons Renedo F., Oliva Del Río B., Agudo Castillo B., et al. (2016). Fatal *Campylobacter jejuni* ileocolitis. *Rev Esp Enferm Dig*. **108**, 662-3.
Mason C.J., Sornsakrin S., Seidman J.C., Srijan A., Serichantalergs O., et al. (2017). Antibiotic resistance in *Campylobacter* and other diarrheal pathogens isolated from US military personnel deployed to Thailand in 2002-2004: a case-control study. *Trop Dis Travel Med Vaccines*. **3**, 13.
Pham N.T., Thongprachum A., Tran D.N., Nishimura S., Shimizu-Onda Y., et al. (2016). Antibiotic resistance of *Campylobacter jejuni* and *C. coli* isolated from children with diarrhea in Thailand and Japan. *Jpn J Infect Dis*. **69**, 77-9.
Post A., Martiny D., van Waterschoot N., Hallin M., Maniewski U., et al. (2017). Antibiotic susceptibility profiles among *Campylobacter* isolates obtained from international travelers between 2007 and 2014. *Eur J Clin Microbiol Infect Dis*. **36**, 2101-7.
Ruiz J., Goñi P., Marco F., Gallardo F., Mirelis B., et al. (1998). Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. *Microbiol Immunol*. **42**, 223-6.
Ruiz J., Marco F., Oliveira I., Vila J., Gascón J. (2007). Trends in antimicrobial resistance in *Campylobacter* spp. causing traveler's diarrhea. *APMIS*. **115**, 218-24.
Sáenz Y., Zarazaga M., Lantero M., Gastanares M.J., Baquero F., et al. (2000). Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrob Agents Chemother*. **44**, 267-71.
Sánchez-Capilla A.D., Sorlózano-Puerto A., Rodríguez-Granger J., Martínez-Brocal A., Navarro-Marí J.M., et al. (2015). Infectious etiology of diarrheas studied in a third-level hospital during a five-year period. *Rev Esp Enferm Dig*. **107**, 89-97.
Sorlózano-Puerto A., Navarro-Marí J.M., Gutiérrez-Fernández J. (2017). Activity of fosfomycin on clinical isolates of *Campylobacter jejuni* and *Campylobacter coli* of enteric origin. *Antimicrob Agents Chemother.* **61**, e02317-16.