Short communication

Potential clinical use of azithromycin against gastroenteritis-causing pathogens other than *Campylobacter*

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Running title: Azithromycin and enteritis.

SUMMARY

The activity of azithromycin against enteritis-producing agents other than *Campylobacter* spp. was studied. The susceptibility to azithromycin, through gradient test, of 88 clinical isolates (51 *Salmonella* spp., 23 *Aeromona*s spp., 10 *Shigella sonnei* and 4 *Yersinia enterocolitica*) for one year was studied prospectively. The results were compared with the activity of ampicillin, trimethoprim-sulfamethoxazole and ciprofloxacin by microdilution. For azithromycin, the minimum inhibitory concentration (MIC) 50 and MIC90 were 4 and 12 mg/l, respectively. Six (6.8%) isolates were simultaneously resistant to ampicillin, trimethoprim-sulfamethoxazole and ciprofloxacin, and 3 (50%) of them presented a MIC>256mg/l. Azithromycin may be a good empirical therapeutic option for the treatment of bacterial enteritis.

Key-words: Intestinal infection; treatment; azithromycin.

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Azithromycin is an erythromycin-derived antibiotic; hence it belongs to the macrolides (Smith *et al*., 2015). Its mechanism of action consists in bacterial protein synthesis inhibition, meaning that it gets attached to the 50s subunit of the bacterial ribosome, blocking the mRNA translation (Bakheit *et al*., 2014). Azithromycin can be used in the treatment, or for the prevention, of certain bacterial infections such as those involving the middle ear or the respiratory tract, also being one of the most used antibiotics in children (Smith *et al*., 2015). Additionally, it is efficient for the treatment of sexually transmitted diseases of nongonococcal origin, like urethritis or chlamydial cervicitis (Bakheit *et al*., 2014). Infectious diarrhea is a common condition, self-limited in most cases, although in communities at risk of complications it may require antimicrobial therapy. The leading cause of diarrhea is *Campylobacter* spp., and the number of cases has considerably increased in recent years. Other causes are *Salmonella*, *Yersinia*, *Shigella* and *Aeromonas* (Sánchez-Capilla *et al*., 2015; Del Valle *et al*., 2019) although their incidence is lower. The most used antibiotics in the treatment of *Campylobacter*-induced diarrhea are the macrolides and the fluoroquinolones. Macrolides are preferred due to their low resistance rate; thus, azithromycin is always the first choice (Sorlózano-Puerto *et al*., 2018). Some *in vivo* studies have described the effect that azithromycin induces on the intestines during episodes of enteritis associated with *Campylobacter* spp (Mourkas *et al*., 2019). The number of enteritis episodes caused by multidrug resistant pathogens is increasing in our environment (Aparicio Gómez *et al*., 2017; Guzmán-Martín *et al*., 2018; Rosales-Castillo *et al*., 2020), and therefore it is important to find alternative therapeutic options.

Not many studies have been published regarding the *in vitro* action of azithromycin on enteritis-producing pathogens other than *Campylobacter* spp. (like *Salmonella*, *Aeromonas*, *Yersinia* or *Shigella*). Some studies concerning *Samonella typhi* infections have emerged, since these organisms are developing resistance to fluoroquinolones and beta-lactams, antimicrobials that used to be considered as first choice in cases of invasive salmonellosis, according to the World Health Organization (WHO) (Misra and Prasad, 2016). Only a few surveys about the effect of azithromycin on *Aeromonas*, *Yersinia* and *Shigella* (Jover-García *et al*., 2017; Martín-Pozo *et al*., 2014) have been published. Due to the limited information concerning the effect of azithromycin on enteritis-producing pathogens other than *Campylobacter*, we considered studying their activity *in vitro,* since it could be a good option for empirical therapy in these cases.

In the University Hospital Virgen de las Nieves of Granada, a prospective study was conducted regarding azithromycin susceptibility. It included 88 pathogenic clinical isolates different from *Campylobacter*, as follows: 27 group D *Salmonella*, 22 group B *Salmonella*, 2 group C *Salmonella*, 15 *Aeromonas caviae*, 7 *Aeromonas veronii*, 1 *Aeromonas hydrophila*, 10 *Shigella sonnei* and 4

Yersinia enterocolitica. They were all isolated from September 2018 to August 2019, all proceeding from fecal cultures corresponding to 50 males and 38 females (23 of them under 3 years of age, 16 between 4-14 years, 36 between 15-65 years and 13 older than 65), and they were all processed following the above described procedures (Del Valle *et al.*, 2020). The minimum inhibitory concentration (MIC) for azithromycin was determined in all the isolates, through gradient test (MIC Test Strip, Liofilchem®, Italy) in Mueller Hinton agar (Beckton Dickinson, Spain), incubated at 37ºC in CO_2 at 5%, adjusting the inoculums in saline serum for a turbidity of 0.5 on MacFarland scale, and expressing the results in mg/L. The results were interpreted after 24h. The susceptibility to ampicillin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole and ciprofloxacin was also studied via the automated microdilution technique (Microscan Walkaway®, Beckham Coulter, USA). The susceptibility of salmonella to ciprofloxacin was also determined via gradient test (MIC Test Strip). The MIC results were interpreted following the *European Committee on Antibiotic Susceptibility Testing* (EUCAST, 2020) guidelines. Finally, a descriptive analysis of the data was performed, in which the absolute and relative frequencies were calculated for the categorical variables. The data was analyzed by IBM SPSS Statistics 19 software.

For azithromycin, the MIC range comprised values between 0.5 and >256mg/L, and the MIC50 and the MIC90 were 4 and 12 mg/L, respectively. The correlation between the MIC values of the azithromycin and the susceptibility to other antibiotics, and the description of the resistant species and their association with the MIC of the azithromycin, are shown in Table 1. Six (6.8%) isolates (4 *S. sonnei*, 1 group D *Salmonella* and 1 *A. veronii*) were simultaneously resistant to ampicillin, trimethoprim-sulfamethoxazole and ciprofloxacin, and three (50%) of these (*S. sonnei*) additionally had a MIC >256 mg/L for azithromycin.

The MIC values for azithromycin obtained in our study were wide, although the majority (93.2%) presented an MIC ≤ 16 mg/L. Even though EUCAST (2020) has not yet established any susceptibility break-points for macrolides in these microbes, it states that azithromycin was used for the treatment of infections caused by *Salmonella* Typhi (MIC ≤16 mg/L for wild-type isolates) and *Shigella* spp.

The frequency of isolates with azithromycin MIC ≤ 16 mg/L is similar to the one that we encountered in some studies performed in *Salmonella*-endemic areas, like Asia (Misra and Prasad, 2016), or in studies on *Aeromonas*, *Shigella* and *Yersinia* in our country, which presented an MIC ≤ 16mg/L in almost 100% of the cases (Jover-García *et al.*, 2017; Martín-Pozo *et al.*, 2014), but inferior to the one that appears in migrants of the Netherlands (Hassing *et al.*, 2014).

Among our isolates, 4 *S. sonnei* specimens were resistant to ciprofloxacin, trimethoprim-sulfamethoxazole and ampicillin, but one of them presented an MIC = 3 mg/L to azithromycin, so this could become suitable as a last resort. In addition to this quality, we must recall its special bioavailability, which offers effectiveness in rather short treatments, as well as its activity against parasites (Maurya *et al.,* 2016).

The main constraint is the lack of studies and data about azithromycin susceptibility in these pathogens, and the absence of established break-points from CLSI and/or EUCAST, which are needed in order to determine the real activity of this antibiotic.

In conclusion, we can state that our area does not have an elevated incidence of clinical isolates different from *Campylobacter* which are resistant to azithromycin; therefore, this could be considered a good option for the empirical treatment of bacterial enteritis, when needed. However, more experiments with the reference broth microdilution method for azithromycin should be carried out on these pathogens in order to confirm these data.

Conflicts of interest

The authors declare that they have no competing interests.

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Table 1. Relation between the obtained MIC values for the azithromycin in the isolates and the susceptibility to other tested antibiotics**.**

MIC Azithromycin	**ANTIBIOTIC' CLINICAL CATEGORY N (%)**								
		Ciprofloxacin		**Trimethoprim-Sulfamethoxazole**		**Ampicillin**		**Ampicillin-Sulbactam**	
	N	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
<=4 mg/L	53	44 (62.9)	9 (50)	46(62.2)	7(50)	16(57.1)	37(61.7)	47(57.3)	6(100)
Aeromonas			2(22.2)		3(42.9)		16(44.4)		6(100)
Salmonella			6(66.7)		1(14.3)		14(38.9)		0(0)
Yersinia			0(0)		0(0		2(5.5)		0(0)
Shigella			1(11.1)		3(42.9)		4(11.1)		0(0)
> 4 mg/L	35	26 (37.1)	9 (50)	28(37.8)	7(50)	12(42.9)	23(38.3)	35(42.7)	0(0)
Aeromonas			1(11.1)		0(0)		7(30.4)		0(0)
Salmonella			4(44.4)		3(42.9)		9(39.1)		0(0)
Yersinia			1(11.1)		0(0)		2(8.7)		0(0)
Shigella			3(33.3)		4(57.1)		5(21.7)		0(0)
Total	**88**	**70(79.5)**	**18(20.5)**	**74(84.1)**	**14(15.9)**	**28(31.8)**	**60(68.2)**	**82(93.2)**	**6(6.8)**
<=8 mg/L	77	63(90)	14(77.8)	68(91.9)	9(64.3)	24(85.7)	53(88.3)	71(86.6)	6(100)
> 8 mg/L	11	7(10)	4(22.2)	6(8,1)	5(35.7)	4(14.3)	7(11.7)	11(13.4)	0(0)
Total	**88**	**70(79.5)**	**18(20.5)**	**74(84.1)**	**14(15.9)**	**28(31.8)**	**60(68.2)**	**82(93,2)**	**6(6.8)**
<=16 mg/L	82	67(95.7)	15(83.3)	72(97.3)	10(71.4)	27(96.4)	55(91.7)	76(92.7)	6(100)
> 16 mg/L	6	3(4.3)	3(16.7)	2(2.7)	4(28.6)	1(3.6)	5(8.3)	6(7.3)	0(0)
Total	**88**	**70(79.5)**	**18(20.5)**	**74(84.1)**	**14(15.9)**	**28(31.8)**	**60(68.2)**	**82(93.2)**	**6(6.8)**