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Emergence of azithromycin resistance mediated by *mph(A)* gene in *Salmonella* Typhimurium clinical isolates in Latin America

RUNING TITLE: Multidrug resistant *Salmonella* Typhimurium isolates.

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Salmonella enterica represents one of the leading causes of food-borne diseases, constituting an important public health problem worldwide. The global burden of disease caused by *Salmonella* infections has been estimated in more than 90 million of human cases per year [1]. In Argentina between 2009 and 2016 *S*. Typhimurium was the most prevalent serotype, with a mean of 25%, followed by *S*. Enteritidis, with a mean of 14% (Personal communication NRL for Salmonella). Ceftriaxone and ciprofloxacin are the treatment of choice for invasive *Salmonella* infections, while azithromycin was suggested as an additional option [1]. The goal of this study is to characterize azithromycin-resistant *S*. Typhimurium clinical isolates harboring the *mph(A)* gene, isolated in Argentina.

Twelve *S.* Typhimurium isolates were recovered from feces (10), psoas abscess (1) and blood (1) samples, and were derived from 6 hospitals of Buenos Aires, Córdoba and

Santa Fe provinces (Table 1). Serovar determination was conducted according to the 9th edition of White-Kauffmann-LeMinor scheme. Susceptibility methods were performed and interpreted according to CLSI M100-S25 guideline. All *S.* Typhimurium isolates showed similar multidrug resistance (MDR) profiles, including high level of azithromycin resistance: $MIC_{50/90}$= 64 µg/mL (MIC range= 32-128 µg/mL). Using the azithromycin break points for *S.* Typhi defined for agar dilution (≤16µg/mL, susceptible; ≥32µg/mL, resistant) by CLSI in 2015, all twelve *S*. Typhimurium isolates were correctly categorized as resistant. All isolates were positive by standard PCR for *mph(A)* gene. High azithromycin MIC (>16 mg/L) was previously reported in *S. enterica*, but only two *S*. Typhimurium isolates harbouring *mph(A)* gene, recovered from the USA and the UK, were described [2,3].

Seven strains were also resistant to cefotaxime and ceftazidime, which correlates with the presence of $bla_{CTX\text{-}M\text{-}14}$ gene (Table 1). Extended-spectrum cephalosporins-resistant *Salmonella* clinical isolates were recognized since the mid-1980 in isolates from Argentina [5], reaching 3.7% (15/402 isolates) in 2015, (WHONET-Argentina network, http://antimicrobianos.com.ar/ATB/wp-content/uploads/2016/12/Informe-Resistencia-ARGENTINA-2015.pdf). Intermediate resistance to ciprofloxacin was observed in five out of 12 isolates, which were positive for *qnrB19* gene (Table 1). Four isolates showed susceptibility only to fosfomycin, tigecycline, colistin and imipenem.

These 12 isolates were grouped by XbaI-PFGE in five clusters (I-V) and compared with the PulseNet National Database (PNND), which include >1600 *S.* Typhimurium patterns since 2004. Four isolates from hospital F from Córdoba province, were grouped as cluster III and related with other isolates in the PNND recovered between 2012 and 2016 from Santa Fe and Buenos Aires provinces. Cluster IV belonged to a pattern shared with other 224 isolates found in the PNND recovered from 10 provinces in the period 2009-2016,

showing that this clone was already circulating across the country. Profiles of clusters I, II and V were not found in the database.

Whole genome sequencing of the first MDR *S.* Typhimurium isolate, M17330, was performed using Illumina compact MiSeq system. Using CLC Genomics Workbench software (CLC bio, Qiagen) a total of 78 contigs were assembled. Contig_33 (15,496 bp) reveals the presence of the *mph(A)-mrx-mphR(A)* cluster and showed 99% of identity with a 16,258 bp fragment of p1 plasmid from *K. pneumoniae* KPN_KPC_HUG_07 (NZ_CP019773). This contig also contains a *catA1* gene and a class I integron with *dfrA12* and Δ*aadA2* (763 bp deletion) cassettes. Others resistance genes were detected in six contigs: $bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$, *sul2, tetB, aph(3'')-Ib, aph(6)-Id, aph(3')-Ia, aac(6')-Iy,* and *qnrB19*, which correlate with its resistance phenotype.

Using sequence data and S1-PFGE analyzes, four plasmids were identified in *S.* Typhimurium M17330: pM17330-1 (ca. 210 Kb), pM17330-2 (ca. 106 Kb), pM17330-3 (ca. 54 Kb), and pM17330-4 (ca. 2.7 Kb). Two *E. coli* transconjugant (TC) showing different susceptibility phenotypes were obtained by biparental conjugation (Table 1). *E. coli* TC17330-1 was selected using sodium azide plus ampicillin and was resistant to cefotaxime, it harbours $bla_{\text{CTX-M-14}}$ gene in the pM17330-2 plasmid. As both *catA1* and *mph(A)* genes were present in the same assembled contig, *E. coli* TC17330-2 was selected using sodium azide plus chloramphenicol. TC17330-2 strain harboured pM17330-1 and pM17330-2 plasmids and showed resistance to azithromycin, ampicillin, cefotaxime, trimethoprim-sulfamethoxazole, kanamycin, streptomycin and tetracycline. Contig_24, corresponding to pM17330-4, shared 100% of similarity with pPAB19-4 plasmid harboring the *qnrB19* gene and previously described in *Salmonella* sp. M9397 isolate from Argentina (JN995611).

S. Typhimurium represents the most frequent serotype in our country, and the presence of *mph(A)* gene in isolates from different locations, different clones, that are located in

conjugative plasmids that harbor additional resistance genes is worrisome for the health system and requires continuous surveillance of azithromycin susceptibility in this *Salmonella* serotype. Since 2015, azithromycin was included to be routinely tested by the National Antimicrobial Surveillance-WHONET-Argentina network. Up to June 2017, 19 extra *S*. Typhimurium clinical isolates were identified as positive for *mph(A)* gene. In the best of our knowledge, these represent the firsts azithromycin-resistant *S.* Typhimurium clinical isolates associated with *mph(A)* gene in the Latin American region. The emergence of *mph(A)* gene and it capacity for horizontal dissemination jeopardizes the use of azithromycin for severe *Salmonella* infections.

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Competing Interests: Nothing to declare.

Ethical Approval: Not required

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Table 1. Epidemiological data, susceptibility profiles and antimicrobial resistance genes

Number	Hospital	Province	Isolation date	Specimen	MIC (µg/mL)							Acquired genes	XbaI-PFGE
					AZM	AMP	CAZ	CTX	IPM	NAL	CIP		
M17330	A	Santa Fe	04/11/14	psoas abscess	64	≥256	4	128	0.25	32	0.5	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$, *qnrB19*	II
M17353	B	Buenos Aires	05/07/14	stool	64	≥256	4	128	0.25	32	0.5	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$, *qnrB19*	V
M17528	D	Santa Fe	06/12/14	stool	128	≥256	4	64	0.25	32	0.5	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$, *qnrB19*	IV
M17891	E	Santa Fe	07/21/14	stool	64	≥256	4	128	0.12	4	0.03	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$	IV
M17671	D	Santa Fe	08/16/14	stool	32	≥256	4	128	0.25	4	0.015	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$	I
M17728	D	Santa Fe	08/30/14	stool	64	≥256	4	128	0.25	4	0.03	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$	V
M17767	F	Córdoba	09/09/14	stool	64	≥256	0.25	0.06	0.25	4	0.03	*mphA*, *bla*$_{TEM-1}$	III
M17768	F	Córdoba	09/10/14	stool	64	≥256	0.25	0.12	0.25	4	0.03	*mphA*, *bla*$_{TEM-1}$	III

M17769	F	Córdoba	09/11/14	stool	64	≥256	0.25	0.12	0.25	4	0.03	*mphA*, *bla*$_{TEM-1}$	III
M17770	F	Córdoba	09/13/14	stool	64	≥256	0.5	0.06	0.25	4	0.03	*mphA*, *bla*$_{TEM-1}$	III
M17945	F	Córdoba	12/07/14	blood	64	≥256	0.25	0.06	0.12	32	0.5	*mphA*, *bla*$_{TEM-1}$, *qnrB19*	I
M17967	C	Buenos Aires	12/07/14	stool	128	≥256	16	≥256	0.25	32	0.5	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$, *qnrB19*	V
TC17330-1	NA	NA	NA	NA	2	≥256	0.25	16	0.12	4	0.03	*bla*$_{CTX-M-14}$	NA
TC17330-2	NA	NA	NA	NA	32	≥256	0.12	16	0.12	4	0.015	*mphA*, *bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$	NA
E. coli J53	NA	NA	NA	NA	2	4	0.12	0.06	0.12	4	0.03	-	NA

FOOTNOTES. All *S*. Typhimurium clinical isolates plus the transconjugant *E. coli* TC17330-1 were also resistant to trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, kanamycin and streptomycin and susceptible to fosfomycin, tigecycline, colistin and imipenem. NA, not applicable.