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Letter to the Editor

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

Sir,

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 at more than 90 million human cases per year [1]. In Argentina between 2009–2016, *S. enterica* serotype Typhimurium was the most prevalent serotype, with a mean of 25%, followed by *S. enterica* serotype Enteritidis, with a mean of 14% (personal communication, National Reference Laboratory for *Salmonella*). Ceftriaxone and ciprofloxacin are the treatments of choice for invasive *Salmonella* infections, whilst azithromycin is suggested as an additional option [1]. The goal of this study was to characterise azithromycin-resistant *S.* Typhimurium clinical isolates harbouring the *mph*(A) gene isolated in Argentina.

A total of 12 *S.* Typhimurium isolates were recovered from faeces (10), psoas abscess (1) and blood (1) samples derived from six hospitals in Buenos Aires, Córdoba and Santa Fe Provinces (Table 1). Serovar determination was conducted according to the 9th edition of the White–Kauffmann–Le Minor scheme. Antimicrobial susceptibility testing was performed and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S25). All *S.* Typhimurium isolates showed similar multidrug-resistant (MDR) profiles, including high-level azithromycin resistance with $MIC_{50/90}$ values of 64/128 μg/mL (MIC range 32–128 μg/mL). Using the azithromycin breakpoints for *S. enterica* serotype Typhi defined for agar dilution by the CLSI in 2015 (≤16 μg/mL, susceptible; ≥32 μg/mL, resistant), all 12 *S.* Typhimurium isolates were correctly categorised as resistant. All isolates were positive by standard PCR for the *mph*(A) gene. High azithromycin MICs (>16 μg/mL) were previously reported in *S. enterica*, but only two *S.* Typhimurium isolates harbouring the *mph*(A) gene, recovered from the USA and the UK, have been described [2,3].

Seven strains were also resistant to cefotaxime and ceftazidime, which correlated with the presence of the $bla_{CTX\text{-}M\text{-}14}$ gene (Table 1). Extended-spectrum cephalosporin-resistant *Salmonella* clinical isolates have been recognised since the mid-1980 in isolates from Argentina [4], 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 5 of the 12 isolates, which were positive for the *qnrB19* gene (Table 1). Four isolates showed susceptibility only to fosfomycin, tigecycline, colistin and imipenem.

The 12 isolates were grouped by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Xba*I into five clusters (I–V) and were 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 were related to other isolates in the PNND recovered between 2012–2016 from Santa Fe and Buenos Aires Provinces. Cluster IV belonged to a pattern shared with another 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 an Illumina compact MiSeq system (Illumina Inc., San Diego, CA). Using CLC Genomics Workbench software (QIAGEN), a total of 78 contigs were assembled. Contig_33 (15 496 bp) revealed the presence of the *mph*(A)–*mrx*–*mphR*(A) cluster and showed 99% identity with a 16 258-bp fragment of p1 plasmid from *Klebsiella pneumoniae* KPN_KPC_HUG_07 (**NZ_CP019773**). This contig also contains a *catA1* gene and a class 1 integron with *dfrA12* and Δ*aadA2* (763 bp deletion) cassettes. Others resistance genes, which correlated with its resistance phenotype, were detected in six contigs: $bla_{CTX\text{-}M\text{-}14}$; $bla_{TEM\text{-}1}$; *sul2*; *tetB*; *aph(3″)-Ib*; *aph(6)-Id*; *aph(3′)-Ia*; *aac(6′)*-Iy; and *qnrB19*.

Using sequence data and *S1* nuclease pulsed-field gel electrophoresis (*S1*-PFGE) analysis, 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 *Escherichia coli* transconjugants (TCs) showing different susceptibility phenotypes were obtained by biparental conjugation (Table 1). *Escherichia coli* TC17330-1 was selected using sodium azide plus ampicillin, was resistant to cefotaxime and harboured the $bla_{CTX\text{-}M\text{-}14}$ 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% similarity with pPAB19-4 plasmid harbouring the *qnrB19* gene and previously described in *Salmonella* sp. M9397 isolate from Argentina (**JN995611**).

Salmonella Typhimurium represents the most frequent serotype in Argentina, and the presence of the *mph*(A) gene in isolates from different locations and different clones, that are located on conjugative plasmids harbouring additional resistance genes is worrisome for the health system and requires continuous surveillance of azithromycin susceptibility in this *Salmonella*

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Table 1
Epidemiological data, susceptibility profiles and antimicrobial resistance genes of 12 *Salmonella enterica* serotype Typhimurium isolates.

Isolate number	Hospital	Province	Isolation date	Specimen	MIC (μg/mL)							Acquired resistance genes	*Xba*I-PFGE profile
					AZM	AMP	CAZ	CTX	IPM	NAL	CIP		
M17330	A	Santa Fe	04/11/2014	Psoas abscess	64	≥256	4	128	0.25	32	0.5	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$, *qnrB19*	II
M17353	B	Buenos Aires	05/07/2014	Stool	64	≥256	4	128	0.25	32	0.5	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$, *qnrB19*	V
M17528	D	Santa Fe	06/12/2014	Stool	128	≥256	4	64	0.25	32	0.5	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$, *qnrB19*	IV
M17891	E	Santa Fe	07/21/2014	Stool	64	≥256	4	128	0.12	4	0.03	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$	IV
M17671	D	Santa Fe	08/16/2014	Stool	32	≥256	4	128	0.25	4	0.015	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$	I
M17728	D	Santa Fe	08/30/2014	Stool	64	≥256	4	128	0.25	4	0.03	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$	V
M17767	F	Córdoba	09/09/2014	Stool	64	≥256	0.25	0.06	0.25	4	0.03	*mph*(A), bla_{TEM-1}	III
M17768	F	Córdoba	09/10/2014	Stool	64	≥256	0.25	0.12	0.25	4	0.03	*mph*(A), bla_{TEM-1}	III
M17769	F	Córdoba	09/11/2014	Stool	64	≥256	0.25	0.12	0.25	4	0.03	*mph*(A), bla_{TEM-1}	III
M17770	F	Córdoba	09/13/2014	Stool	64	≥256	0.5	0.06	0.25	4	0.03	*mph*(A), bla_{TEM-1}	III
M17945	F	Córdoba	12/07/2014	Blood	64	≥256	0.25	0.06	0.12	32	0.5	*mph*(A), bla_{TEM-1}, *qnrB19*	I
M17967	C	Buenos Aires	12/07/2014	Stool	128	≥256	16	≥256	0.25	32	0.5	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$, *qnrB19*	V
TC17330-1	N/A	N/A	N/A	N/A	2	≥256	0.25	16	0.12	4	0.03	$bla_{CTX-M-14}$	N/A
TC17330-2	N/A	N/A	N/A	N/A	32	≥256	0.12	16	0.12	4	0.015	*mph*(A), bla_{TEM-1}, $bla_{CTX-M-14}$	N/A
Escherichia coli J53	N/A	N/A	N/A	N/A	2	4	0.12	0.06	0.12	4	0.03	–	N/A

MIC, minimum inhibitory concentration; AZM, azithromycin; AMP, ampicillin; CAZ, ceftazidime; CTX, cefotaxime; IPM, imipenem; NAL, nalidixic acid; CIP, ciprofloxacin; N/A, not applicable; PFGE, pulsed-field gel electrophoresis.
All *S.* Typhimurium clinical isolates plus the transconjugant *E. coli* TC17330-1 were also resistant to trimethoprim/sulfamethoxazole, chloramphenicol, tetracycline, kanamycin and streptomycin and were susceptible to fosfomycin, tigecycline, colistin and imipenem.

serotype. Since 2015, azithromycin was included to be routinely tested by the National Antimicrobial Surveillance–WHONET–Argentina network. Up to June 2017, 19 additional *S.* Typhimurium clinical isolates were identified as positive for the *mph*(A) gene. To the best of our knowledge, these represent the first azithromycin-resistant *S.* Typhimurium clinical isolates associated with the *mph*(A) gene in the Latin American region. Emergence of the *mph*(A) gene and it capacity for horizontal dissemination jeopardises the use of azithromycin for severe *Salmonella* infections.

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Competing interests

None declared.

Ethical approval

Not required.

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