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SHORT COMMUNICATION

Surveillance of antimicrobial resistance in *Serratia marcescens* in Mexico

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SUMMARY

Antimicrobial resistance is a global public health threat. Therefore, surveillance studies are important tools to help direct antimicrobial use. The aim of this study was to investigate antimicrobial resistance in *Serratia marcescens* isolates collected in 2016-2017 at eight medical centers from two regions of Mexico. Selected *S. marcescens* isolates were further tested by polymerase chain reaction to detect the presence of genes encoding the β-lactamases, SHV, TEM or CTX. Antimicrobial resistance continues to be high in Mexico, particularly to ciprofloxacin and aminoglycosides. Also, a widespread prevalence of bla_{TEM} was detected in *S. marcescens* isolates.

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Serratia marcescens is an important opportunistic pathogen associated with a wide spectrum of clinical diseases, such as pneumonia, keratitis, meningitis, and urinary tract and wound infections (Richards *et al.,* 2000; Dessi *et al.,* 2009). Outbreaks of nosocomial infections by *S. marcescens* have been documented in high-risk settings, mainly affecting immunocompromised patients, the elderly, and newborns (Dessi *et al.,* 2009; Montagnani *et al.,* 2015).

The emergence of *S. marcescens* as a nosocomial pathogen has been attributed to its resistance to several antibiotics, such as β-lactams, cephalosporins, aminoglycosides, ciprofloxacin, chloramphenicol, polymyxins, and tetracycline (Yang *et al.,* 2012). Multiple mechanisms provide antibiotic resistance in *S. marcescens*. In particular, β-lactams resistance in *S. marcescens* (like other Enterobacteriaceae), is commonly associated with the production of β-lactamases. Besides AmpC, an inducible chromosome encoded class C cephalosporinase, several *S. marcescens* isolates also produce extended-spectrum β-lactamases (ESBL), with these ESBL belonging mainly to the SHV, TEM, or CTX type (Mlynarczyk *et al.,* 2009; Yang *et al.,* 2012). More recently, sporadic emergence of carbapenem-hydrolysing β-lactamases (SME, KPC, IMP or VIM type) in *S. marcescens* isolates have been reported, representing a serious threat to therapeutic options against this pathogen (Ghaith *et al.,* 2018).

Global surveillance programs of antimicrobial susceptibility among Gram-negative clinical isolates, such as the Tigecycline Evaluation and Surveillance Trial (TEST), have pointed out that compared to other world regions (especially North America or Europe), the Latin American *S. marcescens* isolates display lower susceptibility rates, particularly to amikacin, cefepime, and ceftriaxone (Bertrand and Dowzicky 2012; Kehl and Dowzicky 2015).

In Mexico, a number of nosocomial outbreaks due to *S. marcescens* have been reported and the prevalence of ESBL encoding genes in some of these strains has also been found (Espinosa de los Monteros *et al.,* 2008; Garza-González *et al.,* 2011). Nevertheless, the surveillance of antimicrobial resistance of *S. marcescens* in Mexico is still limited and more comprehensive epidemiological studies are needed. Accordingly, the aim of this study was to investigate trends in antimicrobial resistance in 193 non-duplicate *S. marcescens* clinical isolates collected in 2016-2017 from eight medical centers at two different regions in Mexico. From the central region: Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra", the Instituto Nacional de Ciencias Médicas y Nutrición "Salva-

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dor Zubirán", and the Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez" located in Mexico City, and the Hospital Regional de Alta Especialidad del Bajío in the state of Guanajuato. And, from the northern region: The Hospital Universitario "Dr. José Eleuterio González", the Hospital San Jose TecSalud, and the Hospital Christus Muguerza in the state of Nuevo Leon, and the Hospital General de Durango in the state of Durango. The study was evaluated and approved by the local Ethics Committee in the Universidad Autónoma de Nuevo León (Approved study number: MB 17-00002).

Each isolate was subcultured onto the appropriate MacConkey agar plates to check for purity; bacterial confirmation was performed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) VITEK Mass Spectrometry (Biomérieux).

Antimicrobial susceptibility was determined by the reference broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI), M100S27. Results were interpreted according to CLSI criteria for all drugs except for moxifloxacin and tigecycline, for which European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used.

Because a ceftriaxone MIC cutoff of 8 µg/mL has been reported as an excellent predictor of ESBL production (Huang *et al.,* 2014), the *S. marcescens* isolates with an MIC ≥8 µg/mL to ceftriaxone were further tested by PCR to detect the presence of genes encoding the β-lactamases TEM, CTX, and SHV as previously reported (Silva *et al.,* 2001; Mlynarczyk *et al.,* 2009). Primers sequences were as follows: TEM-F, 5´-ACTTACTTCTGACAACGATCG-3´ and TEM-R, 5´-GGTCTGACAGTTACCAATGC-3´; CTX-M-F, 5´- TCTTCCAGAATAAGGAATCCC-3´; CTX-M-R, 5´-CCGTTTCCGCTATTACAAAC-3´ (Mlynarczyk *et al.,* 2009); SHV-F, 5´-CGGAATTCAGGAGGTTGACTATGCGTTATATTCGCCTG-3´, SHV-R, 5´-GGTGCGGATCCTTATTAGCGTTGCCAGTG-3´ (Silva *et al.,* 2001). Amplification of 16S rRNA gene was included in every PCR reaction as an internal control (Klindworth *et al.,* 2013).

We collected a total of 193 non-duplicate *S. marcescens* isolates: 151 isolates (78.2%) were from the central region (Mexico City or Guanajuato state) and 42 isolates (21.7%) were from the northern region (Nuevo Leon or Durango state). Isolates were recovered mostly from urine samples (47 isolates, 24.3%), followed by respiratory tract (38 isolates, 19.6%), blood (38 isolates, 19.6%), wound/abscess (37 isolates, 19.1%), and biopsy (25 isolates, 12.9%). The clinical origin of the remaining isolates was cerebrospinal fluid (3 isolates), bile (2 isolates), prosthesis (2 isolates), and pleural fluid (1 isolate). Most of the isolates were from male patients (132 isolates, 66.7%).

The *in vitro* susceptibilities, the MIC range of the isolates for various antimicrobials, the MIC_{50}, and the MIC_{90} are shown in *Table 1*. Among the *S. marcescens* isolates, >92% were susceptible to ertapenem, meropenem, and tigecycline; 90.1% to moxifloxacin; 78.7% to ciprofloxacin; 77.2% to gentamicin; 75.6% to amikacin; and 70.4% to ceftriaxone. In agreement with what is reported in the Latin America region, the *S. marcescens* strains collected here had lower *in vitro* susceptibility rates to different classes of antibiotics. In particular, aminoglycosides susceptibility rates showed the largest differences compared to other regions. For instance, the evaluated strains showed an amikacin susceptibility rate of 75.6%, which is in agreement with the susceptibility rate reported in Latin America during 2004-2009 (75.0%) (Bertrand and Dowzicky 2012). However, for the remaining world regions, including the Asia-Pacific Rim and Africa, amikacin remains highly active against *S. marcescens*, displaying susceptibility rates between 93-100% (Bertrand and Dowzicky 2012; Yang *et al.,* 2017).

Similarly, lower fluoroquinolone susceptibility rates in *S. marcescens* strains from Latin America have been reported (Kehl and Dowzicky 2015; Yang *et al.,* 2017). Herein, we showed that moxifloxacin displays high activity (90.1% susceptibility) against *S. marcescens* isolates, while susceptibility to ciprofloxacin was only 78.7%. This might be related with point mutations at DNA gyrase, since occurrence of *gyrA* substitution at codon Ser83 has recently been confirmed in a Mexican nosocomial strain (Sandner-Miranda *et al.,* 2018).

Tigecycline was also highly active against the *S. marcescens* strains tested (92.7% susceptibility). However, this rate is slightly lower compared to the *S. marcescens* tigecycline susceptibility rate reported globally (95.5%) (Bertrand and Dowzicky 2012) or even in Latin America and Mexico (95.3 and 95.8%, respectively) from 2004-2012 (Kehl and Dowzicky 2015; Vega and Dowzicky 2017). Continuous monitoring in Mexico in the coming years is important, since susceptibility rates to tigecycline have been reported stable in *S. marcescens* isolates across the 2004-2015 time period (Sader *et al.,* 2011; Vega and Dowzicky 2017).

Carbapenem-resistant *S. marcescens* are of particular concern because few treatment options are available for these pathogens. In this study, 0.5 and 5.1% of the *S. marcescens* isolates were resistant to ertapenem and meropenem, respectively. This rate is the same as the Latin American rate of meropenem resistance reported by Vega et al. (Vega and

Table 1 - *MICs and susceptible percentage of Serratia marcescens isolates.*

	MIC (µg/mL)			
Antibiotic	*MIC_{50}*	*MIC_{90}*	*Range*	*% susceptibility*
Amikacin	8	64	2-64	75.6
Ampicillin	64	≥64	16 to ≥64	0
Ceftriaxone	1	8	0.25-32	70.4
Ciprofloxacin	0.5	4	0.06-8	78.7
Ertapenem	0.25	0.5	0.06-2	96.3
Gentamicin	2	16	0.25-32	77.2
Meropenem	1	1	0.125-8	92.7
Moxifloxacin	0.12	0.25	≤0.03-2	90.1
Tigecycline	0.25	0.5	≤0.03-2	92.7

MIC_{50} and MIC_{90}: Minimum inhibitory concentration that inhibits the growth of 50% and 90% of the isolates, respectively.

Table 2 - *Specimen source and bla gene identification of 22 S. marcescens isolates.*

Strain	*Specimen type*	*Medical center / city*	*bla gene*		
			TEM	*CTX*	*SHV*
521E	Respiratory tract	HGD/Durango	+	-	-
4491	Respiratory tract	HSJT/Monterrey	+	-	-
9672	Respiratory tract	HSJT/Monterrey	+	-	-
23334	Respiratory tract	HCM/Monterrey	+	-	-
23820	Respiratory tract	HCM/Monterrey	+	-	-
HU1848	Respiratory tract	HU/Monterrey	-	-	-
SMn04	Respiratory tract	INCMN/Mexico city	+	-	-
267590	Urine	HCM Monterrey	+	-	-
273467	Urine	HCM/Monterrey	+	-	+
1202546	Urine	HU/Monterrey	+	-	-
SMn42	Urine	INCMN/Mexico city	+	-	-
SMn47	Urine	INCMN/Mexico city	+	-	-
SMn57	Urine	INCMN/Mexico city	-	-	-
33177	Wound/Abscess	HU/Monterrey	+	-	-
7027692	Wound/Abscess	HCM/Monterrey	+	-	-
SM032	Wound/Abscess	HSJT/Monterrey	+	-	-
SM054	Wound/Abscess	HSJT/Monterrey	+	-	-
112210	Blood	HSJT/Monterrey	+	-	-
574857	Blood	HSJT/Monterrey	+	-	-
INN007	Blood	INNN/Mexico city	+	-	-
EB2330	Biopsy	INR/Mexico city	+	-	-
INN009	CSF	INNN/Mexico city	+	-	-

bla: β-lactamase. CSF: cerebrospinal fluid. HGD: Hospital General de Durango. HSJT: Hospital San Jose TecSalud. HCM: Hospital Christus Muguerza. HU: Hospital Universitario. INCMN: Instituto Nacional de Ciencias Médicas y Nutrición. INNN: Instituto Nacional de Neurología y Neurocirugía.

Dowzicky 2017) between 2004 and 2015 (5%), and is considerably lower than the meropenem resistant rate reported in the Asia-Pacific region in 2015 (13.6 %) (Yang *et al.,* 2017).

The evaluated *S. marcescens* strains showed a ceftriaxone susceptibility rate of 70.4%, a bit higher than the rate recently reported in Latin America (68.1%) (Vega and Dowzicky 2017). *S. marcescens* isolates displaying an MIC ≥8 µg/mL to ceftriaxone (22 isolates) were then further analyzed by PCR for the presence of β-lactamase encoding genes (*Table 2*). A vast prevalence of bla_{TEM} (20 isolates, 90.9%) was revealed. Similar results were previously reported in clinical isolates of *S. marcescens* from Japan (Zhao *et al.,* 2007). In addition, DNA sequencing of bla_{TEM} amplicons (corresponding to the second half of the gene) indicated that these β-lactamase genes correspond to $bla_{\text{TEM-1}}$ (data not shown). This broad frequency of TEM-1 β-lactamase is significant, as this might contribute to the emergence of new ESBL types. In addition, none of the analyzed strains was detected as a bla_{CTX} gene carrier (*Table 2*); consistently, a low prevalence of CTX ESBLs in *S. marcescens* isolates from Mexico has previously been reported (Garza-González *et al.,* 2011). A single bla_{SHV} positive isolate was found (*Table 2*); DNA sequencing indicates that this amplicon corresponds to the ESBL, SHV-12 (data not shown), which is in agreement with previous reports in Northern Mexico (Garza-González *et al.,* 2011). The presence of *S. marcescens* strains carrying a bla_{SHV} gene might be relevant, since an outbreak of a *S. marcescens* strain producing SHV-5 was reported in a tertiary pediatric hospital in Mexico City (Espinosa de los Monteros *et al.,* 2008).

The limitations of this study include a low number of isolates from the states of Durango and Guanajuato (eight and five, respectively), while most of the isolates (74.2 %) derived from three different centers, all of them located in Mexico City.

Surveillance studies are important tools to help direct antimicrobial use, particularly empiric therapy based on the local or regional resistance patterns of organisms, the implementation of infection control measures, and supporting antibiotic stewardship efforts. From the antibiotics tested in this study, carbapenems and tigecycline were the most effective against *S. marcescens* isolates and should continue to be a suitable option for treatment of this pathogen infection in Mexico. Also, a broad prevalence of bla_{TEM} was found in *S. marcescens* strains. Further studies are needed to increase local epidemiologic knowledge and better ascertain the effectiveness of surveillance programs.

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Competing interests

The authors have no competing interests to declare.

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