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Risk factors and molecular mechanisms associated with trimethoprim–sulfamethoxazole resistance in *Stenotrophomonas maltophilia* in Mexico

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Abstract

Purpose. *Stenotrophomonas maltophilia* is a multidrug-resistant opportunistic pathogen causing an increasing number of nosocomial infections. Our aim was to evaluate the risk factors and mechanisms associated with trimethoprim–sulfamethoxazole (SXT) resistance in *S. maltophilia* infections in Mexico.

Methodology. Clinical isolates and patients' demographic and clinical data were collected from February 2007 to August 2015 in two tertiary-care hospitals in Mexico. Antimicrobial susceptibility and analysis of *sul* and SmeABC and SmeDEF efflux pump overexpression were performed in all isolates.

Results/Key findings. In the 9-year period, 196 patients infected with *S. maltophilia* were identified. Most patients were male, and the mean age was 46.2 years. The mean Charlson score was 1.42, and the most frequent comorbidities were arterial hypertension (26.7 %), type 2 diabetes (21.2 %) and cerebral infarction (11.6 %). High drug resistance to meropenem (93.4 %), gentamicin (55.1 %), ceftazidime (52.3 %), cefotaxime (51.5 %), amikacin (42.3 %) and cefepime (32.1 %), and lower resistance to ciprofloxacin (26.0 %), SXT (25.0 %), chloramphenicol (14.3 %) and levofloxacin (2.6 %) were detected. SXT resistance was not associated with the *sul* genes. SmeABC overexpression was associated with gentamicin (P=0.001) and levofloxacin resistance (P=0.041), whereas SmeDEF overexpression was associated with ceftazidime resistance (P=0.003). Prolonged hospitalization ($\geq$15 days) was an independent risk factor for SXT-resistant *S. maltophilia* infections (OR=3.05; 95 % CI=1.12–8.86; P=0.029).

Conclusion. Given the high SXT resistance rate, SXT is not an effective first-line therapy for our patients; instead, levofloxacin could be used as an appropriate therapeutic option against *S. maltophilia* infections.

INTRODUCTION

Stenotrophomonas maltophilia is a non-fermenting Gram-negative bacillus that has emerged as an opportunistic drug-resistant pathogen that is responsible for an increasing number of nosocomial infections and particularly affects immunocompromised patients, with significant morbidity and mortality [1–3]. The risk factors for *S. maltophilia* infections are a severely compromised health status, malignancies, cystic fibrosis, indwelling devices such as intravascular catheters and ventilation tubes, exposure to broad-spectrum antimicrobials and prolonged hospitalization [2, 4]. However, the impact of acquiring trimethoprim–sulfamethoxazole (SXT)-resistant *S. maltophilia* infections has been poorly studied.

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Keywords: *Stenotrophomonas maltophilia*; risk factors; drug resistance; trimethoprim/sulfamethoxazole.

Abbreviations: CCI, Charlson comorbidity index; CLSI, Clinical and Laboratory Standards Institute; COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; ICU, intensive care unit; ISCR, insertion element common region; LOS, length of stay; MIC, minimum inhibitory concentration; SXT, trimethoprim–sulfamethoxazole.

S. maltophilia strains tend to have high rates of intrinsic or acquired antimicrobial resistance that reduce our therapeutic options [5]. SXT is a common first-line antimicrobial treatment because resistance rates used to be very low (less than 10 %) [5, 6]. In recent years, however, the SXT resistance rate has been gradually increasing, and it has been reported to be over 38.7 % [7].

Several molecular mechanisms have been shown to contribute to the antimicrobial resistance of *S. maltophilia*, e.g. the activity of multidrug efflux pumps, such as SmeABC and SmeDEF [8–12], and the presence of drug-resistance genes, such as the *sul* genes [8, 13].

The aim of this study was to evaluate the risk factors and molecular mechanisms associated with SXT resistance in *S. maltophilia* infections.

METHODS

Study design

The study was conducted at two tertiary-care Mexican hospitals: the Hospital Universitario Dr José Eleuterio González in Nuevo Leon and the Hospital Civil de Guadalajara in Jalisco. Only patients with confirmed *S. maltophilia* infection were included [14]. Data from the first episode were included if patients had multiple infections with *S. maltophilia*. Demographic and clinical data were retrieved from patient charts. The Charlson comorbidity index was used as a surrogate measure for comorbidities [15]. Patients whose medical records were unavailable were not included in the statistical analysis. Patients younger than 18 years old were excluded.

Clinical isolates

Clinical isolates of *S. maltophilia* were collected from February 2007 to August 2015. *S. maltophilia* isolates were identified using Sensititre panels (TREK Diagnostic Systems, Cleveland, OH, USA) according to the manufacturer's instructions, by PCR amplification of a 134 bp fragment of the 16S rRNA gene [16] and by MALDI-TOF mass spectrometry (Bruker Daltonics, Bremen, Germany). *S. maltophilia* ATCC 13637 was used as a wild-type control strain. All of the isolates were stored at −70 °C until use.

Antimicrobial susceptibility

The minimum inhibitory concentration (MIC) was determined by the broth microdilution method. Panels were obtained from Sensititre (TREK Diagnostic Systems Inc.) and used according to the manufacturer's instructions. The antimicrobial agents for susceptibility testing included amikacin, cefepime, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, meropenem and SXT. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria [17]. For antimicrobial agents without specific CLSI criteria for *S. maltophilia*, criteria that were relevant for non-*Enterobacteriaceae* were used.

Antimicrobial resistance mechanisms

Sme efflux pump expression

Total RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Expression of the Sme efflux systems, SmeABC and SmeDEF, was assessed by real-time PCR using previously described specific primers (Table 1). *rDNA* was used as the endogenous control gene [9]. Real-time PCR reactions were performed on the Cepheid SmartCycler II real-time PCR system (Cepheid, Sunnyvale, CA, USA). Amplification mixtures were prepared using the SensiFast SYBR No-ROX One-Step kit (Bioline, Taunton, MA, USA) and contained 2× SensiFast SYBR No-ROX One-Step mix,

Table 1. Primer sequences used in this study

Gene	Designation	Sequence (5′ to 3′)	Annealing temperature	Product (bp)	Reference
Sme efflux pump expression					
smeABC	Forward	ACCGCCCAGCTTTCATACAG	60	69	[9]
	Reverse	GACATGGCCTACCAGGAACAG			[9]
smeDEF	Forward	TCGTCCAGGCTGACATTCAA	60	62	[9]
	Reverse	AACGCGGATCGTGATATCG			[9]
rDNA	Forward	TGACACTGAGGCACGAAAGC	60	30	[9]
	Reverse	CATCGTTTAGGGCGTGGACTA			[9]
SXT resistance mechanisms					
sul1	Forward	ATGGTGACGGTGTTCGGCATTCTGA	50	840	[13]
	Reverse	CTAGGCATGATCTAACCCTCGGTCT			[13]
sul2	Forward	GAATAAATCGCTCATCATTTTCGG	50	810	[13]
	Reverse	CGAATTCTTGCGGTTTCTTTCAGC			[13]
sul3	Forward	GAGCAAGATTTTTGGAATCG	51	752	[24]
	Reverse	CATCTGCAGCTAACCTAGGGCTTTGGA			[24]
ISCR	Forward	GCGAGTCAATCGCCCACT	50		[13]
	Reverse	CGACTCTGTGATGGATCGAA			[13]

1× reverse transcriptase, 1× RiboSafe RNase inhibitor, 400 nM of each primer and 10 ng μl^{-1} of total RNA. After a 5 min retrotranscription step at 45 °C and a 2 min activation step at 95 °C, the PCR process consisted of 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. The expression levels of *S. maltophilia* ATCC 13637 were used to construct the standard curves of *smeABC*, *smeDEF* and *rDNA*, which were used as calibrators to normalize the relative expression levels in clinical isolates. A formula including the Ct values of Sme and the endogenous gene in both the samples and calibrators was used to express *n*-fold differences in the expression of *smeABC* or *smeDEF* genes, in which values of $n<1$ were considered to indicate overexpression of the Sme efflux system [10].

SXT resistance mechanisms

All of the isolates were screened for the presence of *sul1*, *sul2*, and *sul3* genes and insertion element common region (ISCR) elements using previously described primers and PCR conditions (Table 1). Briefly, the reaction mixtures contained 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM concentration of each dNTP, 200 nM of each primer, 1 U of Ampli*Taq* polymerase (Bioline, Taunton, MA, USA) and 200 ng of DNA extracted by thermal lysis. PCR was initiated by denaturation for 5 min at 95 °C, followed by 30 cycles of 1 min denaturation at 95 °C, 1 min annealing at 50 °C and 1 min extension at 68 °C, with a final 5 min extension at 72 °C.

Statistical analysis

Student's *t*-test and one-way ANOVA were used to verify significant differences in efflux pump expression between susceptible and resistant isolates. Categorical variables were compared using Fisher's exact test or Chi-squared distribution; continuous variables were analysed using Student's *t*-test. A multivariate analysis was performed using the logistic regression method to identify independent risk factors associated with SXT-resistant *S. maltophilia* infections. $P<0.05$ was considered to be statistically significant. Odds ratios (ORs) and corresponding 95 % confidence intervals (CIs) were computed using SPSS statistics software version 20.0 (IMB Corporation, Somers, NY, USA).

RESULTS

Clinical isolates

In total, 196 isolates from 196 patients were collected during the 9-year study period: 169 (86.2 %) isolates were from Nuevo Leon and 27 (13.8 %) were from Jalisco. Most of the isolates were from the respiratory tract (63.3 %, *n*=124) followed by blood (17.3 %, *n*=34), wounds (5.1 %, *n*=10), urine (2.0 %, *n*=4), abscesses (1.5 %, *n*=3), pleural fluid (1.5 %, *n*=3), bile (0.5 %, *n*=1), cerebrospinal fluid (0.5 %, *n*=1) and unidentified origin (8.2 %, *n*=16).

Patient characteristics

One hundred and forty-six patients had a complete medical chart and were included in the clinical analysis (Table 2). The majority of patients were male (65.1 %, *n*=95) and the age range was 18–87 years, with a mean of 46.2 years. The most frequent comorbidities were arterial hypertension (26.7 %, *n*=39), type 2 diabetes (21.2 %, *n*=31) and cerebral infarction (11.6 %, *n*=17). The Charlson score mean was 1.42±1.69. Invasive procedures were common: 66.4 % (*n*=97) had a urinary catheter, 64.4 % (*n*=94) had a central catheter (64.4 %, *n*=94) and 53.4 % (*n*=78) received mechanical ventilation. Most patients (*n*=135; 92.5 %) were on antibiotics during sample recollection. The most prescribed antibiotics were carbapenems (92.5 %, *n*=71), vancomycin (38.4 %, *n*=56) and third-generation cephalosporins (25.3 %, *n*=37) (data not shown). Almost two-thirds of the patients (66.4 %, *n*=97) were hospitalized for more than 15 days, more than half (57.5 %, *n*=84) were admitted to the intensive care unit (ICU) and 66 patients (45.2 %) died.

Antimicrobial susceptibility

Table 3 summarizes the susceptibility data. The resistance rates were high for meropenem (93.4 %), gentamicin (55.1 %), ceftazidime (52.3 %), cefotaxime (51.5 %), amikacin (42.3 %) and cefepime (32.1 %). Lower resistance rates were found for ciprofloxacin (26.0 %), SXT (25.0 %), chloramphenicol (14.3 %) and levofloxacin (2.6 %). The comparison of resistant rates per year is shown in Fig. 1, where the resistance rates to SXT showed a decrease through the years.

Antimicrobial resistance mechanisms

Expression of Sme efflux pumps was analysed in isolates resistant to either quinolones, chloramphenicol, or SXT. Among the 91 selected/included isolates, 68 (74.7 %) overexpressed the SmeABC efflux pump, and 60 (65.9 %) overexpressed the SmeDEF efflux pump. Overexpression of the SmeABC efflux pump was significantly associated with resistance to gentamicin ($P=0.001$) and levofloxacin ($P=0.041$) whereas overexpression of the SmeDEF efflux pump was associated with ceftazidime resistance ($P=0.003$) (Table 3).

SXT resistance was not associated with the presence of either the *sul* genes (*sul1*: 4.2 %, *n*=8; *sul2:* 0.5 %, *n*=1; *sul3:* 0.0 %, *n*=0) or the ISCR element (0.0 %, *n*=0).

Risk factors for SXT-resistant strain infection

Patients with gastrostomy/jejunostomy (OR=2.58; 95 % CI=1.11–6.02; $P=0.037$), tracheostomy (OR=2.39; 95 % CI=1.11–5.17; $P=0.039$), length of stay (≥15 days) (OR=2.64; 95 % CI=1.11–6.29; $P=0.032$) and lumbar puncture (OR=3.41; 95 % CI=1.21–9.58; $P=0.022$) had a higher risk of acquiring a SXT-resistant *S. maltophilia* infection (Table 2). An independent risk factor for acquiring SXT-resistant *S. maltophilia* infection was length of stay (≥15 days) (OR=3.05; 95 % CI=1.12–8.86; $P=0.029$) (Table 1).

Risk factors for general mortality in *S. maltophilia* infection

In patients infected with *S. maltophilia*, 30-day mortality was more frequent in patients with arterial hypertension (OR=2.14; 95 % CI=1.02–4.51; $P=0.044$), type 2 diabetes

Table 2. Demographic and clinical characteristics of patients with *Stenotrophomonas maltophilia* infections

Patients infected with SXT-susceptible and SXT-resistant *S. maltophilia* isolates were compared using univariate and multivariate analysis.

Characteristic*	No. (% of patients or range)			SXT-resistant versus susceptible isolates				General mortality	
				Univariate analysis		Multivariate analysis		Univariate analysis	
	Total	SXT		Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*
		Resistant	Susceptible						
No. of patients	146 (100)	41 (28.1)	105 (71.9)						
Mean age ±SD	46.2 ±16.32	43.9±7.3	47.1±15.9		0.465				
Male	95 (65.1)	26 (27.4)	69 (72.6)	1.10 (0.52–2.35)	0.305				
Comorbidity									
Arterial hypertension	39 (26.7)	11 (28.2)	28 (71.8)	1.01 (0.45–2.28)	1.000			2.14 (1.02–4.51)	0.044
Type 2 diabetes	37 (25.3)	6 (16.2)	31 (83.8)	0.41 (0.16–1.07)	0.089			2.73 (1.20–6.22)	0.015
Acute ischemic or haemorrhagic stroke	17 (11.6)	5 (29.4)	12 (70.6)	1.08 (0.35–3.27)	1.000				
Chronic kidney disease	15 (10.3)	3 (20.0)	12 (80.0)	0.61 (0.16–2.29)	0.558				
Acute myocardial infarction	14 (9.6)	2 (14.3)	12 (85.7)	0.40 (0.09–1.86)	0.350			3.39 (1.01–11.38)	0.038
Acute kidney disease	13 (8.9)	4 (30.8)	9 (69.2)	1.15 (0.34–3.97)	0.758				
Heart failure	10 (6.8)	2 (20.0)	8 (80.0)	0.62 (0.13–3.06)	0.726				
Leukaemia	7 (4.8)	2 (28.6)	5 (71.4)	1.03 (0.19–5.51)	1.000			7.90 (0.93–67.38)	0.027
COPD	4 (2.7)	0 (0.0)	4 (100.0)	0.71 (0.64–0.79)	0.577				
Peripheral artery disease	2 (1.4)	0 (0.0)	2 (100.0)	0.72 (0.65–0.79)	1.000				
Connective tissue disease	1 (0.7)	0 (0.0)	1 (100.0)	0.71 (0.65–0.79)	1.000				
Charlson score ± SD	1.42±1.69	0.98 ±1.19	1.60±1.82		0.044				0.002
Invasive procedures									
Urinary catheter	97 (66.4)	29 (29.9)	68 (70.1)	1.32 (0.60–2.87)	0.562			3.87 (1.80–8.32)	≤0.001
CVC	94 (64.4)	28 (29.8)	66 (70.2)	1.27 (0.59–2.74)	0.570			5.26 (2.41–11.49)	≤0.001
Mechanical ventilation	78 (53.4)	23 (29.5)	55 (70.5)	1.16 (0.56–2.40)	0.716			3.91 (1.95–7.85)	≤0.001
Surgery in past 90 days	17 (11.6)	7 (41.2)	10 (58.8)	1.86 (0.66–5.21)	0.263				
Tracheostomy	41 (28.1)	17 (41.5)	24 (58.5)	2.39 (1.11–5.17)	0.039				
Gastrostomy/jejunostomy	29 (19.9)	13 (44.8)	16 (55.2)	2.58 (1.11–6.02)	0.037				
Hemodialysis/peritoneal dialysis	28 (19.2)	6 (21.4)	22 (78.6)	0.65 (0.24–1.73)	0.486			4.87 (1.92–12.37)	≤0.001
Arterial line	26 (17.8)	7 (26.9)	19 (73.1)	0.93 (0.36–2.42)	1.000				
Lumbar puncture	17 (11.6)	9 (52.9)	8 (47.1)	3.41 (1.21–9.58)	0.022				
Cardiac arrest	17 (11.6)	5 (29.4)	12 (70.6)	1.08 (0.35–3.27)	1.000			4.66 (1.44–15.08)	0.008
Pleural catheter	11 (7.5)	2 (18.2)	9 (81.8)	0.55 (0.11–2.65)	0.728				
Administered drugs									
Antibiotics during *S. maltophilia* isolation	135 (92.5)	35 (25.9)	100 (74.1)	0.29 (0.08–1.02)	0.074				
Antibiotics prior to *S. maltophilia* isolation	38 (26.0)	10 (26.3)	28 (73.7)	0.89 (0.39–2.04)	0.837				
Vancomycin	55 (37.7)	19 (34.5)	36 (64.5)	1.65 (0.78–3.46)	0.088			2.48 (1.25–4.91)	0.009
SXT	10 (6.8)	3 (30.0)	7 (70.0)	1.10 (0.22–4.44)	0.444			4.63 (0.93–23.09)	0.043
Antifungals	26 (17.8)	5 (19.2)	21 (80.8)	0.56 (0.19–1.59)	0.340			3.19 (1.33–7.65)	0.007
Corticosteroids	28 (19.2)	5 (17.9)	23 (82.1)	0.50 (0.17–1.41)	0.243			2.63 (1.12–6.18)	0.024
Vasopressors	26 (17.8)	5 (19.2)	21 (80.8)	0.56 (0.19–1.59)	0.340			9.50 (3.07–29.36)	≤0.001
Hospital stay									
LOS (≥15 days)	97 (66.4)	33 (34.0)	64 (55.7)	2.64 (1.11–6.29)	0.032	3.05 (1.12–8.86)	0.029	2.51 (1.22–5.20)	0.012
ICU admission	84 (57.5)	27 (18.5)	57 (67.9)	1.62 (0.77–3.44)	0.264			4.23 (2.07–8.66)	≤0.001
Previous hospitalization	30 (20.5)	11 (36.7)	19 (63.3)	1.66 (0.71–3.89)	0.260				
Outcome									
Overall mortality	66 (45.2)	20 (30.3)	46 (69.7)	1.22 (0.59–2.52)	0.712				

*COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; ICU, intensive care unit; LOS, length of stay; SD, standard deviation; SXT, trimethoprim–sulfamethoxazole.

Table 3. Antimicrobial susceptibility and its correlation with the expression of *smeABC* and *smeDEF* in *S. maltophilia* isolates

Antimicrobial agent*	MIC (μg/mL)†			No. (%) of isolates‡			Sme efflux pumps overexpression Mean MIC (SD)§					
	Range	50 %	90 %	R	I	S	*smeABC* (+) n=68, 74.7 %	*smeABC* (−) n=23, 25.3 %	P value	*smeDEF* (+) n=60, 65.9 %	*smeDEF* (−) n=31, 34.1 %	P value
Amikacin	≤8 ->32	32	>32	83 (42.3)	59 (30.1)	54 (27.6)	41.8 (21.7)	43.4 (21.1)	0.419	40.5 (22.1)	45.6 (20.1)	0.102
Cefepime	2 ->16	16	>16	63 (32.1)	54 (27.6)	79 (40.3)	19.7 (10.2)	19.9 (10.5)	0.924	20.9 (10.2)	17.5 (10.1)	0.187
Cefotaxime	≤8 ->32	>32	>32	130 (66.3)	53 (27.0)	13 (6.6)	51.4 (20.4)	49.8 (21.0)	0.761	51.8 (20.2)	49.3 (21.1)	0.486
Ceftazidime	2 ->16	16	>16	101 (51.5)	23 (11.7)	72 (36.7)	22.7 (11.1)	24.4 (11.7)	0.870	24.5 (10.5)	20.4 (12.2)	0.003
Chloramphenicol	≤4 ->16	16	>16	28 (14.3)	87 (44.4)	81 (41.3)	17.0 (10.0)	20.8 (10.6)	0.201	17.5 (10.3)	18.8 (10.1)	0.877
Ciprofloxacin	≤0.5 ->2	2	>2	51 (26.0)	83 (42.3)	62 (31.6)	2.8 (1.2)	2.9 (1.3)	0.757	2.8 (1.4)	3.0 (1.3)	0.918
Gentamicin	≤0.5 ->32	>8	>8	108 (55.1)	32 (16.3)	56 (28.6)	12.5 (10.7)	19.7 (21.9)	0.001	14.3 (14.5)	14.5 (14.8)	0.863
Levofloxacin	<0.5 ->4	≤2	≤2	5 (2.6)	5 (2.6)	186 (94.8)	2.2 (1.4)	2.7 (2.0)	0.041	2.3 (1.4)	2.4 (1.8)	0.350
Meropenem	2 ->32	>8	>32	183 (93.4)	5 (2.6)	8 (4.0)	19.1 (12.8)	16.0 (11.3)	0.358	18.8 (12.3)	17.3 (13.1)	1.000
SXT	≤0.5 ->16	≤2	>2	49 (25.0)	0 (0.0)	147 (75.0)	2.8 (1.1)	4.3 (6.1)	0.060	2.9 (1.1)	3.8 (5.3)	0.178

*SXT, trimethoprim–sulfamethoxazole.

†MIC, minimum inhibitory concentration.

‡R, resistant; I, Intermediate; S, susceptible.

§SD, standard deviation; (+), positive for Sme efflux pump overexpression; (−), negative for Sme efflux pump overexpression.

(OR=2.73; 95 % CI=1.20–6.22; *P*=0.015), acute myocardial infarction (OR=3.39; 95 % CI=1.01–11.38; *P*=0.038), leukaemia (OR=7.90; 95 % CI=0.93–67.38; *P*=0.027), urinary catheter (OR=3.87; 95 % CI=1.80–8.32; *P*≤0.001), central venous catheter (OR=5.26; 95 % CI=2.41–11.49; *P*≤0.001), mechanical ventilation (OR=3.91; 95 % CI=1.95–7.85; *P*≤0.001), hemodialysis/peritoneal dialysis (OR=4.87; 95 % CI=1.92–12.37; *P*≤0.001), cardiac arrest (OR=4.66; 95 % CI=1.44–15.08; *P*=0.008), length of stay ≥15 days (OR=2.51; 95 % CI=1.22–5.20; *P*=0.012) and ICU admission (OR=4.23; 95 % CI=2.07–8.66; *P*≤0.001).

The use of corticosteroids (*P*=0.024), vasopressors (*P*≤0.001), antifungals (*P*=0.007), vancomycin (*P*=0.009) and SXT (*P*=0.043) were risk factors for 30 day mortality in patients infected with *S. maltophilia*.

DISCUSSION

Clinical and microbiological data from patients infected with *S. maltophilia* strains from two Mexican tertiary-care hospitals were compared. *S. maltophilia* mainly affected patients with respiratory infections who had been admitted

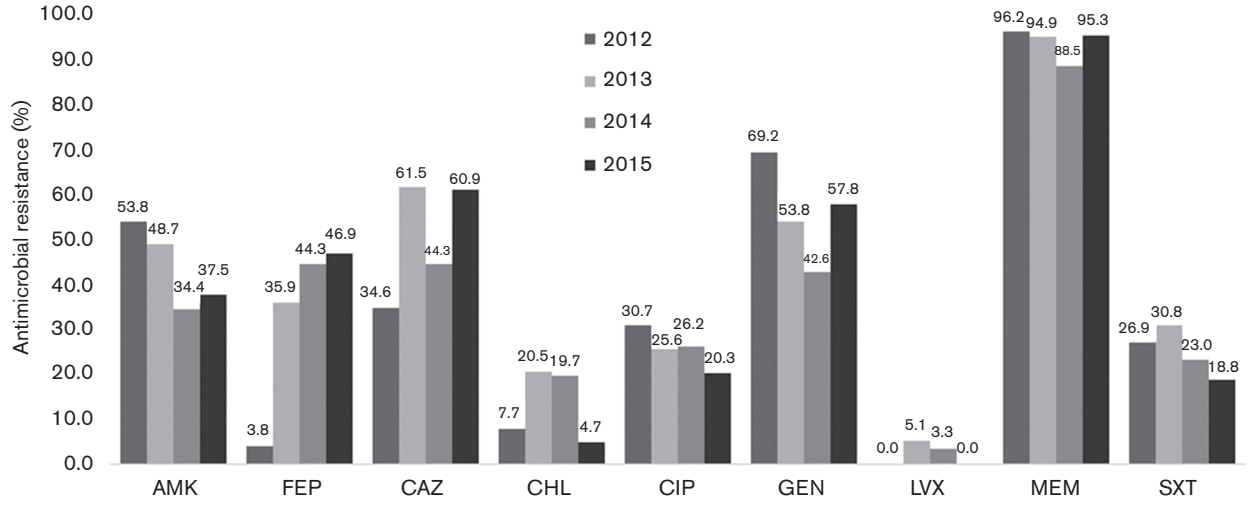

Fig. 1. Comparison of resistance rates of *S. maltophilia* isolates per year. The percentage of isolates resistant to several antimicrobials per year from 2012 to 2015 is shown. The data from the years 2007 to 2011 are not shown because the number of isolates per year is too low (≤3) and they are not considered to be significant. AMK, amikacin; FEP, cefepime, CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; LVX, levofloxacin; MEM, meropenem; and SXT, trimethoprim–sulfamethoxazole.

to the ICU and subjected to multiple invasive procedures. The overall mortality rate of patients with *S. maltophilia* infection was 45.2 %. Patients in the ICU, or under antibacterial therapy, or with arterial hypertension, type 2 diabetes, acute myocardial infarction, leukaemia, intravascular catheters or ventilation tubes, or experiencing a prolonged stay in hospital, were more likely to die.

According to the data shown above, *S. maltophilia* mainly affects patients requiring hospitalization in the ICU and with multiple invasive procedures. Attributable mortality could not be clearly defined, however; the infection may have worsened the clinical situation of patients and contributed to the high overall 30day mortality rate detected in this study.

In our patients, a prolonged length of stay ($\geq$15 days) was an independent risk factor for infections with SXT-resistant strains. A previous study that included patients with bacteremia by SXT-susceptible and SXT-resistant *S. maltophilia* showed that mortality rates do not differ between the two study groups, but patients with SXT-resistant isolates experienced prolonged hospitalization after the onset of bacteremia [18]. According to this study and our results, length of stay seems to be the most important risk factor for infection with SXT-resistant strains.

The usage of antibiotics may have favoured the selection of drug-resistant *S. maltophilia* strains, and a prolonged length of stay may have favoured the dissemination of these drug-resistant strains within the hospital.

It is important to highlight the high resistance rate to carbapenems, aminoglycosides and third-generation cephalosporins, because these drugs are used as empirical therapies in most common nosocomial infections. *S. maltophilia* is intrinsically resistant to several of these groups of antibiotics, including cephalosporins, carbapenems, macrolides and aminoglycosides [5], and consequently treatment of S. *maltophilia* infections with these antimicrobial groups is not adequate. The use of these agents may have favoured the colonization or infection with *S. maltophilia*. Indeed, the use of carbapenems and cephalosporins has been described as a risk factor for the development of *S. maltophilia* bacteremia [19]. These findings underline the importance of monitoring the incidence and the drug susceptibility of *S. maltophilia* and underscore the importance of the de-escalation of drugs used in empirical treatment after the causative agent is defined.

SXT is regarded as a first-line drug for the treatment of *S. maltophilia* infections, because SXT resistance rates used to be less than 10 % in multiple populations [5, 6, 8]. However, SXT resistance rates vary geographically and have been gradually increasing in recent years, reaching values of as high as 32.8 % in our hospitals in 2014 [20], and reaching 38.7 % in Asian countries [7, 8]. Our follow-up study for 9 years of surveillance data showed a 25 % resistance rate for SXT, with a slight decrease per year. It seems that SXT is no longer the best option to combat *S. maltophilia* infections in several populations. For our population, levofloxacin and chloramphenicol were the most active agents against *S. maltophilia* and could be used as appropriate therapeutic options, with special emphasis of levofloxacin against *S. maltophilia* in respiratory infections [21].

Several mechanisms for antimicrobial drug resistance have been reported worldwide in isolates of *S. maltophilia* [8], including the expression of efflux pumps. Overexpression of the SmeABC pump has been associated with resistance to aminoglycosides [8, 10, 22] and fluoroquinolones [8–10]. In our study, we confirmed the association of the overexpression of SmeABC with increased resistance to gentamicin and levofloxacin (Table 4). Furthermore, the overexpression of SmeDEF has been reported to be involved in resistance to quinolones [8, 10, 11, 22], tetracyclines [8, 22], macrolides [8], chloramphenicol [8, 11] and SXT [8, 12, 23]. Interestingly, our results showed that the overexpression of SmeDEF was associated with increased resistance to ceftazidime (Table 4).

The influence of Sme efflux pumps on the antimicrobial resistance patterns of clinical isolates of *S. maltophilia* has mainly been reported in Asian countries, such as Taiwan [10, 23] and Korea [9, 22]. Our results represent the first analysis of Sme efflux pump expression and the antimicrobial resistance patterns of clinical isolates of *S. maltophilia* in Mexico. None of the Sme efflux pumps we analysed were

Table 4. Antimicrobial resistance and sme efflux pump overexpression in several studies

Country	*n*	Overexpression %		Correlation of antimicrobial resistance and sme efflux pumps overexpression		Reference
		SmeABC	SmeDEF	SmeABC	SmeDEF	
Taiwan	93	59	31	CB, CFP, CIP, GEN, TET	MER, CIP	[10]
Taiwan	70	41	63	CAZ, FEP, TIM, TZP, MER, ATM, GEN, CIP, LEV, SXT		[23]
Korea	33	64	58	CIP, LVX		[9]
Korea	102	70–77	59–61	AMK	AMK, LVX, MIN, MXF, TGC	[22]
Mexico	91	75	66	GEN, LVX	CAZ	This study

AMK, amikacin; ATM, aztreonam; FEP, cefepime; CAZ, ceftazidime; CB, carbenicillin; CFP, cefoperazone; CIP, ciprofloxacin; GEN, gentamicin; LVX, levofloxacin; MEM, meropenem; MIN, minocycline; MXF, moxifloxacin; TET, tetracycline; TGC, tigecycline; TIM, ticarcillin/clavulanic acid; TZP, piperacillin/tazobactam and SXT, trimethoprim–sulfamethoxazole.

involved in SXT resistance. SXT resistance has been associated with the presence of class 1 integrons and ISCR linked to the *sul* genes [13]. However, in our strain population, SXT resistance was not associated with the presence of the *sul* genes, suggesting that other underlying mechanisms are involved.

Our study had several limitations. First, our study had an ambispective design, so selection and observational bias may have occurred. Second, not all isolate or patient data were available. Third, the clonal relationship was not analysed for *S. maltophilia* isolates. Previously, we reported high genetic diversity among clinical *S. maltophilia* isolates from Mexico [20], which suggested independent acquisition rather than cross-transmission. However, the impact of patient-to-patient transmission in the present study cannot be excluded. Finally, other potentially active agents against *S. maltophilia*, such as ticarcillin/clavulanic acid and minocycline, were not tested in our hospital, and assessments of their clinical effects are unavailable.

In conclusion, this study was the first to evaluate the risk factors associated with SXT-resistant *S. maltophilia* infections in Mexico. Prolonged length of stay was an independent risk factor for SXT-resistant *S. maltophilia* infections. Infection with SXT-resistant *S. maltophilia* did not increase mortality, but it did lead to a prolonged hospital stay. SXT resistance in *S. maltophilia* was not associated with either SmeABC or SmeDEF pumps, or with *sul* genes or the ISCR element. As *S. maltophilia* isolates from our population had a high resistance rate to SXT, it should no longer be the first-line therapy. Instead, levofloxacin could be used as an appropriate therapeutic option against *S. maltophilia* infections.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was approved by the ethics committees of both the Hospital Universitario 'Dr José Eleuterio González', Universidad Autónoma de Nuevo León (Approval GA15-005) and the Hospital Civil de Guadalajara and Instituto de Patología Infecciosa y Experimental Dr Francisco Ruíz Sánchez (approval 011/14), which waived the need for patients' informed consent.

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