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Sm*qnr* VARIANTS IN CLINICAL ISOLATES OF *Stenotrophomonas maltophilia* IN BRAZIL

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SUMMARY

Stenotrophomonas maltophilia contains a novel chromosomally-encoded *qnr* gene named Sm*qnr* that contributes to low intrinsic resistance to quinolone. We described Sm*qnr* in 13 clinical isolates of *S. maltophilia* from two Brazilian hospitals, over a 2-year period. The strains were identified by API 20 NE (bioMérieux, France). Susceptibility by microdilution method to trimetroprim/sulfamethoxazole, ciprofloxacin, levofloxacin, minocycline, ceftazidime, chloramphenicol and ticarcillin/clavulanate was performed according to CLSI. PCR detection of Sm*qnr* gene was carried out. The sequence of Sm*qnr* was compared with those deposited in GenBank. Pulsed-field gel electrophoresis (PFGE) of all strains was performed. Thirteen Sm*qnr* positives isolates were sequenced and three novel variants of Sm*qnr* were identified. All 13 Sm*qnr* isolates had distinguishable patterns by PFGE. This is the first report of Sm*qnr* in *S. maltophilia* isolated in Brazil.

KEYWORKS: *Stenotrophomonas maltophilia;* Levofloxacin resistance; *qnr* genes.

INTRODUCTION

Stenotrophomonas maltophilia, a non-fermentative Gram-negative bacillus that is ubiquitous in the environment, has emerged as an important opportunistic pathogen[2]. This microorganism exhibits intrinsic and acquired resistance to a wide variety of antimicrobial agents and few options of treatment are available[2,15]. So far, trimethoprim/sulfamethoxazole is the drug of choice to treat infections caused by this microorganism, however, during the past few years increased resistance to this antibiotic has been reported[8,15]. The new fluoroquinolones such as levofloxacin and moxifloxacin showed promising *in vitro* activity against *S. maltophilia*[13]. Resistance to these new fluoroquinolones, among *S. matophilia,* is rare and needs to be further researched.

S. maltophilia contains a novel chromosomally-encoded *S. maltophilia qnr* gene named Sm*qnr* with 219 amino acids with two classic pentapeptide repeat motifs separated by a glycine residue, which confers low level resistance to quinolone antibiotics as showed *in vitro* experiments[12]. The role of Sm*qnr* on quinolones resistance, however, is controversial and there is a lack of research evaluating its association with levofloxacin resistance in *S. maltophilia.*

We describe the characterization of Sm*qnr* genes in clinical isolates of *S. maltophilia* susceptible and resistant to ciprofloxacin and levofloxacin.

MATERIAL AND METHODS

Clinical samples of *S. maltophilia* isolates from two Brazilian teaching hospitals, over a 2-year period were evaluated. Isolates were identified by API 20 NE (bioMérieux, France). Susceptibility by microdilution method to trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, minocycline, ceftazidime, chloramphenicol and ticarcillin/clavulanate was performed according to the CLSI (CLSI 2011)[4]. Tigecycline MIC was interpreted following the Food and Drug Administration (FDA) recommendation for *Enterobacteriaceae*. Endonuclease-digested genomic DNAs were separated by pulsed-field gel electrophoresis (PFGE) using a CHEF-DR III system (Bio-Rad, USA). Genomic DNA was digested with 10U of *Spe*I (fermentas, USA). Running conditions were 21 h at 14 °C, with and initial switching time of one s and final time of 30 s, at 6 V/cm.

PCR for the Sm*qnr* gene was carried out using five different set of specific sequence primers QnrM+ (5'-CTTGGCATGGAATCCC TGAT-3')/QnrM- (5'-TGATGCCTACGGCACCAC-3'), QnrMR55+ (5'-CATGGCATGGAATCCCCGAT-3')/QnrMR55- (5'-TGATG TCTACGGCACCAC-3'), *qnr*A (F:5'-CTCGAATGCCTGGCGCG TGTTT-3') (R: 5'- AAGAGATTTCTCAGCCAGG-3'), *qnr*B (F: 5'-TGCCAGGCACAGATCTTGAC-3') (R: AGGMATHGAAATTCG CCACTG-3') and *qnr*S (F: 5'- TTTGCYGYYCGCCAGTCGAA-3') (R:5':GCAAGTTCATTGAACAGGGT-3') and was performed in accordance with SANCHEZ *et al*. (2008) and ROBICSEK *et al.* (2006)[10,11]. We used five set of primers because the regions around *qnr* are different in the sequences of *S. maltophilia* strains K279a, R551-3 and *qnr* A, B, S of *Enterobacteriaceae* species.

The nucleotide sequences and the deduced amino acid sequence were

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analyzed using the biological sequence aligment editor and CLUSTALW (www.mbio.ncsu.edu/bioedit/bioedit) (CA, USA).

This study was approved by the Ethics Committee of the two hospitals.

RESULTS

Thirteen *S. maltophilia* isolates harboring Sm*qnr* were studied, eight resistant to ciprofloxacin and two to levofloxacin. QnrM gene was detected only using primers derived from *S. maltophilia* strain K279a; *qnr* A, B and S genes of *Enterobacteriaceae* were not detected.

All 13 isolates showed distinguishable patterns by PFGE (Table 1). The distribution of isolates occurred evenly in different units and with different clonal profiles during the study period, which ruled out the possibility of an outbreak.

Two of the 13 isolates were resistant (MIC 8 and 16 mg/L) and two showed increased MIC to levofloxacin (MIC 4 mg/L). Eight isolates were resistant and one exhibited increased MIC to ciprofloxacin (MIC ≥ 2 mg/L). Two isolates were resistant to trimethoprim/sulfamethoxazole (MIC 4 and 8 mg/L). Two isolates were resistant to tigecycline (MIC 4 and 8 mg/L) and all isolates were susceptible to minocycline (MIC ≤ 4 mg/L) (Table 1).

The Sm*qnr* peptide sequences of the 13 isolates were compared with the known Sm*qnr* 1-27 subtypes in GenBank. Sequence analysis showed that seven isolates were identical to the equivalent sequence of Sm*qnr*6 from Japan (AB430849), the other isolates were distributed as followed: one Sm*qnr*4 (GenBank AB430842), one Sm*qnr*12 (GenBank AB430844) and one Sm*qnr*1 (Genbank AB430839) identified in Japan. Three novel variants were observed, the subtype Sm*qnr*LIM31 have six amino acid residues differences, the subtype Smq*nr*LIM39 have four amino acid residues differences and subtype Sm*qnr*LIM45 showed two amino acids alteration (Fig. 1).

DISCUSSION

S. maltophilia strains display high ciprofloxacin resistance, mainly due to several efflux systems[1]. However, *in vitro,* susceptibility testing to levofloxacin is recommended by CLSI (CLSI 2009), and levofloxacin and moxifloxacin are used to treat infections caused by this pathogen. Resistance to levofloxacin and moxifloxacin is still rare among *S. maltophilia*[5,14]. Two recent studies of clinical isolates of *S. maltophilia* that evaluated 102 isolates of bloodstream infection and 377 isolates (majority from the respiratory tract and blood) showed respectively 92.9% and 79.6% of susceptibility to levofloxacin[5,14]. In our study two isolates showed resistance and two increased MIC to levofloxacin. All isolates were susceptible to minocycline and two were resistant to trimethoprim/sulfamethoxazole. Despite good activity *in vitro*, the experience of the clinical use of minocycline to treat infections caused by *S. maltophilia* is restricted to anecdotal reports[7].

The Sm*qnr* plasmid mediated genes are pentapeptides repeat proteins that confer low-level resistance to quinolone by protecting DNA gyrase. The potential source of *qnr* is believe to be horizontal transfer by integrons and mobile genetic elements from chromosome of aquatic or environmental bacterial, such *Shewanella algae, Aeromonas* spp*., Psychromonas* spp *and Vibrionaceae*[14].

Table 1
Characteristics and antimicrobial susceptibilities of 13 clinical isolates of *S. maltophilia*

Isolates	Source	PFGE	MIC (mg/L)							
			SMX	LEV	CIP	MIN	TIG	CAZ	CLO	TIC
LIM7	Blood	A	0.5	1	8	<0.25	0.5	64	8	8
LIM9	Blood	B	2	2	8	0.5	1	32	8	8
LIM11	Blood	C	2	<0.25	1	0.25	0.25	32	8	>128
LIM14	CVC	D	<0.25	<0.25	0.5	0.25	0.25	>128	8	32
LIM31	CVC	E	<0.25	1	2	<0.25	0.5	4	32	32
LIM33	CVC	F	1	16	64	2	4	16	32	64
LIM35	CVC	G	0.5	0.25	4	<0.25	0.25	>128	16	128
LIM37	CVC	H	0.25	0.5	8	<0.25	2	128	16	32
LIM39	CVC	I	0.5	4	16	4	2	8	64	128
LIM41	CVC	J	8	8	32	2	8	64	128	32
LIM45	BAL	K	4	0.5	2	0.5	2	64	>128	128
LIM47	Blood	L	0.5	1	1	<0.25	2	4	32	32
LIM49	Blood	M	1	4	16	<0.25	1	8	128	>128

MIC, microdilutional method; BAL, Bronchoalveolar lavage; CVC, cateter venous central; PFGE, Pulsed field gel electrophoresis; SXT, trimethoprim/sulfamethoxazole; LEV, levofloxacin; CIP, ciprofloxacin; MIN, minocycline; TIG, tigecycline; CAZ, ceftazidime; CLO,chloramphenicol; TIC, ticarcillin/clavulanate. **PFGE: 13** distinguishable patterns (letter A to M).

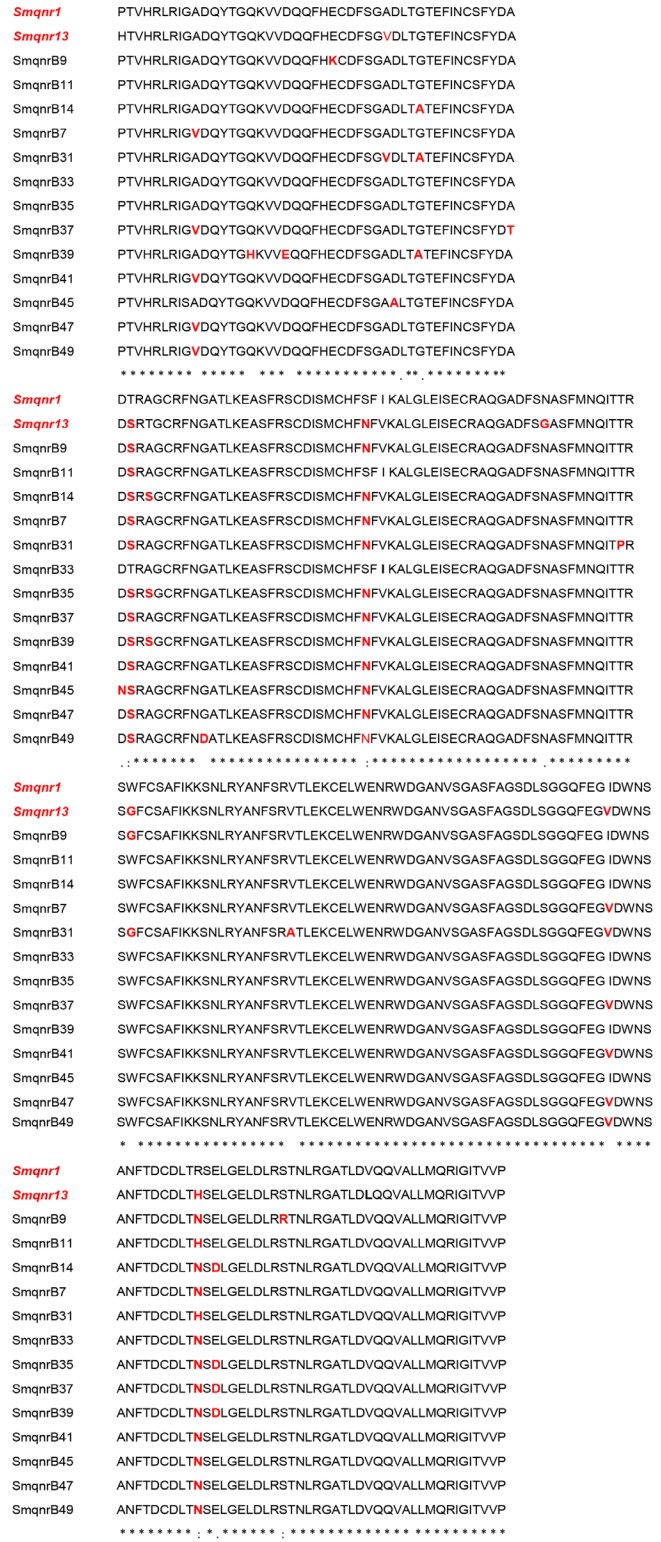

Fig. 1 - Aminoacid sequence alignments of 13 SmQnr proteins from Brazil, SmQnr1 (SHIMIZU *et al.*) and SmQnr 13 (GORDON *et al*). Asterisks, identical aminoacids, colons, strongly similar aminoacids (conserved substitutions); full stops, weakly similar amino acids (semi-conserver substitutions); spaces, variable aminoacids. Amino acid differences are shown in redbold.

The *qnr* genes in *S. maltophilia* isolates have been studied by some authors[3,6,17,18]. In our study, among 13 isolates harboring Sm*qnr*; two were resistant (MIC 8 and 16 mg/L) and two exhibited increased MIC to levofloxacin (MIC 4 mg/L) and eight isolates exhibited resistant to ciprofloxacin. Three new Sm*qnr* variants were identified. Two (LIM31 and LIM45) of them presented high levofloxacin MIC. The isolates were polyclonal, showing that they did not have a clonal relationship. This is the first study that reports Sm*qnr* in *S. maltophilia* clinical isolates in Brazil.

One important limitation of our study is that we were not able to perform cloning and transformation assays to confirm the role of Sm*qnr* on fluorquinolone resistance in *S. maltophilia.*

The role of Sm*qnr* on quinolones resistance among *S. maltophilia*, remains controversial, and appears to be associated with the clonality of strains and varies with the hospital and country. A recent study conducted in China, evaluated 442 clinical isolates of *S. maltophilia* from nine hospitals. The resistance against co-trimoxazole was 48.6%, and a high susceptibility was shown to levofloxacin, only 6.1% of strains were resistant to levofloxacin[18]. Sm*qnr* genes were detected in 114 (26%) isolates in similar frequency in both quinolones sensitive and nonsensitive strains. Twenty new variants of Sm*qnr* genes were identified and called Sm*qnr* (28-47)[18]. An i*n vitro* study, showed that overexpression of Sm*qnr* upon deletion increased modestly the MIC of nalidixic acid and moxifloxacin[3]. And finally, a study conducted in the UK, identified two new variants of Sm*qnr* that when expressed in *E. coli* top10 showed reduced susceptibility to several quinolone including levofloxacin and moxifloxacin[16].

In conclusion, this is the first report of the presence of Sm*qnr* in isolates of S. *matophilia* resistant or with high levofloxacin MIC in Brazil. Three new Sm*qnr* variants were identified. These findings alert the clinicians to the emergence of resistance to this antibiotic that is widely used in the treatment of infections by this agent, and strengthens the role of Sm*qnr* with levofloxacin resistance. In addition, minocycline presented good activity *in vitro* against multidrug resistant strains of *S. maltophilia* and, in the future, may be an option for the treatment of infections caused by this agent.

RESUMO

Variantes de Sm*qnr* de isolados clínicos de *Stenotrophomonas maltophilia* no Brasil

S. maltophilia contem um novo gene *qnr* cromossômico denominado Sm*qnr* que contribui para baixa resistência intrínseca a quinolonas. Descrevemos Sm*qnr* em 13 isolados clínicos de *S. maltophilia* de dois hospitais brasileiros, ao longo do período de dois anos. Os isolados foram identificados pela API 20 NE (bioMérieux, França). Susceptibilidade pelo método de microdiluição dos seguintes antibióticos trimetroprim/sulfametoxazol, ciprofloxacina, levofloxacina, minociclina, ceftazidima, cloranfenicol e ticarcilina/clavulanato foi realizada segundo o CLSI. Detecção do gene de Sm*qnr* foi realizada por PCR. A sequência de Sm*qnr* foi comparada com aquelas depositadas no GenBank. Foi realizada eletroforese em gel de campo pulsado (PFGE) de todos os isolados. Treze isolados contendo Sm*qnr* foram sequenciados e identificados três variantes do gene Sm*qnr*. Todos os 13 isolados de Sm*qnr* apresentaram diferentes padrões por PFGE. Este é o primeiro relato de Sm*qnr* em isolados de *S. maltophilia* no Brasil.

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TRANSPARENCY DECLARATIONS

None to declare.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with the organization that sponsored the research.

REFERENCES

1. Alonso A, Martínez JL. Cloning and charecterization of *SmeDEF*, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother. 2000;44:3079-86.

2. Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clin Microbiol Rev. 2012;25:2-41.

3. Chang YC, Tsai MJ, Huang YW, Chung TC, Yang TC. SmQnrR, a DeoR-type transcriptional regulator, negatively regulates the expression of Smqnr and SmtcrA in *Stenotrophomonas maltophilia*. J Antimicrob Chemother. 2011;66:1024-8.

4. Clinical and Laboratory Standards Institute. Performance standars for antimicrobial susceptibility testing. CLSI. 2011;3:M100-S21.

5. Garazi M, Singer C, Tai J, Ginocchio CC. Bloodstream infections caused by *Stenotrophomonas maltophilia*: a seven-year review. J Hosp Infect. 2012;81:114-8.

6. Gordon NC, Wareham DW. Novel variants of the Smqnr family of quinolone resistance genes in clinical isolates of *Stenotrophomonas maltophilia.* J Antimicrob Chemother. 2010;65:483-9.

7. Harada N, Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Maehara Y. *Stenotrophomonas maltophilia* bacteremia after living donor liver transplantation: report of a case. Surg Today. 2008,38:469-72.

8. Hu LF, Chang X, Ye Y, Wang ZX, Shao YB, Shi W, *et al*. *Stenotrophomonas maltophilia* resistance to trimethoprim/sulfamethoxazole mediated by acquisition of sul and dfrA genes in a plasmid-mediated class 1 integron. Int J Antimicrob Agents. 2011;37:230-4.

9. Nordmann P, Poirel l. Emergency of plasmid-mediated resistance of quinolones in Enterobacteriaceae. J Antimicrob Chemother. 2005;56:463-9.

10. Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother. 2006;50:2872-4.

11. Sánchez MB, Hernández A, Rodríguez-Martínez JM, Martínez-Martínez L, Martínez JL. Predictive analysis of transmissible quinolone resistance indicates *Stenotrophomonas maltophilia* as a potential source of a novel family of Qnr determinants. BMC Microbiol. 2008;8:148-52.

12. Sánchez MB, Martinez JL. Smqnr contributes to intrinsic resistance to quinolones in *Stenotrophomonas maltophilia.* Antimicrob Agents Chemother. 2010;54:580-1.

13. Saugel B, Eschermann K, Hoffmann R, Hapfelmeier A, Schultheiss C, Phillip V, *et al*. *Stenotrophomonas maltophilia* in the respiratory tract of medical intensive care unit patients. Eur J Clin Microbiol Infect Dis. 2012;31:1419-28.

14. Shimizu K, Kikuchi K, Sasaki T, Takahashi N, Ohtsuka M, Ono Y, *et al.* Sm*qnr*, a new chromosome-carried quinolone resistance gene in *Stenotrophomonas maltophilia.* Antimicrob Agents Chemother. 2008;52:3823-5.

15. Toleman MA, Bennett PM, Bennett DMC, Jones RN, Walsh TR. Global emergence of Trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of sul genes. Emerg Infect Dis. 2007;13:559-65.

16. Wareham DW, Gordon NC, Shimizu K. Two new variants of and creation of a repository for *Stenotrophomonas maltophilia* quinolone protection protein (Sm*qnr*) genes. Int J Antimicrob Agents. 2011;37:89-90.

17. Wu H, Wang JT, Shiau YR, Wang HY, Lauderdale TL, Chang SC, *et al. A* multicenter surveillance of antimicrobial resistance on *Stenotrophomonas maltophilia* in Taiwan. J Microbiol Immunol Infect. 2012;45:120-6.

18. Zhang R, Sun Q, Hu YJ, Yu H, Li Y, Shen Q, *et al*. Detection of the Smqnr quinolone protection gene and its prevalence in clinical isolates of *Stenotrophomonas maltophilia* in China. J Med Microbiol. 2012;61(Pt 4):535-9.

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