**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 trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, minocycline, cefazidime, 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.

**KEYWORDS:** *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. This microorganism exhibits intrinsic and acquired resistance to a wide variety of antimicrobial agents and few options of treatment are available. So, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, minocycline, cefazidime, chloramphenicol and ticarcillin/clavulanate was performed according to CLSI. PCR for the Sm*qnr* gene was carried out using five different set of specific sequence primers QnrM+ (5'-CTTGGCATGGAATCCCTGAT-3')/QnrM- (5'-TGATGCTACTAGCCACAC-3'), QnrMR55+ (5'-CATGGCATGGAATCCCTGAT-3')/QnrMR55- (5'-CTGATGCTACTAGCCACAC-3'), qnrA (F:5'-CTCAGATGGCTGGCCGG-3') and qnrS (F:5'-TTTGGGAAAGCCCATCG-3') (R:5'-GACAGTTGACACACGAC-3') and qnrS (F:5'-TTTGGGAAAGCCCATCG-3') (R:5'-GACAGTTGACACACGAC-3') and qnrS (F:5'-TTTGGGAAAGCCCATCG-3') (R:5'-GACAGTTGACACACGAC-3') and was performed in accordance with SANCHEZ et al. (2008) and ROBICSEK et al. (2006) in. 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.

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**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, cefazidime, chloramphenicol and ticarcillin/clavulanate was performed according to CLSI (CLSI 2011). 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 SpeI (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.

The nucleotide sequences and the deduced amino acid sequence were...
analyzed using the biological sequence alignment 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 Smqnr 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 Smqnr peptide sequences of the 13 isolates were compared with the known Smqnr 1-27 subtypes in GenBank. Sequence analysis showed that seven isolates were identical to the equivalent sequence of Smqnr6 from Japan (AB430849), the other isolates were distributed as followed: one Smqnr4 (GenBank AB430842), one Smqnr12 (GenBank AB430844) and one Smqnr1 (GenBank AB430839) identified in Japan. Three novel variants were observed, the subtype SmqqrLIM31 have six amino acid residues differences, the subtype SmqqrLIM39 have four amino acid residues differences and subtype SmqnrLIM45 showed two amino acids alteration (Fig. 1).

DISCUSSION

S. maltophilia strains display high ciprofloxacin resistance, mainly due to several efflux systems. 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. 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. 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.

The Smqnr 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.

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  | 2     | 4   | 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).
The qnr genes in *S. maltophilia* isolates have been studied by some authors. In our study, among 13 isolates harboring Smqnr, two were resistant (MIC 8 and 16 mg/L) and two exhibited increased MIC to levofloxacin (MIC 4 mg/L) and eight isolates exhibited resistance to ciprofloxacin. Three new Smqnr 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 Smqnr 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 Smqnr on fluoroquinolone resistance in *S. maltophilia*.

The role of Smqnr 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. Smqnr genes were detected in 114 (26%) isolates in similar frequency in both quinolones sensitive and nonsensitive strains. Twenty new variants of Smqnr genes were identified and called Smqnr (28-47). An in vitro study, showed that overexpression of Smqnr upon deletion increased modestly the MIC of nalidixic acid and moxifloxacin. And finally, a study conducted in the UK, identified two new variants of Smqnr that when expressed in *E. coli* top10 showed reduced susceptibility to several quinolone including levofloxacin and moxifloxacin.

In conclusion, this is the first report of the presence of Smqnr in *S. maltophilia* resistant or with high levofloxacin MIC in Brazil. Three new Smqnr 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 Smqnr 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 Smqnr de isolados clínicos de *Stenotrophomonas maltophilia* no Brasil

*S. maltophilia* contém um novo gene qnr cromossômico denominado Smqnr que contribui para baixa resistência intrínseca a quinolonas. Descrevemos Smqnr 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 trimetrprim-sulfametoxazol, ciprofloxacina, levofloxacina, minociclina, cefazidima, cloranfenicol e ticarcilina/clavulanato foi realizada segundo o CLSI. Deteção do gene de Smqnr foi realizada por PCR. A sequência de Smqnr foi comparada com aquelas depositadas no GenBank. Foi realizada eletroforese em gel de campo pulsado para PFGE de todos os isolados. Treze isolados contendo Smqnr foram sequenciados e identificados três variantes do gene Smqnr. Todos os 13 isolados de Smqnr apresentaram diferentes padrões para PFGE. Este é o primeiro relato de Smqnr 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.

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