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ORIGINAL ARTICLE

Risk factors for levofloxacin resistance in *Stenotrophomonas maltophilia* from respiratory tract in a regional hospital

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Objectives: *Stenotrophomonas maltophilia* is a bacterial pathogen associated with health-care associated infections, particularly in immunocompromised patients. Members of the fluoroquinolone drug class are frequently used to treat *S. maltophilia* infection; however, *S. maltophilia* resistance to fluoroquinolones, especially levofloxacin, has been increasing.
Methods: We sought to identify risk factors associated with levofloxacin resistance using a case-control study. We examined sputum from 76 *S. maltophilia*-positive patients admitted to our hospital between January 1, 2010 and June 30, 2011. Case groups were defined as patients who had *S. maltophilia* infections resistant to levofloxacin, and control groups were defined as patients who had *S. maltophilia* infections susceptible to levofloxacin treatment. Patient information including demographics, previous antibiotic use, and other traits were

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recorded. In addition, *S. maltophilia* isolates from patient sputum were assessed for antibiotic resistance as well as for the presence of genes associated with drug resistance.
Results: Previous antibiotic treatment with first- or second-generation cephalosporin was found more often in the levofloxacin-susceptible group; by contrast, previous piperacillin/tazobactam treatment occurred more often in the levofloxacin-resistant group. Three genes associated with drug resistance, including *Sme*A, *Sme*D, and *Spg*M were not significantly different between these groups.
Conclusion: Piperacillin/tazobactam treatment is associated with subsequent isolation of levofloxacin-resistant *S. maltophilia* from the respiratory tract.

Introduction

Stenotrophomonas maltophilia is an aerobic glucose non-fermentative Gram-negative bacillus ubiquitously present in the environment. It has emerged as a pathogen in healthcare associated infections (HAIs), especially in immunocompromised patients.[1–3]

S. maltophilia has a variety of clinical presentations, including bacteremia, respiratory tract infections, urinary tract infections, skin and soft tissue infections, endocarditis, and meningitis.[4–7] Respiratory tract infection, especially ventilator-associated pneumonia, is most frequently seen in *S. maltophilia* infections.[8] Risk factors associated with *S. maltophilia* colonization and infection include hematologic malignancy, admission to intensive care units, use of central venous catheters, recent surgery, ventilator usage, and previous therapy with broad-spectrum antibiotics, especially carbapenems.[9–13]

Many antibiotics, including carbapenems, are not effective against *S. maltophilia*, making infections a challenge to treat.[1,14] Trimethoprim–sulfamethoxazole (TMP/SMX) and levofloxacin are the most common antibiotics used to treat the *S. maltophilia* infections; however, according to the Taiwan Surveillance of Antimicrobial Resistance program, resistance to ceftazidime, TMP/SMX, levofloxacin, and ticarcillin/clavulanic acid has been increasing.[15–17] Antibiotic efflux pumps, such as *Sme*ABC and *Sme*DEF, have been reported to play a role in *S. maltophilia* resistance to fluoroquinolones. In addition, *Spg*M, a phosphoglucomutase, has also been associated with fluoroquinolone resistance in *S. maltophilia*.[18,19]

In this study, we assessed the roles of resistant genes for efflux pumps and phosphoglucomutase for levofloxacin resistance among clinical isolates of *S. maltophilia*. We also evaluated the risk factors associated with levofloxacin resistance in *S. maltophilia* infections using a case–control study.

Methods

Setting

The Hsin-Chu branch of the National Taiwan University Hospital is a regional hospital with a capacity of 694 beds. This was a case-control study.

Bacterial isolates

We prospectively collected *S. maltophilia* isolated from the respiratory tracts specimens of adult patients from January 1, 2010 to June 30, 2011 for this case-control study. If several isolates of *S. maltophilia* were obtained from a single patient, only the first to be isolated was included in the study. Patients who were not admitted in our hospital were excluded from the study. All of the isolates were identified by conventional biochemical identification methods and were confirmed by polymerase chain reaction (PCR) analysis of the SM1 and SM4 regions of the *S. maltophilia* 23S rRNA gene.[20]

Susceptibility testing

S. maltophilia is resistant to many drugs, including most of penicillins, cephalosporins, and carbapenems, so we focused on drugs commonly used to treat *S. maltophilia* infections. Because our hospital does not stock them, monobactam-class antibiotics were not used in this study. Susceptibility to various antimicrobial agents, including ciprofloxacin, TMP/SMX, tigecycline, and colistin were determined by minimal inhibition concentrations using the Clinical and Laboratory Standards Institute's reference microbroth dilution method.[21]

Case groups were defined as patients who had levofloxacin-resistant *S. maltophilia* infections. Control groups were defined as patients who had levofloxacin-susceptible *S. maltophilia* infections. Patient medical records collected by chart review included age, sex, underlying disease, previous medical history, and previous antibiotics usage. Previous antibiotics usage was defined as administration of antibiotic less than 15 days prior to when *S. maltophilia* was isolated from the patient's sputum. Drug resistant genes, such as *Sme*A, *Sme*D, and *Spg*M, were analyzed in these bacteria by PCR analysis.[18] Institutional review board approval was obtained for this study (ethical approval number HCGH99IRB-12).

Identification of SmeA, SmeD, and SpgM genes

Cells were prepared and inoculated onto a Mueller–Hinton agar plate as in the agar dilution method. Following overnight culture, cells were collected to make a 1.5 mL suspension of

Table 1 Basic data, underlying diseases, and risk factors of patients

Group	Levofloxacin R	Levofloxacin S	*p*
N	25	51	
Age	77.96 ± 11.93	73.96 ± 15.44	0.219
Male sex	18 (72)	31 (60.8)	0.539
DM	11(44)	12 (23.5)	0.068
ESRD	4 (16)	3 (5.9)	0.152
Malignancy history	3 (12)	6 (11.8)	0.976
COPD	10 (40)	14 (27.5)	0.269
Recent surgery[a]	3 (12)	13 (25.5)	0.175
Recent admission[b]	5 (20)	11 (21.6)	0.875
Previous antibiotics			
3rd generation cephalosporin	10 (40)	24 (47)	0.521
1st or 2nd generation cephalosporin	3 (12)	19 (37.3)	0.023
Augmentin	10 (40)	24 (47)	0.521
Piperacillin/tazobactam	12 (48)	13 (25.5)	0.05
Carbapenem	7 (28)	9 (17.6)	0.298
Quinolone	3 (12)	6 (11.8)	0.976
Aminoglycoside	2 (8)	4 (7.8)	0.981
Drug resistant genes			
*Sme*A positive	6 (24)	15 (29.4)	0.62
*Sme*D positive	23 (92)	47 (92.2)	0.981
*Spg*M positive	23 (92)	44 (86.3)	0.468
Resistance to other antibiotics			
Ciprofloxacin	21 (84)	2 (3.9)	<0.005
TMP/SMX	9 (36)	5 (9.8)	0.006
Tigecycline	4 (16)	0 (0)	0.003
Colistin	20 (80)	24 (47.1)	0.006

[a] Recent surgery: surgery in the past 3 months.
[b] Recent admission: admission in the past month.
Data are presented as *n* (%).
COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; ESRD = end-stage renal disease; TMP/SMX = trimethoprim/sulfamethoxazole.

optical density at 550 nm (OD_{550}) = 1.0. RNA was prepared using an RNA-Be Kit (Tel-Test Inc., Friendswood, TX, USA) and cDNA was obtained with the SuperScript First-Strand Synthesis System for reverse transcription-PCR (Invitrogen Corp., Carlsbad, CA, USA) using random hexamers. Primer pairs 5′-GTCGACCTG GTACAGCA-3′/5′-ACCTTAACCTGTGCCTTG-3′, 5′-CCAAGAGCCTTTC CGTCAT-3′/5′-TCACGCTGAAGTCCGAGA-3′ and 5′-GTGACTTCGACC GTTGCTTC-3′/5′-ATCTTTTCCTTGAT GAACGC-3′ were used for PCR to detect the expression of *Sme*A, *Sme*D, and *Spg*M, respectively, using cDNA of 16S rRNA as an internal control.

Results

Eighty patients were originally recruited for this study. Twenty-seven had levofloxacin-resistant *S. maltophilia* infections. However, four patients were excluded because they were not admitted to the hospital; two of these cases demonstrated levofloxacin-resistance. Therefore, a total of 76 patients were enrolled in the study, 25 with levofloxacin-resistant and 51 with levofloxacin-sensitive *S. maltophilia* infections.

The basic demographic data, underlying diseases, and other potential risk factors for patients in this study are shown in Table 1. Previous antibiotic treatment with first- or second-generation cephalosporin was observed more often in the levofloxacin-susceptible group; by contrast, previous piperacillin/tazobactam use was reported more often in the levofloxacin-resistant group.

Three drug resistance genes were analyzed in patient bacterial isolates, including *Sme*A, *Sme*D, and *Spg*M (Table 1); however, no significant associations were found in either group.

We also tested levofloxacin-resistant *S. maltophilia* for resistance to other antibiotics. The results are shown in Table 1. Similarly, several antibiotics, including ciprofloxacin, TMP/SMX, tigecycline, and colistin had lower resistance rates in bacteria sensitive to levofloxacin. Genes associated with resistance towards other antibiotics are shown in Table 2. Only TMP/SMX treatment showed increased resistance rates in *Sme*D- and *Spg*M-negative groups.

Discussion

Fihman et al[22] reported that risk factors for *S. maltophilia* infection include: immunocompromised status, central venous catheter insertion in intensive care units, and hospitalization within the previous 90 days. Other studies have discussed risk factors for *S. maltophilia* bacteremia;

Table 2 Genes (*Sme*A, *Sme*D, *Spg*M) associated with resistance towards other antibiotics

	*Sme*A +	*Sme*A −	*p*
N	21	55	
Ciprofloxacin	5 (23.8)	18 (32.7)	0.449
TMP/SMX	4 (19.0)	10 (18.2)	0.931
Tigecycline	1 (4.8)	3 (5.5)	0.904
Colistin	11 (52.4)	33 (60)	0.547
	*Sme*D +	*Sme*D −	*p*
N	70	6	
Ciprofloxacin	21 (30.0)	2 (33.3)	0.865
TMP/SMX	10 (14.3)	4 (66.7)	0.001
Tigecycline	3 (4.3)	1 (16.7)	0.192
Colistin	42 (60.0)	2 (33.3)	0.204
	SpqM +	*SpqM* −	*p*
N	67	9	
Ciprofloxacin	20 (29.9)	3 (33.3)	0.831
TMP/SMX	9 (13.4)	5 (55.6)	0.002
Tigecycline	3 (4.5)	1 (11.1)	0.403
Colistin	41 (61.2)	3 (33.3)	0.1

TMP/SMX = trimethoprim/sulfamethoxazole.

however, to our knowledge, none have reported risk factors for fluoroquinolone-resistant *S. maltophilia* infections.[23] In contrast to other studies, we did not find underlying diseases to be a predisposing factor for levofloxacin-resistant *S. maltophilia* infection. Although patients with diabetes mellitus had a higher rate of levofloxacin-resistant *S. maltophilia* infection, this rate was not statistically significant.

Patients who had previously received piperacillin/tazobactam antibiotic treatments had higher rates of levofloxacin resistance; however, previous use of first- or second-generation cephalosporin had lower rates of levofloxacin resistance. The mechanism for resistance to levofloxacin after piperacillin/tazobactam use is unclear. Further studies are necessary for better understanding of the relationship between piperacillin/tazobactam treatment and subsequent resistance to levofloxacin.

The levofloxacin-sensitive group was also sensitive to other antibiotics, especially ciprofloxacin. In our study, nearly all bacteria sensitive to levofloxacin also were sensitive to ciprofloxacin, making it an alternative treatment for *S. maltophilia* infection.[24] *S. maltophilia* isolates resistant to levofloxacin were also positive for genes associated with drug resistance to other antibiotics.

A previous study reported that the *Sme*A, *Sme*D, and *Spg*M genes are associated with multiple drugs resistance in *S. maltophilia*.[18] However, these observations were not consistent with our data. *Sme*A, *Sme*D, and *Spg*M may play minor roles in multiple drugs resistance of *S. maltophilia*, or other mechanisms may have contributed to the drug resistance of *S. maltophilia* found in our hospital. Further testing is necessary to fully elucidate these mechanisms of resistance.

In the previous studies, *S. maltophilia* resistance to TMP/SMX treatment has been associated with efflux pump genes such as *Bpe*EF-*Opr*C.[25] Although no study has yet shown *Spg*M to be related to TMP/SMX resistance, our study indicated that SMX-TMP resistance was associated with a lower frequency of *Sme*D and *Spg*M. *Sme*D and *Spg*M may not induce resistance to SMX/TMP, and other genes may induce resistance to SMX/TMP. When *Sme*D and *Spg*M are expressed, other genes related to SMX/TMP resistance may be suppressed and thus decrease SMX/TMP resistance.

Our study had many limitations. Because the sample size was small and all samples were from the same hospital, many risk factors did not reach statistical significance. Some genes commonly associated with drug resistance, such as *Smqnr*, a gene associated with quinolone resistance in some studies, were not detected in our study.[26,27] We did not find any genes associated with levofloxacin resistance in our study.

In conclusion, except for previous piperacillin/tazobactam antibiotic treatment, we found no significant associations between *S. maltophilia* drug resistance to levofloxacin and other risk factors in our patients. Three genes, including *Sme*A, *Sme*D, and *Spg*M—previously reported to be associated with levofloxacin resistance—were not significantly associated with the resistant group in our study. Other genes may contribute to levofloxacin resistance. More studies including larger case numbers and more drug resistant genes are necessary to understand fully the causes and risk factors of drug resistance in *S. maltophilia*.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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