ORIGINAL ARTICLE

Stenotrophomonas maltophilia: antimicrobial resistance and molecular typing of an emerging pathogen in a Turkish university hospital

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ABSTRACT

Despite its limited pathogenicity, *Stenotrophomonas maltophilia* is an emerging nosocomial pathogen. This study investigated the isolation frequency, antimicrobial resistance and genotypic relationships of 205 *S. maltophilia* isolates from 188 patients in a university hospital between 1998 and 2003. Susceptibility profiles for 11 antimicrobial agents were determined by the NCCLS agar dilution method for non-fermentative bacteria, while enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR and pulsed-field gel electrophoresis (PFGE) were used for genotyping of the isolates. Of the 205 isolates, 56.1% were isolated in the last 2 years of the study. The risk of *S. maltophilia* isolation was higher in intensive care units, *S. maltophilia* was isolated mostly (86.8%) after hospitalisation for ≥48 h, and 90.4% of the patients had underlying diseases. Resistance levels were > 60% for all antimicrobial agents tested except co-trimoxazole. High genetic diversity was found among the *S. maltophilia* isolates, and cross-infection with *S. maltophilia* was not common. Although ERIC-PCR revealed fewer genotypes than PFGE, it proved to be a rapid and easy method for *S. maltophilia* genotyping, and was more economical than PFGE.

Keywords Antibiotic resistance, ERIC-PCR, genotyping, nosocomial infection, PFGE, *Stenotrophomonas maltophilia*

INTRODUCTION

Stenotrophomonas maltophilia is an opportunistic pathogen of increasing importance. The use of broad-spectrum antibiotics and an increase in the number of invasive procedures and immuno-suppressed patients has caused this intrinsically multidrug-resistant microorganism to emerge as an infectious agent in hospitals, especially in intensive care units (ICUs) [1–3]. Its resistance to many antimicrobial agents, including β-lactams and aminoglycosides, allows patient colonisation even when antimicrobial agents are being used [4]. Despite its relatively low virulence, *S. maltophilia* can cause a wide variety of infections, e.g., pneumonia, bacter-aemia, endocarditis, urinary tract infection,

meningitis, cholangitis, soft tissue infection and wound infection [3–7]. Predisposing factors for *S. maltophilia* infection include prolonged hospitalisation, especially in ICUs, consumption of broad-spectrum antibiotics, malignancy, immune suppression, and breakdown of mucocutaneous defence barriers (e.g., following catheterisation, artificial implants, tracheotomy, or peritoneal dialysis) [8,9].

Epidemiological studies of clinical *S. maltophilia* isolates have shown genetic diversity [10,11], probably associated with selection of naturally present *S. maltophilia* from among other bacteria by antibiotic pressure. However, cross-infections between patients, transmitted by healthcare workers, have also been reported [12]. For this reason, detection of antibiotic resistance patterns and typing of *S. maltophilia* isolates is significant in the context of hospital infection control. The present study investigated antibiotic resistance patterns and genotypes among *S. maltophilia* isolates in a hospital for adults during the period 1998–2003.

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MATERIALS AND METHODS

Bacterial isolates

S. maltophilia isolates were obtained from various clinical specimens at the Hacettepe University Faculty of Medicine Adult Hospital, Clinical Pathology Laboratory between 1998 and 2003. In total, 205 isolates from 188 patients were included in the study. Isolates from the same patient were obtained from different anatomical sites. The isolates were identified initially by the Sceptor (Becton-Dickinson, Franklin Lakes, NJ, USA) system, and the identification was confirmed by manual biochemical tests (Gram's stain, catalase, oxidase, aesculin hydrolysis, lysine decarboxylase and DNase).

Antimicrobial susceptibility testing

Susceptibility to 11 antimicrobial agents (imipenem, meropenem, co-trimoxazole, amikacin, gentamicin, ciprofloxacin, ceftazidime, cefepime, cefotaxime, piperacillin and piperacillin–tazobactam) was determined by the NCCLS agar dilution method for non-fermentative bacteria [13]. MICs were determined after incubation for 24 and 48 h on Mueller–Hinton agar plates at 36°C. Intermediately-resistant isolates were considered to be resistant. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218 (for piperacillin–tazobactam) were included as quality control strains in each run.

Enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR typing

The ERIC-PCR method used for genotyping *S. maltophilia* isolates was optimised from previous studies [14,15]. A single colony was inoculated into Mueller–Hinton broth and incubated for 20 h at 37°C. After centrifugation at 10 000 *g* for 10 min, each pellet was washed three times in 750 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and then resuspended in 500 μL TE buffer. The solution was boiled for 20 min and centrifuged at 10 000 *g* for 10 min, and the supernatant was then used as a crude DNA extract in PCRs. Amplification reactions were performed in a final volume of 25 μL, with 15.5 μL H_2O, 2.5 μL 25 mM $MgCl_2$, 2.0 μL each 2.5 mM dATP, dTTP, dGTP and dCTP, 0.3 μL 1 mM ERIC2 primer (5′-AAGTAAGTGACTGGGGTGAGCG-3′) (Trilink Biotechnologies, San Diego, CA, USA), 2.6 μL 10× PCR buffer (Sigma-Aldrich, Munich, Germany), 0.13 μL *Taq* polymerase (Sigma-Aldrich) and 2 μL DNA extract. A negative control with H_2O instead of DNA extract was used in each run. PCRs comprised one cycle for 3 min at 94°C, two cycles of 45 s at 94°C, 1 min at 30°C and 1 min at 72°C, and 44 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C, with a final extension for 4 min at 72°C. The PCR products were analysed by electrophoresis with DNA Molecular Weight Marker XIV (Roche Diagnostics, Istanbul, Turkey) for 2 h at 110 V in an agarose 1.5% w/v gel and staining with ethidium bromide 0.1% w/v. The amplicon patterns were evaluated with a Gel Documentation System (UV Products, Upland, CA, USA). Differences of two or more DNA bands were considered to represent different strains, while isolates differing by only one band were considered to be subtypes [11,16,17].

Pulsed-field gel electrophoresis (PFGE) analysis

Preparation of agarose plugs containing chromosomal DNA for PFGE analysis was performed as described previously [18]. The DNA contained in the plugs was digested with 20 U of *Xba*I (Roche Diagnostics) at 37°C overnight as recommended by the manufacturer. The digested plugs and molecular size markers (Pulse Marker 50–1000 kb; Sigma-Aldrich) were analysed by PFGE in high gel strength agarose (Genaxis Biotechnology, Spechbach, Germany) 1.1% w/v gels in a GN Controller (Amersham Pharmacia Biotech, Freiburg, Germany) with Tris-borate-EDTA (TBE; 0.5 M Tris, 0.5 M boric acid, 0.01 M EDTA, pH 8) buffer. PFGE was for 22 h at 150 V/cm at 12°C, with a pulse time that increased from 10 s to 90 s. The gel was stained with ethidium bromide 0.1% w/v and the DNA patterns were evaluated using the UV Products Gel Documentation System. The band patterns were interpreted according to the criteria of Tenover *et al.* [19], with patterns that differed by two or three bands being defined as closely related subtypes.

Statistics

Data were analysed with SPSS software for Windows (SPSS Inc., Chicago, IL, USA) by the McNemar, chi-square and Fisher–Freeman Halton tests, with $p < 0.05$ being considered significant.

RESULTS

Isolates

The 205 *S. maltophilia* isolates were from 104 male and 84 female patients. There was a gradually increasing frequency of *S. maltophilia* isolation during the study period. Of all non-fermentative bacterial isolates, *S. maltophilia* accounted for 4.0%, 5.8% and 9.7% in 2001, 2002 and 2003, respectively, with 56.1% of the isolates being obtained during the last 2 years of the study. The most frequent site of isolation was the respiratory tract (40%), followed by blood (21.5%) and pus (13.2%). *S. maltophilia* was the only microorganism isolated from 97 (47.3%) specimens. The other infections were polymicrobial. Sixty-two of the specimens yielded two organisms, 39 yielded three, and seven yielded four. The most frequent co-isolated microorganisms were *P. aeruginosa* (24.7%), *Staphylococcus aureus* (20.1%), *Klebsiella* spp. (12.1%) and *Acinetobacter* spp. (10.3%).

Only 12 (6.4%) of the patients were not hospitalised, and 178 (86.8%) of the 205 isolates were obtained from patients after hospitalisation for ≥2 days (Table 1). Eighty-one (46%) patients were in medical wards, 43 (24.4%) in surgical wards, and 52 (29.6%) in ICUs. The greatest risk for *S. maltophilia* isolation was in ICUs if the

Table 1. Distribution of patients and mean duration of hospitalisation before isolation of *Stenotrophomonas maltophilia*

	Patients n (%)	Mean duration (days) of hospitalisation before isolation of S. maltophilia
Medical wards	81 (46.0)	32.5
Surgical wards	43 (24.4)	39.6
Intensive care units (medical and surgical)	52 (29.6)	38.3
Total	176 (100)	36.4

number of beds per unit was taken into consideration. The mean duration of hospitalisation before *S. maltophilia* was isolated was similar for all wards (Table 1).

Patient records were available for all but 13 patients. In total, 170 (90.4%) of the patients from whom *S. maltophilia* was isolated had underlying diseases, and 134 (71.3%) had more than one underlying disease. Malignant diseases were the most common (35.1% of patients), followed by hypertension (22.9%), obstructive lung disease (20.2%) and diabetes mellitus (17.6%). One patient without underlying disease had keratitis caused by contamination of contact lenses.

Antimicrobial resistance

The resistance rates of the *S. maltophilia* isolates were >60% for all antimicrobial agents except co-trimoxazole (Table 2). The differences between resistance rates obtained after incubation for 24 h and 48 h were significant for co-trimoxazole, ciprofloxacin, ceftazidime, cefepime, piperacillin and piperacillin–tazobactam ($p < 0.05$). The differences in the MIC_{50} and MIC_{90} values after incubation for 24 h and 48 h were at most two-fold, except the MIC_{90} value for ciprofloxacin, which increased four-fold. There were no significant changes in the antimicrobial resistance rates during the study period, except for imipenem (p 0.0003), meropenem (p 0.005), co-trimoxazole (p 0.0003), piperacillin (p 0.0001) and piperacillin–tazobactam (p 0.0001) (Table 3).

Genotyping

Among the 205 isolates studied, ERIC-PCR analysis revealed 180 genotypes and PFGE analysis revealed 188 genotypes. Fifteen patients yielded more than one isolate, but only eight of these shared similar ERIC-PCR and PFGE patterns. The isolates with similar patterns were isolated mostly

Table 2. Resistance of *Stenotrophomonas maltophilia* isolates to 11 antimicrobial agents

Antimicrobial agent	MIC range (mg/L)	24 h			48 h		
		Resistant isolates n (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Resistant isolates n (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Imipenem	0.5–1024	202 (98.5)	512	512	203 (99.0)	512	1024
Meropenem	0.5–512	201 (98.0)	128	256	201 (98.0)	128	256
Co-trimoxazole	0.25/4.75–32/608	58 (28.3)	2/38	8/152	73 (35.6)	2/38	8/152
Amikacin	2–1024	174 (84.9)	128	512	176 (85.8)	128	512
Gentamicin	2–2048	194 (94.6)	128	512	196 (95.6)	128	512
Ciprofloxacin	0.5–256	189 (92.2)	4	8	199 (97.1)	4	32
Ceftazidime	1–512	146 (71.2)	32	256	153 (74.6)	64	256
Cefotaxime	1–512	196 (95.6)	128	256	197 (96.1)	128	256
Cefepime	2–128	126 (61.5)	16	32	159 (77.6)	16	32
Piperacillin	4–2048	184 (89.8)	128	1024	197 (96.1)	256	1024
Piperacillin–tazobactam	2/4–1024/4	180 (87.8)	128	512	193 (94.2)	128	512

Table 3. Susceptibility of *Stenotrophomonas maltophilia* isolates to 11 antimicrobial agents during the study period (1998–2003)

Year (n)	IMP (%)	MER (%)	T/S (%)	AK (%)	GEN (%)	CIP (%)	CAZ (%)	CTX (%)	FEP (%)	PIP (%)	P/T (%)
1998 (16)	100.0	100.0	12.5	75.0	100.0	100.0	62.5	93.7	62.5	93.7	87.5
1999 (41)	100.0	100.0	58.5	82.9	90.2	90.2	68.3	97.6	56.1	82.9	97.6
2000 (15)	80.0	86.6	26.6	60.0	86.6	86.6	53.3	93.3	40.0	66.7	86.6
2001 (18)	100.0	100.0	16.6	88.8	94.4	94.4	72.2	83.3	55.5	50.0	50.0
2002 (42)	100.0	100.0	16.6	88.1	90.5	88.1	80.9	97.6	64.3	97.6	92.9
2003 (73)	100.0	100.0	24.6	89.0	98.6	95.9	71.2	97.3	68.5	90.4	76.7
Total	98.5	98.0	28.3	84.9	94.6	92.2	71.2	95.6	61.5	89.8	87.8

IMP, imipenem; MER, meropenem; T/S, co-trimoxazole; AK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; PIP, piperacillin; P/T, piperacillin–tazobactam.

Table 4. Data for 42 isolates of *Stenotrophomonas maltophilia* showing their distribution into 17 ERIC-PCR and 13 PFGE pattern groups, antibiogram similarities, patients, hospital wards and isolation dates

ERIC-PCR pattern no.	PFGE pattern no.	Antibiogram	Isolate no.	Patient no.	Ward	Date
E1	P1	PIP, P/T different	21	H16	ICU	26/2/1999
E1	P1		26	H1	ICU	19/4/1999
E1	P1		51	H1	ICU	20/4/1999
E2	P2	Similar	22	H17	ICU	12/3/1999
E2	P2		23	H18	ICU	15/3/1999
E2	P2		24	H19	ICU	15/3/1999
E3	P14	CAZ different	54	H20	ICU	25/11/1999
E3	P15		56	H21	MW 85	28/8/1999
E4a	P3	Similar	64	H22	SICU	27/10/2000
E4b	P3		65	H23	SW 51	16/11/2000
E5	P16	Similar	70	H24	MW 76	4/5/2000
E5	P17		71	H25	ICU	3/5/2000
E6	P18	Similar	80	H26	SW 72	28/2/2001
E6	P19		81	H27	SW 73	4/1/2001
E7	P20	Similar	87	H28	SW 53	2/11/2001
E7	P21		89	H29	SICU	6/12/2001
E8	P4	Similar	84	H30	ICU	17/4/2001
E8	P4		85	H31	ICU	29/5/2001
E9	P22	GEN, CIP, PIP, P/T different	93	H32	SW 72	9/2/2002
E9	P23		94	H33	SW 51	12/3/2002
E10a	P5	Similar	102	H34	Burn	26/4/2002
E10b	P5		103	H35	SW 73	1/5/2002
E11	P6	Similar	111	H2	SICU	7/9/2002
E11	P6		118	H2	SICU	12/10/2002
E11	P7	Similar	113	H3	MW 75	20/9/2002
E11	P7		116	H3	MW 75	4/10/2002
E11	P7		115	H36	SW 52	4/10/2002
E12	P8	CIP different	122	H37	ICU	16/11/2002
E12	P8		123	H38	ICU	19/11/2002
E13a	P9a	Similar	137	H4	SW 84	10/1/2003
E13b	P9b		141	H4	SW 84	31/1/2003
E13b	P9c		146	H4	SW 84	7/2/2003
E14	P10	T/S, GEN different	154	H5	MW 85	4/4/2003
E14	P10		156	H5	MW 85	7/4/2003
E15	P11	Similar	181	H6	SW 74	20/9/2003
E15	P11		190	H6	SW 74	2/11/2003
E16	P12a	Similar	185	H7	SW 43	27/9/2003
E16	P12b		186	H7	SW 43	27/9/2003
E16	P12b		187	H7	SW 43	27/9/2003
E17a	P13	191, 195 similar	191	H8	ICU	30/10/2003
E17a	P13	194 CAZ different	195	H8	ICU	3/11/2003
E17b	P24		194	H39	MW 86	3/11/2003

ICU, medical intensive care unit; SICU, surgical intensive care unit; MW, medical ward; SW, surgical ward; PIP, piperacillin; P/T, piperacillin/tazobactam; CAZ, ceftazidime; GEN, gentamicin; CIP, ciprofloxacin; T/S, co-trimoxazole.

from ICUs. The isolates from different wards with similar ERIC-PCR patterns showed different PFGE patterns. Antibiogram patterns were found to be unrelated to the genotypes. When MIC values within two dilutions were considered to be similar, the results obtained were inconsistent with those obtained by genotyping. In addition, variations in MICs within a genotype were observed, while isolates with different ERIC-PCR and PFGE patterns sometimes had similar MIC values. Table 4 presents data for 42 isolates with similar ERIC-PCR patterns, together with their PFGE patterns, antibiogram similarities, and data concerning the patients from whom they were isolated (including hospital wards and dates of isolation).

DISCUSSION

S. maltophilia causes infections mainly in hospitals and is a particular risk for debilitated patients. This organism is ubiquitous in the environment and in the hospital setting [4,9]. Since it is able to grow in many different media in the presence of most antimicrobial agents, *S. maltophilia* is isolated with increasing frequency as a nosocomial pathogen. The annual isolation rate per 10 000 patient discharges rose from 7.1 in 1981 to 14.1 in 1984 at a university hospital in the USA [20]. A widespread study between 1997 and 2001, including data from Asia-Pacific, Europe and America, showed that *S. maltophilia* was the third most frequently isolated non-fermentative bacterium, following *P. aeruginosa* and *Acinetobacter*, with a rate of isolation from clinical specimens of 8% [21]. As described above, the isolation frequency of *S. maltophilia* increased during the period of the present study, but further investigations are needed to clarify the underlying reasons for this increase. As in the present study, *S. maltophilia* is isolated most often from respiratory specimens

and blood. Thus, Valdezate *et al.* [22] described 105 *S. maltophilia* isolates obtained between 1995 and 1998, 79 of which were from the respiratory tract and 19 from blood.

Differentiation between *S. maltophilia* colonisation and infection may be difficult when *S. maltophilia* is not the only organism isolated. Sattler *et al.* [23] investigated episodes of infection from non-respiratory sites and reported that 70.6% of *S. maltophilia* isolates were from polymicrobial cultures, which yielded mostly *P. aeruginosa* and *Acinetobacter baumannii*. Isolation of *S. maltophilia* from polymicrobial cultures may be related to a true infection, and is an important consideration in determining initial treatment, since β-lactamases leaking from *S. maltophilia* cells can facilitate the survival of β-lactam-susceptible microorganisms [24]. The present study found that *S. maltophilia* was the only microorganism isolated after cultivation of 97 (47.3%) specimens. The most frequent co-isolated microorganisms from other specimens were *P. aeruginosa* (24.7%), *Staph. aureus* (20.1%), *Klebsiella* spp. (12.1%), and *Acinetobacter* spp. (10.3%). Thus, almost half of the *S. maltophilia* isolates were monobacterial and more likely to be a cause of infection than of colonisation.

The many risk-factors that predispose to the development of *S. maltophilia* infection include prolonged hospitalisation, especially in ICUs, consumption of broad-spectrum antibiotics, malignancy, immune suppression, and a breakdown in mucocutaneous defence barriers (e.g., following catheterisation, artificial implants, tracheostomy, or peritoneal dialysis) [2–4,8,25]. Most of the patients (90.4%) in the present study had underlying diseases, including 35.1% who had malignant diseases. These results are in accordance with previously published data.

S. maltophilia is resistant to a wide spectrum of antimicrobial agents. Berg *et al.* [26] investigated both clinical and environmental isolates, and showed that the resistance profile of a strain did not depend on its source. In a worldwide surveillance study that included 1488 isolates obtained between 1997 and 2001 [21], resistance to the antimicrobial agents tested was > 50%, with the exception of co-trimoxazole (5%), gatifloxacin (5%), levofloxacin (6%), ticarcillin–clavulanate (14%) and ceftazidime (34%). Similarly, the present study found resistance rates of > 60% for all antimicrobial agents except co-trimoxazole.

When an isolate is identified as *S. maltophilia*, co-trimoxazole, ticarcillin–clavulanate, doxycycline, minocycline and the newer quinolones, such as ofloxacin, levofloxacin, sparfloxacin and moxifloxacin, may be possible options for treatment [21,27].

Although the NCCLS [13] suggests the use of dilution methods for testing antimicrobial susceptibilities of *S. maltophilia*, the correlation between in-vitro resistance and the clinical response is unknown. The incubation time and temperature for susceptibility testing remain controversial, with an increase in incubation time influencing the resistance rates of *S. maltophilia* for co-trimoxazole, ciprofloxacin, β-lactams and aminoglycosides [28]. Garrison *et al.* [29] demonstrated that if *S. maltophilia* strains were incubated for > 24 h, mutants resistant to ticarcillin–clavulanate, ciprofloxacin and gentamicin, and which shared PFGE patterns with the susceptible strains, could emerge. In the present study, the differences between resistance rates obtained after 24 and 48 h of incubation were significant for co-trimoxazole, ciprofloxacin, ceftazidime, cefepime, piperacillin and piperacillin–tazobactam ($p < 0.05$).

As was observed in this study, *S. maltophilia* isolates have high genetic diversity, even when isolated in a single hospital [10,11,26]. It has been suggested that most isolates are acquired independently rather than as a consequence of cross-transmission [30]. The present study showed that strains isolated from different wards and sharing the same ERIC-PCR patterns were different by PFGE. Although PFGE is recognised as a more reliable method for genotyping, ERIC-PCR can provide useful results if demographic data are also available. ERIC-PCR is a rapid and easy method with a lower cost than PFGE.

Cross-infections between patients are rare, but cannot be eliminated if the patients sharing isolates with identical PFGE patterns are epidemiologically linked [17]. The present study found that only 42 isolates were genetically related according to ERIC-PCR, and only 31 according to PFGE. In some cases, isolates from the same patient showed different ERIC-PCR and/or PFGE patterns. In seven of the 15 patients yielding more than one isolate from different body sites, the isolates belonged to different genotypes. Isolates belonging to the same genotype were mostly obtained from ICUs. Nosocomial outbreaks of *S. maltophilia* infection have been reported

previously. Garcia de Viedma *et al.* [12] typed isolates from seven patients in a neonatology ward using arbitrary-primed (AP)-PCR, ERIC-PCR and PFGE, and were able to identify an index case. Similarly, Davin-Regli *et al.* [16] found that two patients from different wards with isolates which shared an AP-PCR pattern had been in contact with the same X-ray technician. However, the present study of a large number of isolates from a Turkish hospital found that cross-infections with *S. maltophilia* were uncommon, and that minor outbreaks, especially those occurring in ICUs, can be controlled with standard precautions. Nevertheless, the frequency of isolation of *S. maltophilia* increased during the 6-year period of the study, and the management of infections caused by this bacterium could become a problem because of the multiresistant phenotype of these bacteria.

ACKNOWLEDGEMENTS

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