

Original Article

An Epidemiological Analysis of *Stenotrophomonas maltophilia* Strains in a University Hospital

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SUMMARY: The aim of this investigation was to evaluate the epidemiology of *Stenotrophomonas maltophilia* in a university hospital of Turkey. From June 2000 to December 2001, *S. maltophilia* strains were collected, clinical presentations were noted, and MIC determinations were performed by means of E-test. Enterobacterial repetitive intergenic consensus sequences-PCR (ERIC-PCR) was used for molecular typing of the strains. Forty-four strains of *S. maltophilia* were isolated from 41 hospitalized patients in a teaching hospital. The majority of specimens were from the blood and respiratory tract. Antimicrobial sensitivities of these strains were as follows: 97.7% trimethoprim-sulfamethoxazole, 15.9% ticarcillin, and 95.4% ticarcillin-clavulanate. The strains were evaluated using the ERIC-PCR method. It was of interest to note that epidemiological typing revealed three small outbreaks that were caused by a total of 12 strains. The remaining isolates generated singular DNA patterns. DNA amplification was possible in 38 isolates and yielded 26 different patterns in a period of 20 months, leading to the suggestion that commensal bacteria becomes selected in the presence of a suitable host.

INTRODUCTION

Stenotrophomonas maltophilia, a bacteria that is commonly found almost everywhere in nature, has become a focus of interest because of the increasing frequency of its isolation from hospitalized patients and its broad-spectrum antimicrobial resistance (1-4). Nosocomial colonization and subsequent infection by multi-resistant strains of *S. maltophilia* have resulted in serious outbreaks (5,6). Management of such outbreaks is challenging for clinicians, and demands prompt recognition and epidemiological characterization or typing of the microorganism. Therefore, this study was undertaken to determine whether the increasing frequency of *S. maltophilia* isolation in our hospital was due to epidemiologically related or unrelated strains of *S. maltophilia* infection and whether the strains could be rapidly and accurately genotyped with enterobacterial repetitive intergenic consensus sequences-PCR (ERIC-PCR).

MATERIAL AND METHODS

Patients: Following isolation of *S. maltophilia* from clinical samples supplied by our routine clinical microbiology laboratory, these patients were recorded and evaluated for clinical findings between June 2000 and December 2001 at our teaching hospital (600 beds). Patients from whom *S. maltophilia* was isolated were classified as infected or colonized using the Centers for Disease Control and Prevention criteria for nosocomial infections (7).

Infectious or colonized patients were visited routinely. Age,

previous antimicrobial therapy, underlying diseases, the day of infection after admission, drugs used, and patient response to treatment were all noted.

Cultivation and identification of bacteria: Forty-four *S. maltophilia* isolates were collected from 41 patients. Isolates that grew on sheep blood agar and eosin methylene blue agar were identified with the automatized Sceptor system. All isolates were confirmed to be *S. maltophilia* by standard biochemical methods and PCR using SM1 (5'-CAGCCTGCAAAGTA-3') and SM2 (5'-TTAAGCTTGCCACGACAG-3') oligonucleotide PCR primers designated by Whitby and colleagues (8). DNA crude cell extracts prepared from single colonies were used as templates. For this, single colonies from each bacterial strain were picked from agar plates and resuspended in 100 μ l TE (1 mM Tris HCl, 1 mM EDTA [pH 8.0]). The cell suspensions were heated at 100°C for 10 min. PCRs were performed using the thermocycler (Techne-Progene, Cambridge, UK). All PCRs had an initial denaturation of 95°C for 5 min with a subsequent 30 cycle amplification and contained a 1 μ M concentration of each primer, 10 ng of genomic DNA, a 200 μ M concentration of each of the nucleotides dATP, dTTP, dCTP, and dGTP, and 1.25 U of Taq DNA polymerase (MBI Fermentas, Amherst, N. Y., USA) in a 3 mM MgCl₂ PCR buffer (MBI Fermentas), in a total volume of 50 μ l. The cycle parameters consisted of annealing at 58°C for 10 s, extension at 72°C for 60 s, and denaturation at 95°C for 10 s. For the last cycle, the extension step was 2 min. After amplification, 20 μ l of each reaction mixture was subjected to electrophoresis in a 1.5% agarose gel. Positive results were assessed by the amplification of a 531 bp product.

Antimicrobial susceptibility testing: Antimicrobial susceptibility of isolates was determined by the automatized Sceptor system. MICs of antibiotics were determined in Mueller-Hinton agar (Difco, Detroit, Michigan, USA) by the E-test method (AB Biodisk, Solna, Sweden), according to

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the manufacturer's instructions. All *S. maltophilia* isolates were tested against amikacin, gentamicin, tobramycin, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanate (TIC/CL), ceftriaxone, ceftazidime, ciprofloxacin, imipenem, and trimethoprim-sulfamethoxazole (TMP-SMX).

Isolation of genomic DNA for ERIC-PCR: DNA extracts were prepared from an 18-h culture on sheep blood agar. Colonies were harvested into 500 μ l of TE buffer and adjusted to an optical density at 600 nm with a spectrophotometer and boiled for 15 min, then centrifuged for 5 min at 12,000 xg. Ten microliters of the supernatant was used as the source of target DNA.

ERIC-PCR: For the ERIC-PCR, we used primer ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'). Amplification reactions were performed in a 100 μ l final volume with 200 μ M each deoxynucleotide triphosphate, 10 mM Tris (pH 8.3), 50 mM KCl, 4 mM MgCl₂, 2 U Taq DNA polymerase (MBI Fermentas), and 1.5 μ M ERIC2 primer. PCR was initiated with five cycles of low stringency, which included a denaturation step at 95°C for 1 min, annealing of the primer at 28°C for 1 min, and 2 min of extension at 72°C. After the initial 5 cycles, 55 additional cycles were conducted with annealing of the primer at 50°C. The reaction was terminated with a final extension cycle at 72°C for 10 min. Amplification products were analyzed by electrophoresis using 2% agarose gel (9).

RESULTS

During the period of June 2000 to December 2001, *S. maltophilia* was isolated from 41 patients in Farabi Hospital attached to the Karadeniz Technical University School of Medicine (Trabzon, Turkey). Strains were isolated from patients admitted in the following units: surgical intensive care, 20; internal medicine, 7; pediatrics, 4; neonatal intensive care, 3; orthopedics, 2; infectious diseases, 2; otorhinolaryngology, 1; cardiovascular surgery, 1; and pediatric surgery, 1. General characteristics of patients were as follows: mean ages of adult and pediatric patients were 49.6 \pm 16.4 (20-77) and 4.6 \pm 5.8 (0-15), respectively. The male/female ratio was 31/10. For 23 intensive care unit (ICU) (surgical and neonatal ICU) patients, mean APACHE II Score was 28.34 \pm 5.2 (16-36), and duration of hospitalization was 15.3 \pm 13.3 (3-60) days. For 21 non-ICU patients, the duration of hospitalization was 27.3 \pm 19.5 (3-75) days. All the patients had one or more of various comorbid medical conditions including surgery, trauma, chronic renal failure, hemodialysis, intracranial hemorrhage,

malignancy, prematurity, etc.

All but four patients had a history of exposure to antibiotics. Antibiotics used were extended-spectrum penicillins, 3rd generation cephalosporins, carbapenems, quinolones, aminoglycosides, and glycopeptides. Combined usage of antibiotics was noted in 70.7% (29/41) of patients. Mean duration of antibiotic exposure was 21.4 \pm 13.8 (2-58) days. The frequencies of invasive procedures in the study population were as follows: mechanical ventilation in 41.4%, central venous catheter or dialysis catheter in 90.2%, and tracheostomy in 26.8% of patients.

The majority of specimens comprised isolates from blood (47%) and endotracheal aspirate (34%) of patients colonized or infected with *S. maltophilia*. Other types of specimens from which *S. maltophilia* was isolated included wounds (6.8%), urine (4.5%), CSF (2.2%), ear drainage (2.2%), and peritoneum material (2.2%).

Thirty-three (80.4%) of the patients were infected, while the other 8 (19.5%) patients were only colonized with this bacteria. Infection or colonization of the patients was determined on the basis of clinical examination. Only one patient hospitalized for chronic otitis media surgery fulfilled the criteria for a community acquired infection. Patients' clinical diagnoses were as follows: sepsis, 13; mechanical ventilatory pneumonia, 12; surgical site infections, 3; catheter related bacteremia, 2; meningitis, 1; peritonitis, 1; and chronic otitis media, 1.

Prior to acquisition of *S. maltophilia*, all of the patients had been administered antibiotics that were not effective for this bacteria. After the diagnosis of infection, prior to the initiation of proper treatment, nine patients expired due to the infection. Despite induction of proper treatment, an additional six patients expired. The mortality rate was 36.5% (15/44) among all patients, and 45.4% (15/33) in infected patients. All but one of the surgical intensive care unit patients ($n = 6$) were connected to mechanical ventilators, and all the patients had central venous and urinary catheters.

According to the E-test results, the susceptibilities and MIC values of the *S. maltophilia* strains in response to the different antibiotics are presented in Table 1. Only one isolate was resistant to TMP-SMX, and two of the 44 isolates were resistant to TIC/CL. All of the strains showed high resistance to most of the antibiotics.

ERIC-PCR results: Three small outbreaks of *S. maltophilia* consisting of 12 strains were identified in the hospital. The remaining 26 isolates had singular DNA patterns on ERIC-PCR. Six isolates showed no amplification. The representa-

Table 1. Susceptibility results of antibiotics for 44 *S. maltophilia* strains

Antibiotic	MIC (μ g/ml) range of susceptible isolates	Isolates susceptible (%)
Amikacin	\leq 16	43.1
Gentamicin	\leq 4	38.6
Tobramycin	\leq 4	13.6
Piperacillin	\leq 64	18.1
Piperacillin-tazobactam	\leq 16/4	40.9
Ticarcillin	\leq 16	15.9
Ticarcillin-clavulanate (TIC/CL)	\leq 64/2	95.4
Ceftriaxone	\leq 8	18.1
Ceftazidime	\leq 8	29.5
Ciprofloxacin	\leq 1	25.0
Imipenem	\leq 4	18.1
Trimethoprim-sulfamethoxazole (TMP-SMX)	\leq 2/38	97.7

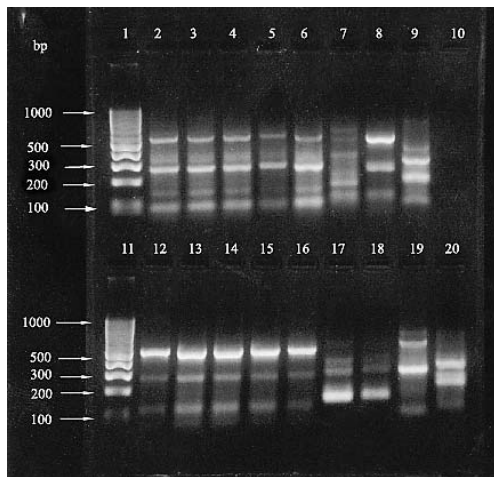


Fig. 1. Representative ERIC-PCR amplification patterns of *S. maltophilia* isolates. Lane 1, 11, molecular weight marker; Lanes 2-6, Epidemic Pattern A; Lanes 7-9, 19, 20, non-epidemic strains; Lane 10, negative control; Lanes 12-16, Epidemic Pattern B; Lanes 17,18, Epidemic Pattern C.

tive isolates are shown in Figure 1. Isolates obtained from five patients who were hospitalized within a 10-month period (November 2000 - August 2001) showed the same ERIC-PCR pattern (Pattern A). Three isolates of Pattern A were obtained from patients who were hospitalized in the surgical ICU and one each from the pediatrics unit and the neonatal ICU. Four of the isolates were obtained from blood cultures and one from an endotracheal aspirate sample.

Pattern B consisted of the same bands that were obtained from the other five isolates, from three samples from patients in the internal medicine service and two patients in the surgical ICU. Two patients in this group were diagnosed with chronic renal failure and were on hemodialysis. Pattern B bands were isolated within a 1-year period (July 2000 - July 2001). The patients in this group were hospitalized nonsynchronously; i.e., they were admitted to the hospital at different periods of time. Three of these isolates were obtained from blood samples, the other two (isolates from patients in ICU) obtained from endotracheal aspirates.

A different amplification, termed Pattern C, was noted in two samples. These two strains were isolated from samples (urine and endotracheal aspirate) of two patients who were hospitalized in the surgical ICU, and the samples were taken on the same day (November, 2001).

Multiple isolates from different units at different times (weeks to several months) showed individually distinct DNA patterns.

DISCUSSION

S. maltophilia has become an increasingly important pathogen in nosocomial infections. Colonization and infections occur in patients with impaired host defence mechanisms and indwelling catheters, in hemodialysis patients, and in association with certain surgical procedures. Recent reports have found an increased risk of acquiring *S. maltophilia* in patients treated with broad spectrum antimicrobial agents (10).

Infections with *S. maltophilia* were quite rare (only two cases) in our hospital prior to June 2000 (3,4). During the period from June 2000 - December 2001, 41 patients acquired this organism. This marked increase in infections led to

epidemiological analysis using molecular typing methods to characterize the strains and to evaluate an infection control program. Numerous typing methods such as ribotyping, multilocus enzyme electrophoresis, and random amplification of polymorphic DNA for comparison of bacterial isolates have been developed (11,12). These procedures are important for investigating strain origin, clonal relatedness among strains, and epidemiology. Although PFGE is the gold standard in molecular typing, the usefulness of ERIC-PCR has also been demonstrated in detecting the epidemiology of *S. maltophilia* infections (9,13).

S. maltophilia isolates were shown by ERIC-PCR to be very heterogeneous. Although three different groups of *S. maltophilia* isolates showing the same DNA amplification patterns were identified, 29 different amplification patterns were seen in our hospital. Isolates of Patterns A and B belonged to patients in different wards of the hospital during different time periods. Isolates of Pattern C belonged to patients in the same ward and the same room in the same period.

Cross-contamination may have been responsible for the three small outbreaks. However, 10- and 12-month strain isolation periods also suggest that *S. maltophilia* may survive in hospital surroundings for a very long time, causing isolation of isogenic strains in different parts of the hospital, which has been previously shown to occur (13,14). The identification of two strains of Pattern C during the study period necessitated prompt education of the staff of the related units by the Hospital Infection Control Committee. Education of the staff and effective infection control strategies might have had an effect on limiting the spread of the outbreak.

Van Couwenberghe et al. found antibiotic exposure to be the primary risk factor for isolation of *S. maltophilia* (10). Almost all of our patients were on antibiotic treatment for long periods of time; only 9.7% (4/41) of our patients were not receiving antibiotics. It is noteworthy that none of the antibiotics received are effective against *S. maltophilia*. Extended-spectrum penicillins, quinolones, and especially imipenem were identified as predisposing patients to *S. maltophilia* infections or colonization (13).

In accordance with findings in previous studies, TMP-SMX and TIC/CL appear to be the most active antimicrobial agents (13,15,16). Because of high piperacillin-, ticarcillin-, first, second and third line cephalosporin-, and imipenem-resistance, TMP-SMX and TIC/CL are used alone or in combination to combat *S. maltophilia* infections. Resistance to TIC/CL in relation to cross-contamination or widespread use of betalactam antibiotics may cause major difficulties in the treatment (13). In view of the high rates of resistance of this bacterium to the betalactam antibiotics, the presence of *S. maltophilia* should be considered in patients not responding to such antibiotic treatment.

This organism is clearly a cause of significant nosocomial morbidity and mortality not only in university hospitals or cancer centers but also in community hospitals. In our study, the mortality rate was as high as 45.4% in infected patients, and 36.5% among all patients. Similar high rates were also encountered in other reports (5,10).

The non-epidemic strains representing the majority of the strains in our study highly suggest that the selection of the commensal organism occurs due to the antibiotic effect. In selected departments where immune compromised patients are admitted and treated for very long periods with antibiotics, resistant strains are likely to occur. Therefore,

continued monitoring of antimicrobial resistance, prudent antimicrobial use policies, and application of infection control practices will be required to control the spread of this nosocomial pathogen.

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