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Molecular epidemiology of *Stenotrophomonas maltophilia* in a university hospital

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Summary: The aim of this investigation was to study the molecular epidemiology of *Stenotrophomonas maltophilia* in a university hospital in Italy. Sixty-one clinical isolates were collected from 43 patients during a two-year period. The majority of specimens were from the respiratory tract (41 of 43) of patients in the adult intensive care unit (ICU) (19 of 43) or cystic fibrosis (CF) patients (13 of 43). Genotypic analysis by pulsed-field gel electrophoresis (PFGE) of clinical isolates identified 31 different PFGE patterns. Although most patients were infected or colonized by different *S. maltophilia* clones, clones with identical genotype were isolated in patients from ICU, where two separate outbreaks were identified. Antimicrobial susceptibility identified a multi-resistant phenotype in all *S. maltophilia* PFGE clones. The majority of PFGE clones identified (six of seven clones from patients in the ICU) were susceptible to fluoroquinolones. Mechanical ventilation was associated with *S. maltophilia* acquisition in the ICU.

Keywords: *Stenotrophomonas maltophilia*; molecular epidemiology; PFGE typing; antimicrobial resistance.

Introduction

Stenotrophomonas maltophilia is a nonfermentative Gram-negative bacillus, usually considered to be a colonizer or an opportunistic pathogen in immunocompromised patients.[1] The most common *S. maltophilia* infections cause pneumonia in cancer and cystic fibrosis (CF) paients.[1–3] In recent years, hospital-acquired infections due to *S. maltophilia* have been on the increase.[1,2,4–6] The respiratory tract is the most common site of isolation for hospitalized patients, accounting for 56–69% of all isolates.[1–3] Risk factors for *S. maltophilia* colonization and infection include mechanical ventilation, prolonged hospitalization, and the use of equipment in contact with the respiratory tract, such as nebulizers.[1–3,7] Treatment of *S. maltophilia* infections is problematic because of the multiple antibiotic resistance exhibited by this organism.[6,8,9]

In our hospital, an increase in the number of cases of *S. maltophilia* has been observed over the past few years, with an unusual accumulation of isolates in our adult intensive care unit (ICU) during 1999 and 2000. The purpose of the present study was to investigate the molecular epidemiology of *S. maltophilia* in different wards of our university hospital and to determine whether this increase was due to spread of epidemic strains.

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Materials and methods

Patients

Between January 1999 and December 2000, 61 clinical isolates of *S. maltophilia* were collected as part of routine diagnostic microbiology services from 43 patients in the teaching hospital of University 'Federico II' (approximately 1500 beds) in Napoli, Italy. Patients' charts were reviewed to look for clusters of *S. maltophilia* acquisition associated with a hospital unit, previous antimicrobial therapy, and underlying disease. Cases of *S. maltophilia* infection were classified as hospital acquired if they were not present at the time of admission and developed 72 h after admission to the hospital or if they developed at the incision site within 30 days of a surgical procedure. Patients were also categorized as colonized or infected using the Centers for Diseases Control and Prevention (CDC) definitions for hospital-acquired infections.[10]

Isolation of clinical specimens

S. maltophilia strains isolated from clinical specimens by standard methods were collected, and stored at −80°C in glycerol for susequent typing. Isolates were identified as *S. maltophilia* spp. by using the VITEK system (bioMérieux, Morey-l'Etoile, France).

Molecular typing by pulsed-field gel electrophoresis (PFGE)

PFGE profiles were evaluated as described previously.[3] Interpretation of chromosomal DNA restriction patterns was based on the criteria of Tenover *et al.*[11] Briefly, strains showing more than three fragment variations were assumed to represent major PFGE patterns, while one to three fragment differences were considered to represent PFGE pattern subtypes.

Antimicrobial susceptibilities

Antimicrobial resistance was determined by the disk diffusion method, according to the National committee for Clinical Laboratory Standards document M7-A4.[12] Isolates showing an intermediate level of susceptibility were classified as resistant.

Results

Between January 1999 and December 2000, 61 clinical isolates of *S. maltophilia* were collected as part of routine diagnostic microbiology services from 43 patients in the teaching hospital of University 'Federico II' of Napoli. Features of clinical isolates from 43 patients colonized or infected with *S. maltophilia* from different wards are shown in Table I. Patients were classified as either infected or colonized on the basis of the evaluation of the clinical chart. The majority of specimens were isolated from patients in the adult ICU (19 of 43) or CF patients (13 of 43). Twenty-five *S. maltophilia* strains were isolated between November 1999 and July 2000, and were responsible for 14 respiratory infections (12 from ICU). The most frequent site of isolation was the respiratory tract (41 of 43), with the upper and the lower respiratory tract involved in 19 and 22 cases, respectively. The lower respiratory tract was the only site of isolation for ICU patients and was always associated with clinical infection. All 19 patients in ICU underwent mechanical ventilation. Specimens from the upper respiratory tract, either pharyngeal swabs or expectorations, were more frequently isolated from CF and haematological patients and were associated with infection in seven of 12 and in five of seven cases, respectively. Other infrequent sources were wounds from surgical patients (two of four).

To determine whether the increase of *S. maltophilia* isolation in our hospital was due to spread of epidemic or endemic strains, all *S. maltophilia* isolates were genotyped with *Xba*I digestion and PFGE.

Table I *Features of clinical isolates from 43 patients colonized or infected with* S. maltophilia *from different wards*

	Wards									
	S		H		ICU		CFP		Total	
Site of isolation	C	I	C	I	C	I	C	I	C	I
Upper respiratory tract			2	5			5	7	7	12
Lower respiratory tract		2				19		1		22
Wound		2								2

S, surgery; H, haematology; ICU, adult intensive care unit; CFP, cystic fibrosis patients; C, colonized patients; I, infected patients.

Genotypic analysis of all clinical isolates identified 31 distinct PFGE patterns, that differed in migration of at least four DNA fragments. Among the distinct PFGE clones 25 *S. maltophilia* strains isolated from 19 patients in the ICU were resolved into seven distinct PFGE patterns which we named from A to G. Of these seven, two patterns predominated, pattern A (12 strains) and pattern B (five strains) isolated from nine and four different patients, respectively (Figure 1). Interestingly, these two PFGE patterns occurred in very well-defined temporal clusters, PFGE pattern A being isolated between November 1999 and April 2000, and PFGE pattern B between January and February 2000. The 24 *S. maltophilia* strains isolated from different patients in the other wards of the hospital showed distinct PFGE patterns, no one having a PFGE pattern identical to those observed in the ICU (Figure 1). Multiple isolates from the same patient always showed identical PFGE patterns.

It has previously been shown that *S. maltophilia* infections can be selected because of the broad antibiotic resistance exhibited by this organism.[6,8,9] We therefore considered whether the spread of epidemic clones of *S. maltophilia* in our ICU would have been favoured by a particular multi-resistant phenotype. To addess this issue, we analysed the antibiotype of *S. maltophilia* strains isolated in different wards of the hospital. Susceptibilities of 14 antimicrobials against 31 *S. maltophilia* strains with different PFGE profiles is shown in Table II. As expected, all strains were resistant to imipenem and most were resistant or of intermediate susceptibility to piperacillin, ticarcillin, ceftriaxone and ceftazidime. Clavulanate reverted ticarcillin resistance in 65% of the total number of strains, but only in 57 and 46% of strains isolated in ICU and CF patients, respectively. Ciprofloxacin and ofloxacin fluoroquinolones were the most active agents, with 32 and 22% of resistant strains isolated in all different wards, respectively. All strains were resistant to tetracycline and up to 60% to gentamicin, tobramycin and amikacin, with highest percentages of resistant strains isolated in CF patients. Resistance of *S. maltophilia* strains to trimethoprim–sulfamethoxazole varied on different wards. The strains from surgery and ICU wards tested to be intermediate and those from haematology and CF patients tested to be resistant. We then analysed the association between antibiotype and genotype for the seven PFGE patterns identified from the 25 *S. maltophilia* ICU strains. Antibiotypes corresponding to different PFGE patterns showed a multi-resistant phenotype, resistant to beta-lactams and of variable resistance to ticarcillin-clavulanate, chloramphenicol and trimethoprim–sulfamethoxazole (Table II). Also, five of seven

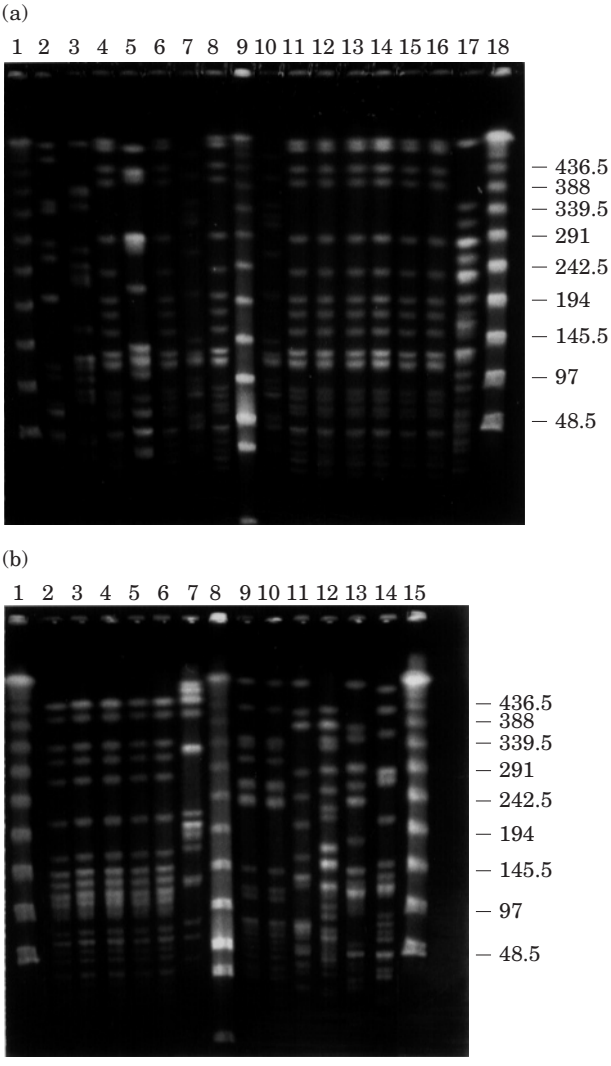

Figure 1 PFGE profiles of *S. maltophilia* strains isolated from different patients. (A) Lanes: 2 and 17, clinical strains from different CF patients; 3, 4, 6–8, 10–16, clinical strains from different patients in ICU (strains in lanes 4, 6, 8, 11–16, PFGE pattern A); 4 and 5, clinical strains from patients in Haematology; 1 and 18, multimers of phage lambda DNA (48.5 kb) molecular mass markers; 9, low-range DNA molecular mass markers. (B) Lanes: lanes 2–6 (PFGE pattern B) and 13, clinical strains from patients in ICU (strains in lane 5 and 6 are two different clinical isolates from the same patient); lanes 7 and 14, clinical strains from different CF patients; lanes 9–11, clinical strains from patients in Haematology (strains in lane 9 and 10 are two different clinical isolates from the same patient); lane 12, clinical strain from patient in Surgery; 1, and 15, multimers of phage lambda DNA (48.5 kb) molecular mass markers; 8, low-range DNA molecular mass markers. Sizes of lambda DNA molecular mass markers are indicated on the right of each panel.

Table II *Susceptibilities of 14 antimicrobials against 31* S. maltophilia *strains with different PFGE profiles*

	Percentage of resistant strains in different wards				
Antimicrobial agent	S	H	ICU	CFP	Total
Piperacillin	75	85.7	100	92.3	90.3
Ticarcillin	75	71.4	100	100	90.3
Ticarcillin–clavulanate	33.3	14.2	42.8	53.8	35.4
Ceftriaxone	100	85.7	100	100	96.7
Ceftazidime	33.3	57.1	100	92.3	77.4
Imipenem	100	100	100	100	100
Gentamicin	50	42.8	42.8	69.2	54.8
Tobramycin	75	57.1	57.1	69.2	64.5
Amikacin	25	57.1	42.8	46	45.1
Tetracycline	100	100	100	100	100
Ciprofloxacin	50	42.8	28.5	23	32.2
Ofloxacin	50	42.8	14.2	15.3	22.5
Chloramphenicol	50	42.8	28.5	30.7	35.4
TMP–SMX	25	85.7	57.1	92.3	80.6

S, surgery; H, haematology; ICU, adult intensive care unit; CFP, cystic fibrosis patients; TMP–SMX, trimethoprim–sulfamethoxazole.

and six of seven PFGE clones were susceptible to ciprofloxacin and ofloxacin, respectively. Interestingly, PFGE clones A and B responsible for the two independent outbreaks in the ICU were both resistant to gentamicin, tobramycin and amikacin, while four of five other PFGE clones were not. Also, PFGE clone A was resistant to both fluoroquinolones tested. All *S. maltophilia* strains of identical PFGE profile showed the same antibiotype (data not shown). Thus, at least for ICU *S. maltophilia* isolates, phenotypic analysis of antimicrobial susceptibilities gave identical results to those obtained with PFGE genomic typing.

Discussion

S. maltophilia is an increasingly important hospital-acquired organism. Colonization and infection are particularly likely to occur in patients with impaired host defense mechanisms who are taking broad-spectrum antimicrobial therapy.[1,6,8,9]

In the present study, we describe the occurrence of *S. maltophilia* infections during a two-year period in a 1500-bed university hospital. The majority of clinical specimens were from ICU or CF patients, whilst a few strains were isolated from haematology and surgery wards. The respiratory tract has been identified as the most common site of *S. maltophilia* isolation for hospitalized patients, accounting for 56 to 69% of all isolates.[1–3,7] Accordingly, we found that the respiratory tract was the site of *S. maltophilia* isolation in 95% of our patients. This organism was isolated from the lower respiratory tract in all ICU patients and was always associated with clinical infection. The upper respiratory tract represented the most frequent site of isolation in CF and haematological patients and was associated with infection in 58 and in 71% of cases, respectively. It has previously been reported that the majority of patients (53 to 71%) with *S. maltophilia*-positive respiratory tract cultures are colonized rather than infected,[1–3] although the rate of infection is higher in ICU patients than others (64 versus 32%, respectively).[2]

Additional epidemiological information was provided by the molecular typing of our *S. maltophilia* strains. Analysis of the macrorestriction pattern of genomic DNA by PFGE demonstrated that different *S. maltophilia* clones infected or colonized CF, haematological and surgical patients. On the contrary, two distinct *S. maltophilia* clones were isolated from nine and four patients of the ICU, respectively, allowing us to define two distinct outbreaks over a six-month period in this area. The ICU strains were not isolated from patients on other wards. Because all 19 patients in our ICU underwent mechanical ventilation, it is likely that this is a risk factor for the acquisition of this organism.

The majority of strains of *S. maltophilia* are characterized by their resistance to many currently available antibiotics, notably the carbapenem class.[3,6–9,13,14] All our strains were resistant to imipenem and most were resistant or of intermediate susceptibility to beta-lactams. All CF patients and

nine of 16 patients from ICU were treated with carbapenems before *S. maltophilia* isolation. In keeping with other work,[8] the majority of *S. maltophilia* strains isolated from patients outside ICU were susceptible to fluoroquinolones.

The analysis of the antibiotype of *S. maltophilia* clones from ICU indicated greater resistance then elsewhere, including fluoroquinolones and trimethoprim–sulfamethoxazole and aminoglycosides. It may be that equipment such as nebulizers,[7] in contact with aerosolized aminoglycosides,[14] is a risk factor for *S. maltophilia* colonization and infection.

In conclusion, the present study has analysed the molecular epidemiology of *S. maltophilia* in different wards of a university hospital, confirming several characteristics of *S. maltophilia* hospital-acquired infection and identifying other novel and interesting aspects. *S. maltophilia* infection tends to occur in immunocompromised or debilitated patients in different wards of the hospital, but patient to patient cross-infection is confined to the ICU. Mechanical ventilation and broad-spectrum antibiotics facilitate the dissemination of highly resistant epidemic strains. Molecular typing of clinical isolates and analysis of their antimicrobial susceptibilities are important procedures to reduce acquired infections and control outbreaks by *S. maltophilia* in ICU patients.

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