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DNA Restriction Fragment Length Polymorphism Differentiates Crossed from Independent Infections in Nosocomial *Xanthomonas maltophilia* Bacteremia

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Restriction fragment length polymorphisms of total DNA and rDNA were used to study the relationship between 11 isolates of *Xanthomonas maltophilia*, obtained from seven patients with nosocomial bacteremia in four distinct wards of a single hospital, and the type strain of the species, ATCC 13637. Our results indicated that there were episodes of cross-infection among the patients of two wards, but there were also independent infectious episodes in the two other wards.

Xanthomonas maltophilia, formerly named *Pseudomonas maltophilia* (17), is a nonfermentative, gram-negative rod that is infrequently found in clinical microbiology laboratories. This organism has been recognized as an opportunistic pathogen that affects patients compromised by debilitating illness, surgical procedures, or indwelling catheters (3, 4, 7, 9, 20). The nosocomial spread of outbreak strains of *X. maltophilia* has been suggested (4, 9, 14, 18). However, the lack of any adequate typing systems to establish strain relatedness has always been an obstacle to addressing such an epidemiological issue. Indeed, *X. maltophilia* strains are biochemically closely related to each other (10), and all strains present with a similar pattern of a high level of resistance to the currently available antibiotics (20). A serological classification of *X. maltophilia* based on heat-stable O-antigen typing has been used with some success, but it requires access to specific reagents of restricted availability (13). Furthermore, it has been shown, in the case of *Pseudomonas aeruginosa*, that genetically indistinguishable strains may present with different serotypes (11). Actually, genotypic analysis by restriction fragment length polymorphism (RFLP) has been proposed for other species to circumvent the problems of isolate identification solely on the basis of phenotypic characteristics (2, 5, 6, 8, 12, 16).

Between September 1989 and October 1990, we isolated *X. maltophilia* in nine episodes of nosocomial bacteremia in seven children on four distinct wards in a 500-bed pediatric hospital (Hôpital Robert Debré, Paris, France). To determine the relationship between 11 strains isolated from these patients, we studied the RFLPs of total DNA, after ethidium bromide staining, and rDNA regions (ribotyping).

MATERIALS AND METHODS

Patients. All patients had nosocomial bacteremia associated with the presence of a central venous catheter. Recognition of the catheter-related bacteremia was ascertained by simultaneous quantitative cultures of central and peripheral venous blood samples. The finding of a significantly larger concentration of bacteria in blood obtained via the catheter than in blood collected from a peripheral vein and the presence of no other focus of infection was indicative of catheter-related bacteremia. Of the seven infected patients (ages, 2 to 20 years), two suffered from hematologic malignancy, three from primary intestinal illness (chronic enteritis in two patients, small bowel fistula in one patient), one from multiple trauma, and one from anaerobic appendicular peritonitis. One patient had three bacteremic episodes over a 3-month period. The second and third episodes occurred 49 and 79 days after the initial bacteremia, respectively.

Bacterial strains. Eleven *X. maltophilia* clinical isolates and the type strain of the species ATCC 13637 were studied (Table 1). In two patients, we compared isolates recovered from blood that was simultaneously drawn via the catheter and from a peripheral vein. In addition, five epidemiologically unrelated clinical strains from various regions of France were also studied for comparison. Clinical isolates were identified by the analytical profile index procedure API NE (API, La Balme les Grottes, France). Susceptibilities of the organisms to antimicrobial agents were studied by the disk diffusion method (1).

Genotyping techniques. Bacterial DNA was prepared as described elsewhere (12). DNA (1 to 5 μg) was digested with *Bam*HI and *Bcl*I restriction endonucleases (Boehringer, Mannheim, Germany) according to the specifications of the manufacturer and analyzed by submarine electrophoresis on 0.8% agarose gels containing ethidium bromide. Bacteriophage lambda DNA, which was digested with *Eco*RI and *Hin*dIII, was used as a size marker. Size-separated DNA restriction fragments were transferred to a nylon membrane (Gene Screen Plus; New England Nuclear, Boston, Mass.) by the method of Southern (15). The 16S-23S rRNA from *Escherichia coli* (Boehringer) was labeled by random oligopriming by using a mixture of hexanucleotides (Pharmacia, Uppsala, Sweden) and cloned reverse transcriptase from Moloney murine leukemia virus (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in the presence of [^{32}P] dCTP (2′-deoxycytidine-5′-triphosphate; 800 Ci/mol; Amersham International, Amersham, United Kingdom) (12). Hybridization, washing, and autoradiographic procedures were performed as described previously (12).

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TABLE 1. Origins and phenotypic and genotypic characteristics of the 11 *X. maltophilia* epidemic strains and the type strain of the species

Patient	Ward	Strain	Blood origin	Date of isolation (mo/day/yr)	API profile	Chloramphenicol susceptibility[a]	Total DNA RFLP pattern type	rDNA RFLP pattern		Ribotype
								*Bam*HI	*Bcl*I	
		ATCC 13637				S	I	a	a	A
N	Gastroenterology	415	Catheter	2/1/90	1452341	S	II	d	c	B
N	Gastroenterology	426	Peripheral	2/1/90	1452341	S	II	d	c	B
D	Gastroenterology	552	Catheter	7/21/90	1452341	S	II	d	c	B
D	Gastroenterology	587	Catheter	9/8/90	1452341	S	II	d	c	B
D	Gastroenterology	622	Catheter	10/7/90	1452341	S	II	d	c	B
A	Gastroenterology	583	Peripheral	9/5/90	1452341	S	II	d	c	B
A	Gastroenterology	586	Catheter	9/5/90	1452341	S	II	d	c	B
B	Intensive care unit	284	Catheter	7/22/90	1452341	S	II	d	c	B
E	Surgery	310	Catheter	9/4/89	1452341	R	III	b	b	C
Z	Hematology	317	Catheter	9/25/89	1452341	R	IV	c	a	D
C	Hematology	694	Catheter	10/11/90	1452341	S	V	a	c	E

[a] S, susceptible; R, resistant.

RESULTS AND DISCUSSION

The biochemical characteristics of all the clinical isolates correlated well with those of the reference strain studied under the same conditions. A single biochemical pattern was observed for all strains (API profile, 1452341) (Table 1). All isolates were susceptible to moxalactam, trimethoprim-sulfamethoxazole, and ticarcillin-clavulanic acid and were resistant to cefotaxime, ceftazidime, and imipenem. Epidemiologically related strains, however, could be separated into two groups on the basis of their susceptibility or resistance to chloramphenicol. Susceptibility was observed for all seven isolates from the gastroenterology ward, one isolate from the intensive care unit, and one isolate from the hematology ward. Resistance was limited to one strain from the surgery ward and one strain from the hematology ward (Table 1).

Within the 11 epidemic isolates and the type strain of the species, RFLP analysis of total DNA by ethidium bromide staining revealed five different patterns (patterns I to V) for each of the two restriction endonucleases that were used (Table 1). Figure 1 shows four of these patterns (lanes 1, 2, 3, and 4 through 10). Only strains isolated from the gastroenterology and intensive care units exhibited indistinguishable patterns (pattern II, lanes 4 through 10). This technique, however, is of limited application because the large number of bands and the relatively poor resolution render comparison of multiple strains difficult. RFLP analysis of the rDNA regions has recently been applied with success to the genotyping of other bacterial species (2, 5, 12, 16). One advantage to this approach is that because rDNA sequences are highly conserved in the eubacterial kingdom (19), a unique hybridization probe, i.e., *E. coli* rRNA, can be used for any bacterial species.

*Bcl*I and *Bam*HI gave three and four different rDNA RFLP patterns, respectively, for the 11 epidemic strains and the type strain of the species (Fig. 2). Each distinct combination of patterns was used to define a ribotype. For example, ribotype A generated by the type strain of the species, ATCC 13637, is defined by the combination of pattern a with *Bam*HI and pattern a with *Bcl*I. Five distinct ribotypes (A to E) were identified by using these two enzymes (Table 1). Digestion with a third enzyme (*Eco*RI) did not provide any additional information (data not shown).

In our study, RFLP analysis of total DNA directly correlated with ribotyping, in that the total number of DNA RFLP patterns was equivalent to that of ribotype patterns for all strains studied.

On the basis of phenotypic characteristics only, our conclusion would have been that the occurrence of *X. maltophilia* bacteremia is the result of the spread of two epidemiological strains in the whole hospital. However, our genotypic results showed that patients from the gastroenterology and intensive care units harbored only genotypically related strains, indeed suggesting nosocomial transmission of *X. maltophilia* and cross-infection between and within those two wards. Those isolates, on the contrary, were genotypically unrelated to the bacterial strains found in the hematology and surgery wards. Furthermore, the three strains isolated in these two latter wards were not related to each other, which excludes the possibility of nosocomial transmission. Furthermore, either by ethidium bromide staining or rDNA probing, all five unrelated, nonepidemic control isolates gave specific patterns, each one different from that of the nosocomial strains, thereby confirming the uniqueness of these strains (data not shown).

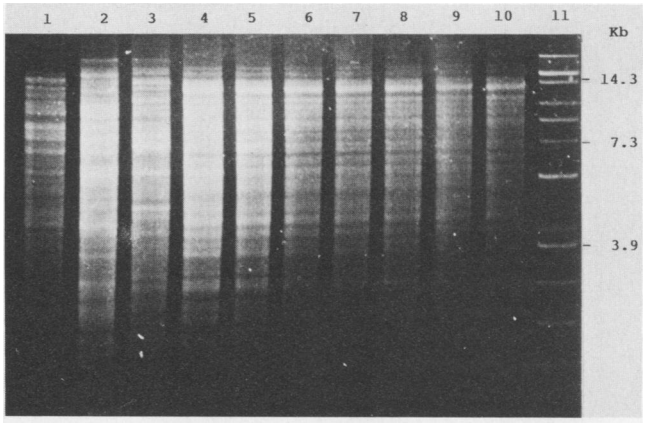

FIG. 1. *X. maltophilia* RFLP patterns of total DNA obtained after *Bcl*I digestion and ethidium bromide staining. Lane 1, ATCC 13637 (pattern I); lane 2, 310 (pattern III); lane 3, 317 (pattern IV); lane 4, 284 (pattern II); lane 5, 552 (pattern II); lane 6, 587 (pattern II); lane 7, 586 (pattern II); lane 8, 583 (pattern II); lane 9, 426 (pattern II); lane 10, 415 (pattern II); lane 11, size marker (see Table 1 for origins of the strains).

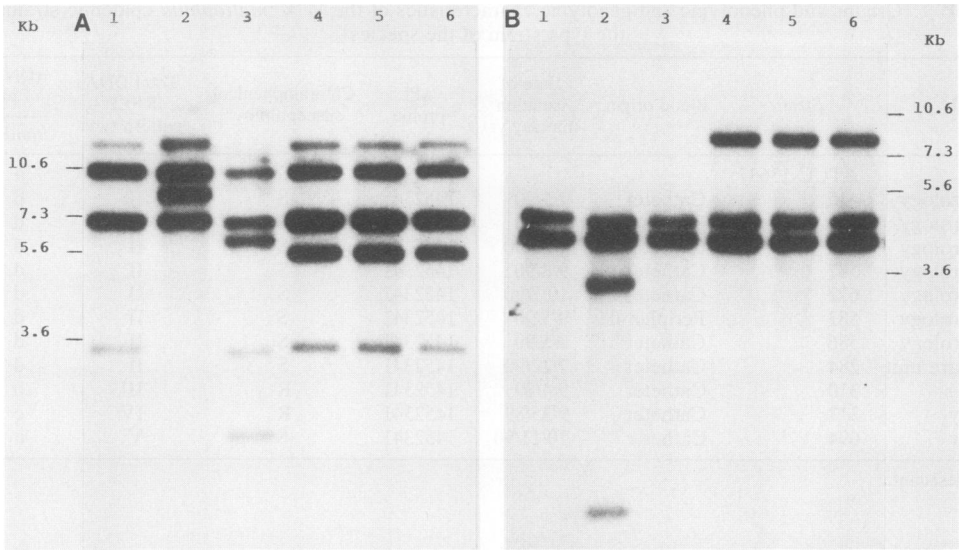

FIG. 2. *X. maltophilia* RFLP patterns of the rDNA regions. (A) *Bam*HI digestion. Lane 1, ATCC 13637 (pattern a); lane 2, 310 (pattern b); lane 3, 317 (pattern c); lane 4, 284 (pattern d); lane 5, 415 (pattern d); lane 6, 587 (pattern d). (B) *Bcl*I digestion. Lane 1, ATCC 13637 (pattern a); lane 2, 310 (pattern b); lane 3, 317 (pattern a); lane 4, 284 (pattern c); lane 5, 415 (pattern c); lane 6, 587 (pattern c) (see Table 1 for origins of the stains).

No potential environmental sources of infection have yet been identified that might provide further insights concerning the precise modes and risks of nosocomial *X. maltophilia* transmission.

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