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## Brief reports

# Pulsed-field gel electrophoresis of restriction-digested genomic DNA and antimicrobial susceptibility of *Xanthomonas maltophilia* strains from Brazil, Switzerland and the USA

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### Introduction

*Xanthomonas maltophilia* is a nonfermentative Gram-negative bacillus that is infrequently isolated in clinical microbiology laboratories. This organism is an opportunistic pathogen that principally affects patients compromised by debilitating illness, surgical procedures, or indwelling catheters (Elting *et al.*, 1990). The incidence of clinical isolation of *X. maltophilia* is increasing, possibly in part because of the selective pressure from some new antimicrobial agents (Marshall *et al.*, 1989). Strains of *X. maltophilia* are usually resistant to aminoglycosides and most β-lactams, show variable susceptibility to fluoroquinolones and are generally susceptible to trimethoprim/sulphamethoxazole (Neu, Saha & Chin, 1989; Lecso-Bornet *et al.*, 1992). Low outer membrane permeability and constitutive overproduction of β-lactamase appears to be responsible for the generally high degree of antibiotic resistance (Mett *et al.*, 1988).

A reliable typing system would help in the better understanding of the epidemiology of *X. maltophilia* infections. In addition, the nosocomial spread of outbreak isolates has been suggested, but the lack of a satisfactory typing method is an important obstacle to addressing such an epidemiological issue (Khardori *et al.*, 1990). The purpose of the present study was to determine the in-vitro susceptibility patterns to some new fluoroquinolones and β-lactams of 30 strains of *X. maltophilia* collected from three countries and evaluate the potential of restriction fragment length polymorphisms (RFLP) of chromosomal DNA (chrDNA) for epidemiological typing.

### Materials and methods

We tested 30 clinical isolates from three countries: 11 from the USA (University of Iowa Hospitals and Clinics, Iowa City, IA), eight from Brazil (Escola Paulista de Medicina, Sao Paulo) and 11 from Switzerland (Kantonsspital Basel University Clinics, Basel). With the exception of four Brazilian isolates (numbers 14 to 17) which were collected from an outbreak in a dialysis unit, all other isolates were epidemiologically



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unrelated. All isolates were identified by conventional tests for motility, oxidase, dihydrolase, and by AP120E system (Analytab Products, Plainview, USA).

MICs were determined by broth microdilution using cation-adjusted Mueller-Hinton broth as described by the National Committee for Clinical Laboratory Standards (1990). The agents tested were: ciprofloxacin (Miles Pharmaceuticals, West Haven, USA); levofloxacin, ofloxacin and FK-037 (Neu, Chin & Huang, 1993) (R. W. Johnson Research Institute, Raritan, USA); OPC-17116 (Sader, Erwin & Jones, 1992) (Otsuka America Pharmaceuticals, Rockville, USA); cefepime (Bristol-Myers Squibb Co., Syracuse, USA); ceftazidime (Glaxo Laboratories, Research Triangle Park, USA); biapenem (Lederle Laboratories, Pear River, USA); imipenem (MSD Research Laboratories, West Point, USA); meropenem (ICI Pharmaceuticals, Wilmington, USA).

RFLP of chrDNA was determined using the procedure previously used for *Pseudomonas aeruginosa* (Sader *et al.*, 1993). After growing the cells in trypticase soy broth to the exponential phase, they were pelleted by centrifugation, washed and mixed with 2% agarose (SeaPlaque GTG, FMC, Rockland, USA) to a final 1% agarose concentration. The plugs were then incubated in 100 mM EDTA-1M NaCl-6 mM Tris, pH 7·5 overnight at 37°C and then equilibrated with 100 mM Tris-100 mM EDTA, pH 7·5 (TE). The plugs were then incubated in 1% sarkosyl-0·4 M EDTA, pH 9·3, overnight at 50°C, equilibrated with TE and stored at 5°C until used. Restriction digestion of genomic DNA was performed with either *Xba*I or *Spe*I (New England Biolabs, Beverly, USA). Plugs containing digested DNA were loaded into 1% agarose gel and run in 50 mM Tris, 100 mM EDTA, 50 mM borate buffer. Restriction fragments were separated by pulsed-field gel electrophoresis using a CHEF-DR II System (Bio-Rad Laboratories, Richmond, USA). Pulse time was ramped from 10 to 90 sec for 24 h.

## Results and discussion

All isolates were susceptible to trimethoprin/sulphamethoxazole (data not shown). The Table summarizes the in-vitro activity of the four fluoroquinolones and six β-lactams

**Table.** In-vitro activity of broad-spectrum β-lactams and fluoroquinolones against 30 *X. maltophilia* isolates

| Antimicrobial agents | MIC (mg/L) range | $MIC_{50}$ | $MIC_{90}$ | Percent susceptible (breakpoint[a] (mg/L)) |
|---|---|---|---|---|
| OPC-17116 | 0·06 – > 4 | 0·5 | 4 | 83 ( ⩽ 2) |
| Levofloxacin | 0·25 – > 4 | 2 | 4 | 63 ( ⩽ 2) |
| Ofloxacin | 1 – > 4 | 4 | > 4 | 43 ( ⩽ 2) |
| Ciprofloxacin | 1 – > 2 | > 2 | > 2 | 3 ( ⩽ 1) |
| Ceftazidime | 1 – > 16 | 16 | > 16 | 37 ( ⩽ 8) |
| Cefepime | 2 – > 16 | > 16 | > 16 | 30 ( ⩽ 8) |
| FK 037 | 4 – > 16 | > 16 | > 16 | 7 ( ⩽ 8) |
| Meropenem | 2 – > 8 | > 8 | > 8 | 3 ( ⩽ 4) |
| Biapenem | > 8 | > 8 | > 8 | 0 ( ⩽ 4) |
| Imipenem | > 8 | > 8 | > 8 | 0 ( ⩽ 4) |

[a]The breakpoints for ciprofloxacin, ofloxacin, ceftazidime and imipenem were according to NCCLS (1990) recommendations. The breakpoints for the newer agents were based on breakpoints established for compounds with similar chemical and pharmacokinetic characteristics.

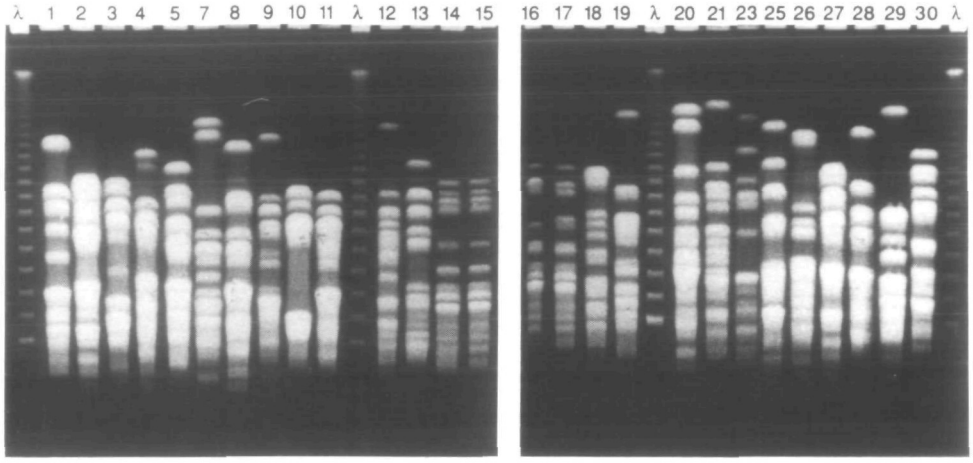


**Figure.** Restriction fragment length polymorphism analysis of chromosomal DNA after digestion with *Xba*I. Epidemiologically unrelated strains of *X. maltophilia* show a great variety of patterns. Four Brazilian strains isolated from an outbreak (strains 14 to 17) share a similar pattern, two of them being identical (strains 14 and 15)

tested against the 30 *X. maltophilia* isolates. This genomically diverse sample from three geographically distinct areas provided a broad view of the antimicrobial susceptibility of the species. OPC-17116 was the most active new agent tested, with 25 isolates having an MIC $\leq$ 2 mg/L. However, the activity of the fluoroquinolones varied, with only one isolate having a ciprofloxacin MIC $<$ 2 mg/L. Among currently marketed drugs, ofloxacin was the most active. The four Brazilian isolates from the outbreak shared a very similar antimicrobial susceptibility pattern within one $\log_2$ dilution for all compounds except levofloxacin (data not shown).

Our results agreed with other studies (Mett *et al.*, 1988; Neu *et al.*, 1989) that demonstrated the poor activity of the broad-spectrum β-lactams against *X. maltophilia*. Among the β-lactams tested, ceftazidime was the most active compound; however, only 11 of 30 isolates were susceptible (MIC $\leq$ 8 mg/L). The carbapenems also demonstrated poor activity with all but one isolate being resistant to the three compounds tested. Because carbapenems have a broad spectrum of activity, colonization or superinfection with resistant bacterial species is usually rare. However, exposure to imipenem has been demonstrated to be a risk factor for acquisition of *X. maltophilia* infection (Elting *et al.*, 1990). Our results suggest that biapenem and meropenem may also present a similar risk factor for *X. maltophilia* superinfection.

The Figure shows the RFLP patterns demonstrated by the *X. maltophilia* isolates after digestion with *Xba*I. The epidemiologically unrelated isolates from three different countries demonstrated a remarkable variety of patterns. In contrast the four Brazilian isolates from a single outbreak presented a similar pattern, with two of them being identical (isolates 14 and 15). Digestion with *Spe*I produced essentially the same results (data not shown). The RFLP of chrDNA thus shows potential as a method for epidemiological *X. maltophilia* typing. This technique has produced excellent results for epidemiological typing of other species (Sader *et al.*, 1993). The reproducibility appears to be very high, since the number and locations of the restriction sites depend only on the genomic nucleotide sequence. The sensitivity and specificity can be adjusted by

varying the enzyme used for cleavage. The use of low-frequency-cleavage enzymes gives a small number of bands, allowing a more precise comparison of electrophoretic patterns, but reducing the ability to demonstrate small genomic differences between isolates. On the other hand, high-frequency-cleavage endonuclease increases the number of bands, but the electrophoretic patterns can become too complex for routine analysis. The pattern of the bands can also be adjusted by varying the instruments switch time. Larger DNA molecules take more time to realign after the fields are switched than do the smaller ones. Consequently, short switch times affect the migration of larger molecules more than they affect smaller ones, increasing the distance between them. This helps arrange the pattern in a more suitable way for comparison.

In conclusion, our results show the potential of RFLP for epidemiological typing *X. maltophilia* species and illustrate the poor activity of the new broad-spectrum β-lactams against this species.

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