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Epidemiological analysis of carbapenem-sensitive and -resistant *Pseudomonas aeruginosa*

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Summary *Pseudomonas aeruginosa* with decreased levels of meropenem susceptibility were identified in the Royal Infirmary Edinburgh in 2002. Within the affected group of patients, none had meropenem-resistant *P. aeruginosa* when they arrived in the intensive care unit (ICU). Seven isolates from the ICU were collected five months after the decreased susceptibility to meropenem was identified. In order to investigate if resistance was a problem in *P. aeruginosa* throughout Edinburgh, both in hospital- and community-acquired isolates, a prospective study was performed. The susceptibilities of 104 *P. aeruginosa* to imipenem, meropenem, ceftazidime, piperacillin/tazobactam and ciprofloxacin were investigated. Meropenem had the highest activity against these isolates and the lowest MIC_{90} (2 mg/L), followed by imipenem (4 mg/L), ciprofloxacin (8 mg/L), piperacillin/tazobactam (16 mg/L) and ceftazidime (32 mg/L). These isolates were also analysed genotypically by pulsed-field gel electrophoresis. Five of the seven ICU isolates were identified, one isolate was 98% similar and the other was 85% similar to the ICU isolates. One isolate from the prospective study had approximately 90% genotype similarity to the six ICU isolates with $\geq$98% similarity. There was no clonality within the strains from the prospective study and clusters with $>$90% similarity comprised at five or less isolates. Isolates with the same resistance patterns did not necessarily have the same genotypic profile. Strains isolated from different patients on the same day were also not necessarily related. The conclusions of this study were that while the seven ICU isolates were clonal or highly related, they were not widespread throughout Edinburgh and the *P. aeruginosa* within Edinburgh were highly varied.

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Table I Susceptibility profiles of the outbreak isolates

Strain ID	Imipenem MIC (mg/L)	Meropenem MIC (mg/L)	Ceftazidime MIC (mg/L)	Piperacillin/ tazobactam MIC (mg/L)	Ciprofloxacin MIC (mg/L)
O1	16 R	16 R	4	32 R	16 R
O2	8 R	4	32 R	8	8 R
O3	4	4	2	4	8 R
O4	2	0.5	4	4	0.12
O5	4	8 R	2	8	16 R
O6	0.5	0.25	2	4	8 R
O7	4	8 R	2	8	16 R

R, resistant; MIC, minimum inhibitory concentration.

Introduction

Pseudomonas aeruginosa is the second most frequently reported pathogen overall in intensive-care-unit (ICU)-acquired infections in Europe.[1] It is of particular concern for intubated and/or immunocompromised patients. The antimicrobial agents most commonly used to treat *P. aeruginosa* infections are meropenem, imipenem, ceftazadime, piperacillin/tazobactam and ciprofloxacin. However, resistance to imipenem is increasing; in recent years, it has increased to between 12% and 18%.[1] Resistance to other therapeutic agents varied from 9.8% for meropenem to 25% for ciprofloxacin.

There is an awareness of the ever-decreasing number of available therapeutic options. Therefore, it is important to understand the origins of resistance in order to minimize its transmission and spread. When an outbreak of resistant isolates occurs within a hospital, it is important to understand whether the outbreak is caused by a single clone or multiple isolates, and whether they have resistance to all available antimicrobial agents or specifically to one antimicrobial agent. There are various techniques available to analyse similarity or diversity of bacterial isolates. Pulsed-field gel electrophoresis (PFGE) is one such method, which is preferred to other typing methods by many laboratories for the epidemiological study of bacteria, such as *P. aeruginosa*.[2]

The aims of this study were firstly to identify whether resistant *P. aeruginosa* were widespread in hospitals and the community in Edinburgh. Secondly, as resistance in the ICU appeared within days, we decided to analyse the Edinburgh cohort genotypically to investigate whether there was a clonal relationship between *P. aeruginosa* with the same and different resistance patterns, i.e. to elucidate whether sensitive isolates were of the same genotype as resistant isolates, and to investigate whether there was a genotypic link between hospital- and community-acquired *P. aeruginosa*.

Materials and methods

Bacterial strains and susceptibility testing

Seven resistant *P. aeruginosa* isolates from the ICU with the initial meropenem resistance problem were collected in December and early January of 2002 and 2003, respectively. These isolates were identified as meropenem resistant by the Royal Infirmary Edinburgh. One hundred and four *P. aeruginosa* strains, 23 from community-acquired infections and 81 from hospital-acquired infections, identified by the diagnostic laboratories at the Royal Infirmary Edinburgh were collected between January and May 2003. The antimicrobial agents were obtained from their respective manufacturers and were stored and prepared according to the manufacturers' guidelines. The susceptibilities of the *P. aeruginosa* to imipenem, meropenem, ceftazidime, piperacillin/tazobactam and ciprofloxacin were determined by agar dilution in vitro, according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines.[3] The antimicrobial breakpoints were assigned at the following minimum inhibitory concentration (MIC) values according to the BSAC guidelines: imipenem $\geq$8 mg/L; meropenem $\geq$8 mg/L; ceftazidime $\geq$16 mg/L; piperacillin/tazobactam $\geq$32 mg/L; and ciprofloxacin $\geq$2 mg/L.[4]

PFGE analysis

All isolates were genotyped by PFGE (CHEF DR II system, BioRad, Hemel Hempstead, Hertfordshire, UK) following total bacterial DNA digestion with the

Table II Susceptibility profiles of the 104 prospective study isolates

Antimicrobial agents	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Imipenem	0.5–32	2	4
Meropenem	0.032–16	0.25	2
Ceftazidime	0.5–32	2	32
Piperacillin/ tazobactam	0.5–128	4	16
Ciprofloxacin	0.016–32	0.25	8

MIC, minimum inhibitory concentration.

endonuclease *Xba* I. The method of Cheng *et al.* was used with the following alteration: a suspension of a single colony of each isolate was made in buffer (4.38% NaCl, 9.3% $EDTANa_2$, 86.32% distilled water) and mixed with molten 1% low-melting-point preparative-grade agarose instead of overnight broth culture.[5] The macrorestriction patterns were compared by Bionumerics® software (Applied Maths, Sint-Martens-Latem, Belgium). The percent relatedness was calculated using the Dice coefficient, and the unweighted pair group method with arithmetic averages was used for cluster analysis to produce a dendogram with band optimization settings of 1.00% and a band tolerance position of 1.0–2.0%.

Results

Phenotypic characteristics

The susceptibility profiles of the seven outbreak isolates are shown in Table I.

The prospective study had the following rates of

Table III Clustered susceptibility profiles of the 104 prospective study isolates

Isolate	Imipenem MIC (mg/L)	Meropenem MIC (mg/L)	Ceftazidime MIC (mg/L)	Piperacillin/ tazobactam MIC (mg/L)	Ciprofloxacin MIC (mg/L)
PaFW 46	32 R	4	32 R	32 R	2 R
PaFW 10	2	0.25	32 R	64 R	8 R
PaFW 14	16 R	16 R	8	16	8 R
PaFW 50	4	0.5	32 R	32 R	4 R
PaFW 110	16 R	16 R	2	4	8 R
PaFW 3	2	1	32 R	4	32 R
PaFW 19	1	2	32 R	4	32 R
PaFW 21	2	0.5	32 R	32 R	0.12
PaFW 29	16 R	4	32 R	4	0.12
PaFW 65	1	0.12	32 R	128 R	1
PaFW 95	8 R	4	8	16	2 R
PaFW 7	1	2	32 R	8	1
PaFW 16	1	0.5	4	16	32 R
PaFW 23	0.5	0.25	2	4	8 R
PaFW 24	2	1	32 R	4	0.12
PaFW 25	2	2	32 R	16	0.5
PaFW 26	1	0.25	8	16	4 R
PaFW 34	1	0.5	32 R	8	0.25
PaFW 37	4	2	2	4	8 R
PaFW 59	0.5	0.032	1	0.5	2 R
PaFW 60	4	0.25	2	0.5	2 R
PaFW 61	2	0.5	8	4	2 R
PaFW 62	2	1	16 R	4	1
PaFW 63	2	0.25	4	8	8 R
PaFW 71	2	0.25	2	4	4 R
PaFW 80	4	0.12	4	8	8 R
PaFW 85	2	0.12	2	4	4 R
PaFW 93	32 R	4	1	2	0.12
PaFW 102	2	0.5	8	8	16 R
PaFW 108	4	0.25	16 R	16	0.25
PaFW 111	2	0.25	2	4	4 R

R, resistant; MIC, minimum inhibitory concentration.

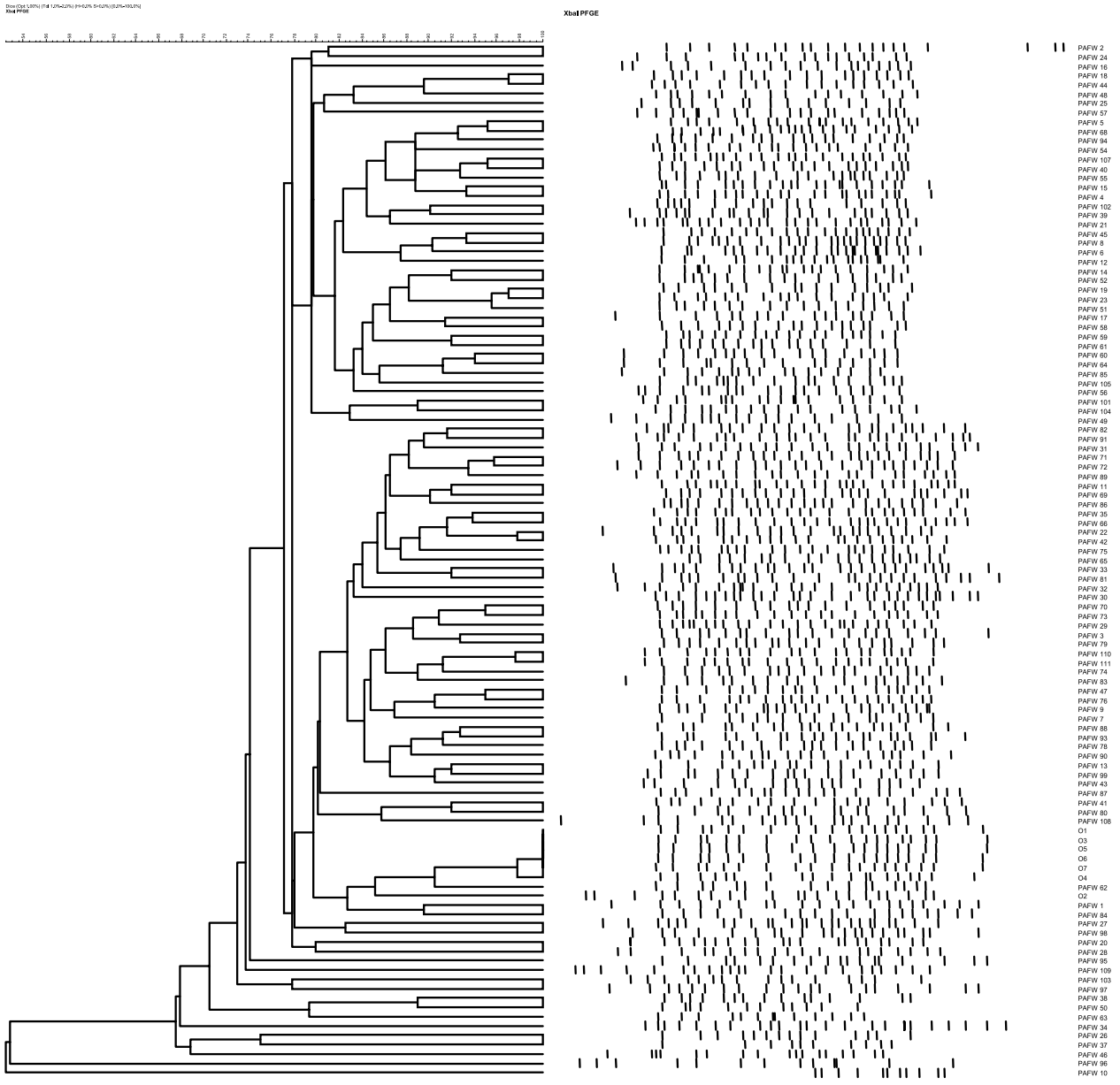

Figure 1 Cluster analysis dendogram of *Pseudomonas aeruginosa* isolates showing the percent similarities and resistance profiles of the isolates.

resistance: ciprofloxacin 20%; ceftazidime 13.5%; imipenem 5.8%; piperacillin/tazobactam 4.8%; and meropenem 1.9%. The MIC range, MIC_{50} and MIC_{90} of meropenem was the lowest of the five antimicrobial agents against the 104 isolates as indicated in Table II. The isolates were numbered in the order in which they were collected, i.e. PaFW 1 was the isolate collected first. The MICs for each cluster of isolates against the five antimicrobial agents are described in Table III.

PFGE analysis

PFGE analysis of all of the isolates showed rates of similarity from 52% to 100% on the dendogram using the BioNumerics software analysis (Figure 1). Most of the isolates had $>75\%$ similarity. Five of the seven initial isolates had 100% similarity, one isolate was 98% similar and the other was 85% similar to the other outbreak isolates. Isolate PaFW 62 had approximately 90% genotype similarity to the six outbreak isolates with similarities of $\geq 98\%$. These seven isolates formed the only major cluster of the isolates genotyped. No other cluster had comparable percent similarities. While most of the isolates had $>75\%$ similarity, clusters with $>90\%$ similarity contained at five or less isolates. Within

these small groups, the isolates were not necessarily isolated at the same time; PaFW 9, PaFW 47 and PaFW 76 had 90% similarity. Isolates with the same patterns of resistance were also not clustered together. There was no genotypic similarity pattern to suggest that resistant or susceptible isolates have spread from the community to the hospital or vice versa. There was also little similarity to the outbreak isolates; the sequence similarity of the 104 prospective study isolates was $<82\%$ similar to the outbreak isolates.

Discussion

This study provides the first published data on the genetic relatedness of hospital isolates of *P. aeruginosa* resistant to antibacterial agents and susceptible *P. aeruginosa* from different and the same sites of isolation in the UK. PFGE has been shown to be an excellent epidemiological tool for the discrimination of related and unrelated isolates of *P. aeruginosa*.[6,7] Tenover *et al.* defined categories of genetic and epidemiological relatedness of isolates using PFGE.[8] However, they suggested that these guidelines should be used to examine relatively small sets of isolates (typically ≤ 30) related to putative outbreaks of disease. Therefore, these guidelines were not used to analyse the 111 isolates of this study.

The results of this study have shown that only seven of the isolates were closely related, six of which were associated with the outbreak and one was a non-outbreak isolate. One of the outbreak isolates was genetically distinct from the others. This indicates that while the outbreak was most probably caused by one clone, at least one other type of strain/clone was also involved. These isolates also had varying resistance profiles. The reasons for this could be that either the resistance associated with the original clone was modified as the selective pressure of the antimicrobial agent was removed, or isolates with the same genetics had different resistance mechanisms. All the isolates had a level of relatedness associated as being part of the same species, but were not related to such an extent that they were clonal. Therefore, the outbreak did not persist and normal infection control measures have eradicated the problem.

No clonality existed within either the resistant or sensitive *P. aeruginosa* isolated. This study suggests that none of the genotypes present were predisposed to the acquisition of resistance genes, nor did one type persist more than the others. Thus, there has not been a specific type or clone that has established itself within Edinburgh. The conclusions of this study were that while the outbreak isolates were clonal or highly related, they did not persist or spread to other areas within Edinburgh, as indicated by the prospective study. The prospective study isolates were very heterogeneous and resistant clones were not selected from the strains within this environment.

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