ORIGINAL ARTICLE

Eradication of a resistant *Pseudomonas aeruginosa* strain after a cluster of infections in a hematology/oncology unit

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Objective This report chronicles an outbreak of a multiply resistant strain of *Pseudomonas aeruginosa* and the measures required to contain this outbreak.

Methods Laboratory-based ward-liaison surveillance allowed the detection of a multiply resistant strain of *P. aeruginosa* infecting patients in our hematology/oncology unit. Sampling of the immediate environment was carried out. Pulsed field gel electrophoresis was used to compare the patients' organisms with those found in the environment. Extensive dismantling of the drainage system, repeated cleaning and disinfection, and a review of the departmental antibiotic policy were some of the infection control measures instigated.

Results During a period of 11 months, three patients in the hematology department and two patients in the oncology department were infected with multiply resistant *P. aeruginosa*. There were two cases of pneumonia, one of which was fatal, and two cases of neutropenic septicaemia. Pulsed field gel electrophoresis performed on the isolates showed that the isolates from geographically separate areas could be divided into two strains that were closely related but distinct. Two genotypically identical strains were also isolated from the plumbing systems in the areas of each ward where patients had been treated.

Conclusion The potential for serious nosocomial infections with *P. aeruginosa* is well recognized. Eradication of the organism from the environment may require the co-ordinated efforts of clinicians, nurses, pharmacy and hospital engineers, working in collaboration with the hospital infection control team. To date, the same strains have not been isolated despite repeated surveillance over the past 18 months and therefore these measures have, in our opinion, successfully removed the potential for nosocomial infection with this resistant organism in our hospital.

Keywords *Pseudomonas aeruginosa*, multiple resistance, nosocomial infection, surveillance

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INTRODUCTION

As the number of immunosuppressed patients being treated in hospital continues to increase, effective infection control measures must be present to ensure that the level of nosocomial infections is kept to a minimum. The empirical use of broad-spectrum antibiotics in patients with febrile neutropenia may lead to the selection of more resistant populations of organisms and therefore adequate surveillance must be in place to detect the emergence of such resistant pathogens. *Pseudomonas aeruginosa* is one such organism which may become resistant to commonly used empirical antibiotics. The laboratory observation of similar antibiograms in two or more separate isolates is often the first indication that an episode of cross-infection has occurred or that a common source for the organism exists. Further confirmation that identical strains are present relies on more complex typing methods involving the determination of phenotypic and, more recently, genotypic markers. DNA fragmentation by endonuclease digestion followed by pulsed field gel electrophoresis (PFGE) has been shown to be a highly discriminatory method of typing various organisms including *P. aeruginosa* [1,2].

The environment (particularly sink drains and water outlets) has previously been reported as a possible source in outbreaks of infection with *P. aeruginosa* in intensive care and hematology

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wards [3]. Acquisition of environmental strains by the patient may be associated with a poor prognosis. We report a cluster of cases of infection with a multiply resistant organism which was isolated from the patients' close environment. This occurred in a large teaching hospital over several months and affected two separate wards which shared common features of immunocompromised patients and an empirical antibiotic policy. The episode highlights the fact that the eradication of problematic bacterial strains may require the co-ordinated efforts of medical, nursing and infection control staff and hospital engineers.

METHODS

Clinical areas

Two separate wards were involved in this cluster of infections. The hematology unit is a recently refurbished ward with single rooms for source and protective isolation. Within this unit, only two rooms were involved and both of these may be supplied with HEPA filtered air (although this is not always used). The oncology unit is situated in a different part of the hospital. No local plumbing or ventilation system serves both areas. None of the patients on these wards require mechanical ventilation.

Surveillance

A system of laboratory-based ward-liaison surveillance is in place, where laboratory medical staff authorize all positive reports before visiting the clinical areas.

Bacteriological culture

Clinical samples were collected and cultured by routine bacteriological methods. Isolates of *P. aeruginosa* were confirmed by growth at 42 °C and biochemical reactions (oxidase test and API identification system (bioMerieux, Lyon, France)).

Disc sensitivity testing of isolates was performed on Iso-Sensitest agar. Antibiotic discs were obtained from Mast Diagnostics, Bootle, UK. After incubation for 18 h in air, plates were read using a Radius semi-automated plate scanner (Mast Diagnostics, Bootle, UK). Antibiotic sensitivity was determined by the Kirby–Bauer method using NCCLS guidelines. The initial antibiotics tested were ciprofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, gentamicin, and tobramycin. Resistant isolates were then further tested against aztreonam, piperacillin, amikacin and colistin. The minimum inhibitory concentrations were tested for meropenem and ceftazidime by Epsilometer-test (E test; Cambridge Diagnostics, Cambridge, UK).

Isolates which were indistinguishable by their antibiograms and biochemical API profile were stored on nutrient agar slopes at 4 °C until further study.

Patient screening

Following detection of the resistant strain in clinical samples, routine surveillance of patient samples was instituted in the hematology department after an initial meeting between the microbiology consultant, the infection control nurse and the consultants and nursing staff in the hematology department. A sample of faeces on admission and weekly samples while the patient was in the ward were cultured on Pseudomonas isolation agar (Difco Labs, Detroit, MI, USA) in air overnight at 37 °C. The colonies of *P. aeruginosa* were then screened for resistance to piperacillin-tazobactam on Columbia blood agar on which had been laid a piperacillin-tazobactam impregnated disc (75/10 μg). Isolates showing resistance to this agent were then tested against the other antibiotics listed above.

Environmental sampling

The method of detection of *P. aeruginosa* was similar to that used for the patients' samples. Plain cotton-tipped swabs without transport medium were used. These were moistened in sterile saline if the site being swabbed was found to be dry. The outflows of all sinks, drains and shower-heads and taps were sampled. Initially, swabs were taken from all such sites in both wards. If the resistant strain had been detected by environmental sampling, the patient in that room was screened to exclude colonization with the same resistant organism. Sampling was repeated 1 month later in the oncology department. Thereafter, sampling was confined to sites which had previously yielded a positive result. In hematology, samples taken weekly from the main ward area failed to grow the same resistant strain of *P. aeruginosa* on three successive occasions. Thereafter, further environmental sampling on a weekly basis was confined to the two isolation rooms which had yielded positive results. At this stage, environmental sampling of the main ward area was discontinued. A total of 195 environmental specimens were collected during the incident.

Pulsed-field gel electrophoresis

Isolates of appropriately resistant *P. aeruginosa* from patients' clinical samples and from environmental sampling of both wards were genotyped by PFGE using restriction enzyme XbaI. This was performed by Dr J. R. W.Govan and Mrs C. Doherty of the Department of Medical Microbiology, The University of Edinburgh Medical School.

Disinfection

Sinks and drains found to be positive were dismantled, cleaned with hot water and detergent, then dried before disinfection. One litre of 10 000 p.p.m. sodium hypochlorite was poured into the drain and left for 30 min then flushed with running water. Further rinses were performed hourly for the next 8 h.

RESULTS

None of the patients' faecal samples on admission yielded *P. aeruginosa* with a similar antibiogram. The five patients from whom multiply resistant *P. aeruginosa* was isolated are listed in Table 1. The two patients in ward 5 were not nursed in the same room. Several months elapsed between the first detection of the organism and subsequent isolation from other patients. Environmental sampling was instigated after two patients who had been in the same room in ward 8 (room 6) were found to have been infected with apparently identical strains of *P. aeruginosa*.

The occasions when environmental surveillance of ward 8 detected a piperacillin-tazobactam resistant *P. aeruginosa* with the same antibiogram are shown in Table 2. Attempted disinfection of the drains was being performed throughout this time on the dates listed.

Environmental sampling of ward 5 was started after the isolation of the similar multiply resistant strain from two patients. Piperacillin-tazobactam resistant *P. aeruginosa* detected by environmental sampling in ward 5 are listed in Table 3. Again, disinfection of the drains was carried out after a positive result from an environmental sample.

Sensitivity testing

All piperacillin-tazobactam resistant isolates were sensitive to ceftazidime, meropenem and amikacin, but were resistant to all other agents tested.

The minimum inhibitory concentration of ceftazidime by E test was increased (2 mg/L) in comparison with a fully sensitive control (*P. aeruginosa* NCTC 10662) but this minor alteration was not detectable by disc sensitivity testing. The sensitivity to meropenem was unchanged by disc sensitivity testing and by E test.

Typing results

PFGE allowed the isolates to be separated into two distinct groups (data not shown). Those from ward 8 patients and from the environmental samples from rooms 5 and 6 in ward 8 could be grouped together with identical PFGE profiles. Another group, consisting of the patient isolates and the environmental isolates from ward 5, possessed a dissimilar PFGE profile which differed from that of the former group by three bands. This is consistent with two closely related but separate strains being present, as a single point mutation could produce a three-band difference [4]. The remaining piperacillin-tazobactam resistant isolates had unique PFGE profiles which were distinct from either of the other two strains.

DISCUSSION

The strain of *P. aeruginosa* involved in this investigation was able to cause serious morbidity and mortality in this neutropenic population; being isolated from blood cultures, sputum, an intravenous catheter tip and being the cause of a fatal nosocomial pneumonia in one patient. The recurrent isolation of the same multiply resistant strain of *P. aeruginosa* over several months led us to suspect that an environmental site was the continuing source of this organism. Results obtained from preliminary environmental swabbing indicated that the *P. aeruginosa* strain could be isolated from the drainage system but not from the water supply or fittings. Previously, when drains have been implicated as a source of the offending organism, splash-

Table 1 Details of patients from whom multiply resistant *P. aeruginosa* was isolated

Patient	Initials	Ward/room	Date first isolated	Site	Clinical progress
1	M.H.	8/6	2 February 1997	Sputum	Allogeneic bone marrow transplant (BMT) for chronic myeloid leukaemia – profoundly neutropenic nosocomial pneumonia – died
2	K.W.	ward 5	18 July 1997	Blood	Neutropenic sepsis – recovered on appropriate therapy
3	S.M.	8/6 → room 5 on	26 July 1997 30 July 1997	Blood	Allogeneic BMT for very severe aplastic anaemia – profoundly neutropenic, line-associated septicaemia – recovered on appropriate therapy
4	E.W.	ward 5	6 August 1997	Line tip	Hickman line colonization – detected upon removal
5	P.B.	8/5	24 August 1997	Sputum	Autologous PBSC transplant for mantle cell lymphoma – profoundly neutropenic, nosocomial pneumonia – recovered on appropriate therapy

Table 2 Results of screening the environment in ward 8

Date	Ward/room	Site	Result
30 July 1997	8/6	Main room sink outlet	PTZ-R Paer[a]
(patient S.M. moved to room 5)		En-suite sink outlet	PTZ-R Paer
5 August 1997	Main room and en-suite sink outlets dismantled, cleaned and disinfected		
27 August 1997	8/6	Main room sink outlet	PTZ-R Paer
29 August 1997	Main room and en-suite sink outlets dismantled, cleaned and disinfected		
3 September 1997	8/5	Toilet bowl	PTZ-R Paer
9 September 1997	Room 5 Toilet bowl dismantled, cleaned and disinfected		
29 October 1997	8/6	En-suite sink outlet	PTZ-R Paer
6 November 1997	8	Nurses' sink drain in ward[b]	PTZ-R Paer
6 November 1997	Nurses' sink and en-suite sink outlets dismantled, cleaned and disinfected		
26 November 1997	Nurses' sink and en-suite sink outlets dismantled, cleaned and disinfected		
15 January 1998	8/6	En-suite sink outlet	PTZ-R Paer
17 January 1998	Nurses' sink and en-suite sink outlets dismantled, cleaned and disinfected		
January–July 1998	Continued monthly sampling of ward and weekly sampling of positive areas		
2 July 1998	8		PTZ-R Paer
5 July 1998	Drain outside room 6 cleaned and disinfected		
20 July 1998	8	Drain outside room 6	PTZ-R Paer
	8/6	En-suite sink outlet	PTZ-R Paer
23 July 1998	Both drain pipes replaced and straightened		

[a] PTZ-R Paer, piperacillin-tazobactam-resistant *P. aeruginosa*.
[b] The nurses' sink in the main ward and the en-suite sink in room 6 shared a common outflow.

Table 3 Environmental samples from ward 5

Date	Room	Site	Result
22 August 1997	3–6	Shower drain	PTZ-R Paer[a]
	6	Sink drain	PTZ-R Paer
	Main ward	Sluice sink	PTZ-R Paer (different PFGE profile)
	Main ward	Sluice sink	PTZ-R Paer
	Main ward	Sink	PTZ-R Paer
	Main ward	Disabled toilet sink	PTZ-R Paer
	Main ward	Shower drain	PTZ-R Paer
29 August 1997	3–6		No PTZ-R Paer
5 September 1997	All areas		No PTZ-R Paer
7 October 1997	All areas		No PTZ-R Paer
4 November 1997	Main ward	Shower drain	PTZ-R Paer (different PFGE profile)
November 1997–September 1998	Weekly sampling until January 1998 then continued monthly		
22 September 1998	Ten various sites positive in ward and room drains		PTZ-R Paer
30 September 1998	All positive sites treated with 10 000 p.p.m. hypochlorite		
8 October 1998	Six various sites positive		PTZ-R Paer (two isolates' sensitivity pattern differed only with respect to meropenem resistance, but all with same PFGE profile
15 October 1998	All positive sites treated with 10 000 p.p.m. hypochlorite		
4 November 1998 and subsequently	All sites negative		

[a] PTZ-R Paer, piperacillin-tazobactam-resistant *P. aeruginosa*.

back and regurgitation have been assumed to be the cross-infection hazard [3]. In our cluster, these events were not observed to occur. Indeed, there is no unequivocal proof that the environment did not become colonized from patients and a separate, undetected mechanism of cross-infection was occurring. However, a patient (S.M.) in ward 8, who was known to be excreting the organism, was moved from room 6 to room 5, 2 months before another patient in room 5 became infected (P.B.). Environmental screening of hospital wards has previously detected resistant micro-organisms which have been implicated in episodes of nosocomial infection [5]. The eradication of these organisms from a particular site may require, as in this instance, the co-ordinated efforts of the infection control team, ward staff and engineers. Because of the possibility of further episodes of nosocomial infection, it was considered justifiable to attempt to remove the potential environmental source.

The initial failure to eradicate the organism by cleaning and disinfection was assumed to be due to persistence of drainage sludge providing a protected environment for the organism. As a result, a structural review of the plumbing system and repeated dismantling and disinfection of drains by the hospital works department was performed in both wards. Throughout this time, the overall cost of laboratory consumables required in the processing of surveillance samples received was increased. Simultaneously, a review of the departmental antibiotic policy was made. Regular rotation of the antibiotics contained within a hospital's (or a department's) antibiotic policy has been recommended previously [6], although antibiotic rotation may not prevent the selection of resistant bacterial strains if genetically linked cross-resistance across different antibiotic classes is present. Although this organism was found to be resistant to both of the antibiotics used as first line in the department, the decision was made to alter only one of the antibiotics as the isolation of the organism from patients was uncommon, and the majority of other Gram-negatives isolated in the unit remained sensitive to both.

The demonstration by PFGE of two closely related, but distinct, strains of *P. aeruginosa* from the two physically separate ward areas is interesting and suggests the interaction of cross-infection and antibiotic-driven selection of resistance. As the two affected areas are geographically unlinked, an unidentified member of the hospital staff may have carried the organism between the two wards. We suspect that a mutation in one of the strains could then have conferred the minor difference in PFGE profiles. The use of prophylactic ciprofloxacin during neutropenia and similar first-line antibiotic agents in both clinical areas (piperacillin-tazobactam + gentamicin) subsequently produced similar selection pressures resulting in the selection of the related ancestral strains in both units. Meropenem sensitivity was not consistent within one of the clones, a later isolate from the environment in ward 5 having developed resistance to this agent. Fortunately, a strain with this particular sensitivity pattern was not isolated from a patient as the range of available treatment options would be narrow.

Resistance in this organism involved several structurally unrelated classes of antibiotics. This may possibly result from an alteration in the permeability of the porin channels on the outer membrane or an increased efflux of antibiotics via the MexA-MexB-OprM pump (or a combination of both) [7,8]. The pattern and degree of resistance to the beta-lactam agents would be consistent with the selection of a strain possessing a partially induced or stably derepressed-chromosomal AmpC beta-lactamase [9]. It is possible that several distinct mechanisms to include the other antibiotic classes may be involved. Although a single plasmid which possesses several genes that confer resistance to different classes of antibiotics might be present we feel this unlikely as plasmid-mediated quinolone resistance has not been described and is unlikely since the wild-type gene for DNA gyrase would still be able to function. The pattern of resistance is similar to that possessed by a strain of *P. aeruginosa* reported in an outbreak involving patients and the environment in a French hospital in 1998 [10]. In that example the mechanism of resistance to a number of antibiotics was found to be associated with an outer membrane protein causing increased efflux of drugs from the peri-plasmic space.

This report cannot prove that the infection control measures taken were solely responsible for the eradication of this resistant strain, although we feel that this is likely. Our experience with this cluster of isolates highlights the importance of constant surveillance of the patterns of resistance amongst organisms in a hospital and the value of accurate typing methods to determine cross-relatedness of bacterial strains. The potential of environmental organisms to infect susceptible patients should not be forgotten and ward procedures should be implemented to prevent cross-infection where possible. With increasing use of broad-spectrum antibiotics, surveillance activity to detect multiply resistant strains must continue to be the normal practice of infection control staff. The continuing value of laboratory-based ward-liaison surveillance should not be underestimated.

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