Molecular epidemiology of *Pseudomonas aeruginosa* bloodstream infection isolates in a non-outbreak setting

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**Abstract**

**Purpose.** The molecular epidemiology of *Pseudomonas aeruginosa* bloodstream infection (BSI) isolates has received limited attention. This study aims to characterize the molecular relationship of *P. aeruginosa* BSI isolates in the non-outbreak setting at a single tertiary healthcare facility.

**Methodology.** *P. aeruginosa* BSI isolates from patients who were admitted to the Royal Brisbane and Women’s Hospital over a 13 month period from November 2009 were identified retrospectively from the Pathology Queensland Clinical and Scientific Information System. The isolates were typed by the iPLEX MassARRAY matrix assisted lazer desorption/isonisation time of flight (MALDI-TOF) MS genotyping. The DiversiLab automated rapid strain typing platform (bioMérieux) was used to assess the genotypic relationships between study isolates that showed indistinguishable iPLEX20SNP profiles. Clinical data was also collected retrospectively from patient notes.

**Results.** Fifty-three *P. aeruginosa* BSI episodes were available for study. Thirty-five different clones or clonal complexes were identified by the iPLEX MassARRAY MALDI-TOF MS genotyping. Seventeen BSI isolates with indistinguishable iPLEX20SNP profiles underwent further DiversiLab genotyping and were found to belong to a further 13 different genotypes. There was no relationship between clonality and acquisition type, source of infection or length of stay in the setting of hospital-acquired infection.

**Conclusion.** The non-clonal population structure suggests that there is ongoing environmental exposure of inpatients to *P. aeruginosa*. In clinical areas dealing with at-risk patients, routine attention to mechanism of environmental colonization is important and should be addressed even in the non-outbreak setting.

**INTRODUCTION**

*Pseudomonas aeruginosa* is an important opportunistic pathogen that is capable of causing life-threatening illness, including bloodstream infection (BSI), in the vulnerable host. The majority of infections are hospital acquired (HAI). However, both healthcare-acquired infection (HCAI) and community-acquired infection (CAI) have also been described. Respiratory, urinary tract, soft tissue and central line-associated infections have all been described as important sources of BSI. The mortality from this BSI has been described up to 42%, depending on the population studied [1].

Molecular typing of *P. aeruginosa* has been predominantly studied in three distinct settings: cystic fibrosis, during nosocomial outbreaks and when comparing environmental and a range of clinical isolates, sampled from different geographical locations. In the latter setting, there appears to be a limited association between genotype and the ecological setting. Also described as a non-clonal epidemic population structure [2, 3]. Most cystic fibrosis outbreak strains and nosocomial outbreak strains represent a random sample of the broader *P. aeruginosa* population diversity punctuated by highly successful clones or clonal complexes [2–5]. A recent study in the nosocomial setting showed for the first time, successful international spread of a sequence type (ST)
235 clonal type with a VIM-2 metallo-β-lactamase in Russia, Belarus and Kazakhstan [6].

To date, the molecular epidemiology of *P. aeruginosa* BSI isolates has received little attention. In this study, we sought to characterize the molecular relationships between *P. aeruginosa* BSI isolates cultured in a non-outbreak setting at a large tertiary healthcare facility.

**METHODS**

**Study population**

*P. aeruginosa* BSI isolates from patients who were admitted to the Royal Brisbane and Women’s Hospital, Queensland, Australia, from 1 November 2009 to 30 November 2010 were retrospectively identified from the Pathology Queensland Clinical and Scientific Information System. Each BSI episode was defined as the 14 day period after the collection date of the sentinel-positive blood culture. Permission to carry out the study was obtained from the Pathology Queensland and also the Royal Brisbane and Women’s Hospital Ethics Committee.

**P. aeruginosa culture, identification and susceptibility testing**

Blood cultures were undertaken by a routine diagnostic laboratory using the BD BACTEC blood culture system (BD) with an incubation period of up to 5 days. Upon detection, each culture-positive blood sample was inoculated onto blood, chocolate and MacConkey agars and then incubated at 35°C in either 5% CO₂ or aerobic conditions. After overnight incubation, the *P. aeruginosa* isolates were identified by a positive cytochrome c oxidase test and the VITEK 2 system (bioMérieux). Sentinel isolates from each BSI episode were stored at −80°C using the Protect Microorganism Preservation System (Thermo Fisher Scientific). For the purposes of this study, each isolate was resuscitated from −80°C storage on Mueller–Hinton agar plates at 37°C for 24 h. Disc diffusion susceptibility testing to aztreonam, ceftazidime, ciprofloxacin, and piperacillin–tazobactam and meropenem was performed on all isolates according to the European Committee on Antimicrobial Susceptibility Testing guidelines and interpretative breakpoints [7, 8]. A standardized definition for multidrug-resistant (MDR) bacteria was used [9].

**P. aeruginosa** iPLEX MassARRAY matrix assisted laser desorption/ionization time of flight (MALDI-TOF) MS genotyping

Heat-denatured suspensions of each strain were prepared by heating 1 ml of a 1.0 McFarland suspension in sterile H₂O at 100°C for 10 min. Samples were then vortexed and subsequently centrifuged at 3000 g for 5 min with the supernatants thereafter used for genotyping. Genotyping was undertaken on all isolates using a single nucleotide polymorphism (SNP)-based typing assay (i.e. iPLEX20SNP assay) based on the Sequenom iPLEX MassARRAY MALDI-TOF MS platform as described previously [10]. Briefly, the iPLEX20SNP assay comprises 20 informative SNPs characterized from multilocus sequence typing (MLST) housekeeping genes. Previous *in silico* analyses have demonstrated that compared to MLST, the iPLEX20SNP assay shows a high discriminatory power and is capable of distinguishing most MLST STs either individually or as belonging to closely related single- or double-locus variant groups [10]. ST assignment of the 20-SNP profiles generated in the current study was performed by utilization of the MLST database as described previously [10]. If a 20-SNP profile had not been previously described, it was classified as a novel ST.

**DiversiLab genotyping**

The DiversiLab automated rapid strain typing platform (bioMérieux) was used to assess the genotypic relationships between study isolates that showed indistinguishable iPLEX20SNP profiles. DiversiLab in our local laboratory experience has had good performance as an indirect typing tool for *P. aeruginosa*. Genomic DNA was extracted using the MO BIO Ultraclean Microbial DNA isolation kit (MB-12224-250; MO BIO Laboratories) as per the manufacturer’s instructions. Repetitive element palindromic PCR (rep-PCR) was performed with the DiversiLab Pseudomonas kit using primer mix O. The PCR conditions were initial denaturation at 94°C for 2 min, annealing temperature of 50°C for 30 s and extension at 70°C for 30 s and followed by a final extension at 70°C for 3 min. Genetic relatedness was assessed using DiversiLab software version 3.6 (bioMérieux). Isolates producing fingerprints related by ≥95% were allocated to the same rep-PCR type.

**Clinical data and case definitions**

Detailed clinical data including age, gender, clinically suspected source of the BSI, admission date, discharge date and admitting speciality were retrospectively collected from the patient case notes. Each BSI was classified into acquisition type based on the modified Centers for Disease Control and Prevention definitions. A HAIs is defined as a positive blood culture obtained from a patient after 48 h of hospitalization or within 48 h of discharge. A HCAI is defined as a positive culture obtained at the time of hospital admission or within 48 h of admission if the BSI fulfilled any of the following criteria: (1) A complication of an invasive medical device; (2) Occurs within 30 days of a surgical procedure where the BSI is related to a surgical site infection; (3) An invasive instrumentation or incision related to a BSI was performed within 48 h before the onset of infection if the timing of onset was longer than 48 h, there must be compelling evidence that the infection was related to an invasive device or procedure; (4) Received therapy with temporary intravenous access in the 48 h before the onset of infection. If the timing of onset was longer than 48 h, there must be compelling evidence that the infection was related to the invasive device or procedure; (5) Associated with neutropenia (<0.5 × 10⁹ l⁻¹) contributed to by cytotoxic therapy; or (6) The patient resided in a nursing home. A CAI is defined as a positive culture obtained at the time of hospital admission or within the first 48 h after admission which does not fit the criteria for a HCAI [11, 12]. The suspected source of the BSI was classified
according to clinical judgement at the time of the BSI. Patient bed movement data for patient admissions to the healthcare facility prior to, during and after the admission including the BSI episode studied were obtained from the hospital-based corporate information system database.

**RESULTS**

Over the 13 month period, we identified a total of 72 episodes of *P. aeruginosa* BSI from 67 patients admitted to the Royal Brisbane and Women’s Hospital. Fifty-three (74%) of the 72 BSI isolates were available for further testing. The 53 BSI isolates studied represented 48 different patients (Table S1, available in the online Supplementary Material). Four of the patients had a further recurrent BSI episode. One patient had two recurrent BSI episodes. Thirty-five of the *P. aeruginosa* BSI isolates were from a HCAI 4, from CAI and 2 were an unknown acquisition type. The BSI isolates were highly susceptible to the antibiotics tested with only one isolate. Excluding the recurrent infection episodes, the majority of patients with a BSI were male (65%), and the average age was 60 years (range, 20–82 years). The most frequently suspected clinical sources of all BSIs were central line-associated infections (19 episodes), urinary tract infections (13 episodes) and pneumonia (8 episodes), while surgical site, skin or soft tissue and port exit site infections each accounted for only one BSI episode. Haematology was the predominant speciality responsible for patient care. In regard to HAI episodes, excluding the episodes involving hospital readmission, the range of time from admission to collection of the sentinel blood culture containing the *P. aeruginosa* isolate was 3 to 114 days, with a median of 16 days.

The iPLEX20SNP genotyping of the 53 BSI isolates revealed 35 different 20-SNP types. Five isolates were deemed untypeable. When the 35 20-SNP profiles (48 isolates) were used to predict a MLST type, 29/35 matched profiles obtained from the MLST website to provide a predicted MLST type or types. Six 20-SNP profiles (six isolates) did not match with any of the listed 20-SNP profiles meaning that a MLST type could not be assigned. Twenty-eight (80%) of the 35 20-SNP profiles pertained to individual isolates. The remaining seven 20-SNP profiles had more than one isolate assigned to them. Of these seven 20-SNP profiles, the predicted MSLT types represented six different clonal clusters and one singular clone. A number of the MLST types identified have been previously described in the greater Brisbane environment or in clinical strains [3]. The strain types associated with drug resistance in the hospital setting, ST111, ST175 and ST235, were not seen in the current evaluation [6, 13–21].

The most common iPLEX20SNP genotype was 3, which was detected in BSI isolates from four patients. iPLEX20SNP genotypes 5 and 7 were detected in BSI isolates from three patients each, while genotypes 6, 8, 10 and 24 were detected in BSI isolates from two patients each. iPLEXSNP genotype 5 represented a single MLST type 222. This is in contrast to the other five genotypes described that represented MLST clonal clusters. The 17 BSI isolates that were assigned to these 5 iPLEXSNP genotypes underwent further DiversiLab genotyping (Fig. 1). These isolates belonged to 15 different patients. Thirteen different DiversiLab genotypes were identified. In detail, there was no genetic variation found on DiversiLab typing among the two isolates belonging to the assigned iPLEXSNP genotype 10. Three of the four isolates assigned to the iPLEXSNP genotype 3 also showed an indistinguishable rep-PCR type. Two of the three isolates assigned to the iPLEXSNP genotype 6 were assigned also to the same DiversiLab genotype. The two isolates assigned to the iPLEXSNP genotype 8 were assigned to different DiversiLab genotypes. Each of the four isolates assigned to the iPLEXSNP genotype 7 was assigned to a different DiversiLab genotype. The two isolates assigned to the iPLEXSNP genotype 24 were assigned to different DiversiLab genotypes.

Overall, there was no relationship between the source of infection and clonality. There was limited cross-over between clone/cluster types and acquisition type. Three of the HCAI isolates belonged to the same iPLEXSNP genotype 5 which represented a single MLST ST222. Each patient was managed by a different hospital team, and there was no cross-over of patient movement during the studied or in previous hospital admissions. Of the three DiversiLab genotypes that involved more than one BSI isolate, all involved a HAI acquisition type. The two isolates assigned to DiversiLab genotype A represented a CAI and a HAI BSI acquisition. The three isolates assigned to DiversiLab genotype B involved both HCAI and HAI BSI acquisitions. On review of patient movement data for these patients, there was again no evidence of commonality of ward location at any time point during any relevant hospital admissions. The two isolates assigned to DiversiLab genotype C represented a recurrent BSI in one patient. There was no relationship between clonality and length of stay for HAI BSI isolates. Overall, there was no evidence of cross-transmission of any of the common clonal types seen by any of the typing methods, on review of patient movement data.

**DISCUSSION**

This study found that there was a non-clonal population structure of the *P. aeruginosa* isolates causing BSI in a single tertiary institution when studied over a 13 month period. There was no association between acquisition type, origin of infection or the length of stay in the HAI group and the genotypes seen. The isolates were highly susceptible, and the strain types previously associated with drug resistance, ST111, ST175 and ST235, were not seen. There was not a singular successful clone causing invasive BSI but many successful clonal types. The diversity of clonality of the BSI isolates seen most likely reflects environmental exposure of the vulnerable host. This may reflect both carriage prior to the hospital admission and water exposure in the hospital setting. Molecular typing of a broader sample of clinical isolates in the hospital setting and appropriate environmental
sampling may help better understand hospital sources of exposure and appropriate infection control strategies.

In the hospital setting, epidemic clones of *P. aeruginosa* causing outbreaks of BSI infection are well published. Identified sources have included hospital staff in the setting of artificial fingernails, onychomycosis or intermittent otitis externa [22–24]. Other identified hospital sources have included contaminated tubing used for irrigation of burn patients, contaminated endoscopes and contaminated water basins [25–27]. Epidemic clonal transmission has also been described in the setting of both BSI and other sites of *P. aeruginosa* infection in the setting of carbapenem resistance conferred by metallo-β-lactamases. The majority of isolates belong to a few successful clones such as ST111, ST175 or ST235. This has been seen worldwide [6, 13–21].

The molecular epidemiology of *P. aeruginosa* BSI in the non-outbreak setting has not been well described. Pirnay et al. [2], Kidd et al. [3] and Gomila et al. [17] have looked at a limited number of BSI isolates in the setting of a broader sampling of clinical isolates. In detail, Pirnay et al. [2] characterized 328 diverse and unrelated *P. aeruginosa* strains from both the clinical setting and external environment by MLST. Clinical isolates were indistinguishable from environmental isolates. Interestingly, the isolates were widespread in the environment without specific habitat selection [2]. This non-clonal population structure was also found by Kidd et al. in their characterization of environmental and a range of clinical *P. aeruginosa* isolates in South East Queensland [3].

In our study focusing on BSI isolates, 42 different clones/clonal complexes were identified from the 53 isolates characterized. The lack of genotypic relationship between the *P. aeruginosa* isolates studied suggests an environmental source of infection, with many successful clonal types causing invasive infection. Also supporting an environmental origin is a lack of association with the clone/clonal complex and whether the infection was acquired in the hospital or the community setting. In addition, some of the singleton clonal types have been previously described by Kidd et al. in the Brisbane environmental setting: 266 and 870 from the Brisbane River and 222 from the water of a municipal pool.

<table>
<thead>
<tr>
<th>Key</th>
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<tr>
<td>1</td>
<td>Pa-AE23</td>
<td>179, 180, 353, 1494, 1496, 1543, 1745</td>
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<tr>
<td>2</td>
<td>Pa-AF14</td>
<td>179, 180, 353, 1494, 1496, 1543, 1745</td>
</tr>
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<td>3</td>
<td>Pa-AD77</td>
<td>272, 348, 416, 1170, 1213, 1320</td>
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<tr>
<td>4</td>
<td>Pa-AE37</td>
<td>17, 318, 322, 380, 636, 688, 845 etc.¹</td>
</tr>
<tr>
<td>5</td>
<td>Pa-AE19</td>
<td>17, 318, 322, 380, 636, 688, 845 etc.¹</td>
</tr>
<tr>
<td>6</td>
<td>Pa-AD99</td>
<td>398, 399, 401, 810, 1517</td>
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<tr>
<td>7</td>
<td>Pa-AE31</td>
<td>61, 223, 309, 311, 316, 325, 361, etc.²</td>
</tr>
<tr>
<td>8</td>
<td>Pa-AE09</td>
<td>61, 223, 309, 311, 316, 325, 361, etc.²</td>
</tr>
<tr>
<td>9</td>
<td>Pa-AD97</td>
<td>61, 223, 309, 311, 316, 325, 361, etc.²</td>
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<tr>
<td>10</td>
<td>Pa-AF47</td>
<td>65, 107, 109, 253, 297, 338, 342, etc.³</td>
</tr>
<tr>
<td>11</td>
<td>Pa-AF46</td>
<td>65, 107, 109, 253, 297, 338, 342, etc.³</td>
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<td>17</td>
<td>Pa-AE15</td>
<td>398, 399, 401, 810, 1517</td>
</tr>
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Notes:
1. 958, 1255, 1313, 1806
2. 383, 458, 1251, 1310, 1803
3. 377, 532, 773, 815, 923, 1110, 1363, 1570, 1607, 1619

![Fig. 1. DiversiLab genotyping results of BSI study isolates that showed indistinguishable iPLEX20SNP profiles.](image-url)
[3]. There was no suggestion of cross-transmission between the patients with hospital-acquired BSI.

In terms of the hospital environment, components of the tap (faucet), particularly the body, supporting metal collars and flow straighteners, have been found to be reservoirs of *P. aeruginosa* [28–30]. This has also been found for sinks, tubing used for local irrigation and shower heads [25, 29, 31]. Quick et al. [29] found that, even in a new hospital, *P. aeruginosa* can become rapidly endemic in the hospital plumbing. This group also showed that the water supply was the origin of the clones in their hospital [29]. Reuter et al. [28] studied the source of the *P. aeruginosa* patient colonizations and infections in their intensive care unit. Their longitudinal sampling suggested that there was evidence of transmission from patients to the faucets resulting in colonization. Both short- and long-term colonization of differing genotypes was found. In the United Kingdom, recently introduced national guidelines have been put in place for routine water sampling in augmented care units with directed interventions such as disinfection and replacement of high-risk plumbing parts as required [32].

It is also known that the greater the length of hospitalization of a patient, the greater the risk of colonization with *P. aeruginosa* [31, 33, 34]. The majority of infections in this study were HAIs. However, colonization of the host by *P. aeruginosa* prior to admission is a possible source of subsequent infection. Lepelletier et al. [34] looked at patient gastrointestinal colonization in five different wards at a French university hospital. Newly admitted patients had a rectal swab as part of the study. In the routine wards, the proportion of culture-positive patients was 1.9% to 8.7% [34]. Food may also be a source of colonization in the at-risk patient. Washed or unwashed vegetables and fruit may potentially be another mechanism of transmission to the at-risk patient [35]. Wright et al. [36] identified *P. aeruginosa* from the vegetable salads served to patients at their hospital.

It must be remembered that BSI are only one of many infections that *P. aeruginosa* causes. We have not typed isolates from other infections over the period studied nor isolates causing patient colonization. This would give us a more complete picture of the clonal types in this institution. We cannot exclude some cross-transmission between patients being the source of colonization and subsequent BSI. However, the diversity of clonal and cluster types we have seen suggests that environmental exposure is the main source of these isolates.

In summary, the *P. aeruginosa* BSI isolates studied over 13 months at a single institution were diverse in their clonal structure. There was evidence of many successful *P. aeruginosa* clonal types. As the majority of infections were HAIs, this suggests that there is ongoing environmental exposure of inpatients to *P. aeruginosa*. *P. aeruginosa* BSI is an infection with considerable morbidity and mortality. This would suggest that, in areas of the hospital dealing with the at-risk patient, routine attention to mechanism of environmental colonization is important and should be addressed even in the non-outbreak setting.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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