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Epidemiol. Infect. (2017), **145**, 3040–3046. © Cambridge University Press 2017
doi:10.1017/S0950268817001832

SHORT REPORT
Pseudomonas aeruginosa blood stream infection isolates from patients with recurrent blood stream infection: Is it the same genotype?

K. L. McCARTHY[1]*, T. J. KIDD[2] AND D. L. PATERSON[1]

[1] *The University of Queensland, UQ Centre for Clinical Research, Brisbane, Australia*
[2] *The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Australia*

Received 2 June 2017; Final revision 19 July 2017; Accepted 20 July 2017; first published online 22 August 2017

SUMMARY

The type identity of strains of *Pseudomonas aeruginosa* from primary and recurrent blood stream infection (BSI) has not been widely studied. Twenty-eight patients were identified retrospectively from 2008 to 2013 from five different laboratories; available epidemiological, clinical and microbiological data were obtained for each patient. Isolates were genotyped by iPLEX MassARRAY MALDI-TOF MS and rep-PCR. This showed that recurrent *P. aeruginosa* BSI was more commonly due to the same genotypically related strain as that from the primary episode. Relapse due to a genotypically related strain occurred earlier in time than a relapsing infection from an unrelated strain (median time: 26 *vs.* 91 days, respectively). Line related infections were the most common source of suspected BSI and almost half of all BSI episodes were associated with neutropenia, possibly indicating translocation of the organism from the patient's gut in this setting. Development of meropenem resistance occurred in two relapse isolates, which may suggest that prior antibiotic therapy for the primary BSI was a driver for the subsequent development of resistance in the recurrent isolate.

Key words: Bacterial typing, hospital infection, medical microbiology, molecular epidemiology, public health microbiology.

Recurrent *Pseudomonas aeruginosa* blood stream infections (BSI) have been reported in 6% of hospitalised patients with a primary *P. aeruginosa* BSI [1]. A haematological co-morbidity or having received steroids in the preceding 30 days, have been identified as patient risk factors for relapse [1] and other high risk populations include liver, and haematopoietic stem cell transplantation recipients, and acquired immunodeficiency syndrome (AIDS) patients [2–4]. In these cohorts rates of BSI recurrence of up 33% have been described [2–4] and higher mortality is associated with relapse in comparison with the primary BSI episode [1].

Much of the literature on relapsing Gram negative BSI has focused on coliform bacteria but the correspondence of strain types of *P. aeruginosa* isolated from primary and recurrent BSI has not been studied. It is therefore unclear if repeated BSI is due to recurrence of the original infecting strain or to a different emergent strain of *P. aeruginosa*. This study therefore aimed to further characterise the genotypic relationship between the isolates of *P. aeruginosa* from primary and relapsing BSI episodes in an individual patient.

P. aeruginosa BSI isolates from patients who had blood cultures collected over a 5-year period

* Author for correspondence: Dr K McCarthy, University of Queensland Centre for Clinical Research, Building 71/918, Royal Brisbane and Women's Hospital Campus, Herston, QLD 4029, Australia.
(Email: kate.mccarthy1@uqconnect.edu.au)

https://doi.org/10.1017/S0950268817001832 Published online by Cambridge University Press

(January 2008–January 2013) were retrospectively identified from five public or private diagnostic pathology laboratories that service eight tertiary care hospitals and one secondary care hospital in metropolitan Brisbane, Australia. These hospitals provide specialised and broad medical, surgical and intensive care for an urban population of 2·24 million.

Time periods of storage of BSI isolates varied between the laboratories and all stored isolates were included in the initial analyses, with BSI episodes among individual patients defined as the 14 day period after the collection of the sentinel-positive blood culture [5]. A relapsing BSI episode was defined as a subsequent *P. aeruginosa*-positive blood culture occurring within the study period and outside of the preceding BSI episode. Patients with a relapsing BSI episode or episodes for which both of the BSI isolates were stored were included in the study. Patient data, including name, age, gender, date of birth and hospital of admission, was collected and correlated across the various pathology providers to ensure that cases of recurrent infection were not overlooked. The source of the initial BSI was determined by the treating clinician at the time of notification of the positive blood culture. Permission to undertake the study was obtained from all laboratories involved.

Blood cultures, isolate identification and susceptibility testing were undertaken as previously described [5]. Briefly, each blood culture was incubated using the BD BACTEC™ blood culture system (BD, North Ryde, Australia) with an incubation period of up to 5 days. Bacterial identification and susceptibility testing was achieved using the VITEK 2 system (bioMérieux Australia Pty Ltd, Queensland, Australia). Annual Clinical and Laboratory Standards Institute (CLSI) breakpoints were used to define susceptibility or resistance to the antimicrobial agents tested [6]. From January 2012 in one pathology service and July 2012 in another, antibiotic breakpoint references were changed to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [7]. A standardised definition for multidrug resistant (MDR) bacteria was applied [8]. Confirmed *P. aeruginosa* isolates were stored at −80 °C using the Protect Microorganism Preservation System (Thermo Fisher Scientific Australia Pty Ltd, Thebarton, Australia) until transportation to a research laboratory. Upon receipt each isolate was resuscitated from −80 °C storage on Mueller-Hinton agar at 37 °C for 24 h.

All isolates were genotyped with a single nucleotide polymorphism (SNP)-based assay as outlined previously [5]. Briefly, heat denatured DNA preparations from each isolate were analysed using an iPLEX20SNP assay based on the iPLEX MassARRAY matrix-assisted laser desorption ionisation with time of flight mass spectrometry (MALDI-TOF MS) platform (Agena Bioscience, Brisbane, Australia). The iPLEX20SNP assay, comprising 20 informative multilocus sequence typing (MLST) housekeeping gene SNPs, is capable of distinguishing most MLST sequence types (STs) either individually or as belonging to closely related single- or double-locus variant groups [9]. ST assignment of the 20-SNP profiles generated in the current study was performed by utilisation of the MLST database as described previously [9]. Twenty-SNP profiles, which did not match with any of the listed 20-SNP profiles (i.e. an MLST type could not be assigned) were assigned as novel genotypes. If an isolate had a 20-SNP profile with a singular non-typable allele and a historical isolate was available with an otherwise identical 20-SNP profile, the former isolate was assigned to the same sequence type.

The DiversiLab automated rapid strain typing platform (bioMérieux) was used to assess the genotypic relationships between study isolates showing indistinguishable 20-SNP profiles. Genomic DNA was extracted using the MO BIO Ultraclean Microbial DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, USA) as per the manufacturer's instructions and repetitive element palindromic polymerase chain reaction (rep-PCR) was performed with the DiversiLab Pseudomonas kit using the previously outlined primer mix and PCR conditions [5]. Genetic relatedness was assessed using DiversiLab software version 3·6 (bioMérieux) with isolates showing fingerprints related by $\geqslant 95\%$ being allocated to the same rep-PCR type.

If a patient's BSI isolates were assigned to the same 20-SNP profile that corresponded to a single ST, or the same novel 20-SNP genotype the isolates were considered related. For isolates where the 20-SNP profiles corresponded to $\geqslant 2$ STs, genetic relatedness was confirmed provided the isolates were also allocated to the same DiversiLab rep-PCR type.

An unpaired Mann–Whitney test was utilised to compare continuous variables and all calculations were performed with GraphPad Prism 6 for Windows.

Over the study period there was a total of 942 BSI isolates of *P. aeruginosa* identified that had been stored by the laboratories. Twenty-eight patients, from nine different healthcare settings, experienced one or more relapsing BSI episodes; 25 had one

https://doi.org/10.1017/S0950268817001832 Published online by Cambridge University Press

relapse and three had two relapses. Apart from one individual, the primary and secondary episodes occurred within the same healthcare setting. Community-acquired acquisition was not seen. Further comments will relate to the isolates recovered from the primary and the first relapse episodes only for each patient (56 isolates).

All isolates were susceptible to piperacillin-tazobactam. Four isolates (7%) from three patients showed evidence of meropenem resistance; in two of these patients the relapse isolate exhibited resistance to meropenem compared with the primary isolate, which was sensitive. Overall, only two isolates from a single patient exhibited a MDR phenotype that did not include resistance to meropenem.

The iPLEX20SNP and DiversiLab genotyping results of the primary and recurrent BSI isolates are presented in Table 1. Fourteen patients (50%) experienced recurrent infection with an isogenic (or related) strain of *P. aeruginosa* and nine (32%) patients were reinfected with a genotypically unrelated strain. The genetic relationship between the initial and relapse isolates from the remaining five patients could not be determined due to the limitations of the typing systems.

A suspected source of infection was identified for 46 (82%) of the 56 *P. aeruginosa* isolates arising from the primary and secondary cases of BSI (Table 1). Indwelling line-related infections were by far the most common source of infection, being implicated in 35 of 46 (76%) positive blood cultures. Other identified sources of isolates included urinary (5), skin/soft tissue (3), abdominal (1) and cardiovascular (2). There were 17 subjects in whom a suspected source of infection was identified for the primary and secondary BSI with both genotyped isolates; 11 of these patients experienced relapsing line-related infections with six showing a related *P. aeruginosa* strain in both blood cultures. Relapsing BSI arising from the urinary tract and skin/soft tissue sources was also observed in two subjects (C and H, Table 1) but both cases involved unrelated genotypes. Of the four subjects in whom there were apparently differing sources of infection in the first and relapsing BSI episodes, an isogenic strain was identified in three (O, S and AA), while unrelated genotypes were recovered from patient E.

The genotypic relationships of isolates in the context of clinical and laboratory variables for patients are summarised in Table 2. Blood cell count parameters were also available for 50/56 (89%) BSI episodes and showed that 21 (42%) of these originated from subjects who were neutropenic at the time of blood culture collection. The median time to a primary BSI relapse was 49 days (interquartile [IQR] range: 26–101 days). For the 14 patients showing relapsing BSIs with an isogenic *P. aeruginosa* strain the median time to recurrence was 26 days (IQR: 21–48 days). Conversely, if the primary recurrent BSI involved an unrelated *P. aeruginosa* strain (nine patients) the median time to relapse of BSI was 91 days (IQR: 70–176 days; $P = 0{\cdot}013$). The three subjects (i.e. G, J, T) who experienced a second relapse BSI had *P. aeruginosa* cultured from their blood at 70, 322, and 56 days after the primary BSI recurrence, respectively.

The key finding of this study is that recurrent *P. aeruginosa* BSI can occur from the same or different genotype, with recurrence from a genetically related strain occurring more frequently. Moreover, reinfection from a genetically related strain was associated with a markedly shorter median time to recurrence of 26 days, compared with 91 days for BSI recurrence with an unrelated strain. The most common source for all the BSI episodes studied was line-related infection, and almost half of BSI episodes occurred in neutropenic patients, which suggests that gut translocation may be another potential source of the recurrent BSI.

Although Gram-negative bacteria comprise a substantial component of normal human host microflora, *P. aeruginosa* is infrequently reported, but may occur at sites other than the bowel, including the perineum, throat, nose and axilla [10]. The hospitalised host on the other hand, appears to have increased susceptibility to colonisation by *P. aeruginosa* often with high rates (>50%) being recorded in intensive care units [11]. Colonisation with one or more distinct strain genotypes may occur [12, 13], and give rise to a secondary site of infection and subsequent bacteraemia [12, 14]. The finding here that relapsing BSI due to the same strain of *P. aeruginosa* occurred earlier than episodes due to an unrelated strain may reflect changes in strain populations colonisation of the host due to exposure to the ubiquity and genetic heterogeneity of this species in the natural environment [15].

In our study, 21 patients were neutropenic at the time of one of the BSI episodes, suggesting that endogenous translocation from the gastrointestinal tract may have been a potential source for some of these infections. Line-related infection causing both primary and relapsing episodes was the most common cause of all BSI cases and the most common source

https://doi.org/10.1017/S0950268817001832 Published online by Cambridge University Press

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Table 1. *Infection source, genotypic relationships and time to recurrence of P. aeruginosa isolates from 28 subjects showing relapsing blood stream infection*

Subject	Primary BSI			Secondary BSI			rep-PCR relatedness of isolates[b]	Overall relatedness[b]	Time to recurrence (days)
($n = 28$)	Infection source[a]	20-SNP type	Predicted ST	Infection source[a]	20-SNP type	Predicted ST			
A	Line	1	313, 648, 1462	Line	1	313, 648, 1462	R	R	25
B	Line	NT	–	Line	2	589, 791, 803	–	I	257
C	Skin/ST	3	242, 996	Skin/ST	3	242, 996	U	U	165
D	Line	4	272, 348, 416, 1170, 1213, 1320	Unknown	4	272, 348, 416, 1170, 1213, 1320	R	R	47
E	Urinary	5	89, 307, 308, 662, 1028, 1410, 1488, 1746, 1794	Skin/ST	6	408	–	U	64
F	Line	7	N0VEL-O1	Unknown	8	343, 381, 1256, 1516	–	U	92
G[c]	Line	NT	–	Line	9	910, 1689	–	I	104
H	Urinary	10	779, 1626	Urinary	11	65, 107, 109, 253, 297, 338, 342, 377, 532, 773, 815, 923, 1110, 1363, 1570, 1607, 1619	–	U	557
I	Line	12	259, 1295, 1392	Line	12	259, 1295, 1392	R	R	49
J[d]	Line	4	272, 348, 416, 1170, 1213, 1320	Line	4	272, 348, 416, 1170, 1213, 1320	U	U	15
K	Unknown	NT	–	Cardiovas.	NT	–	–	I	32
L	Line	13	1684	Line	14	222	–	U	187
M	Unknown	15	1650	Line	15	1650	–	R	21
N	Line	NT	–	Line	NT	–	–	I	45
O	Urinary	16	822, 1239	Line	16	822, 1239	R	R	85
P	Line	NT	–	Abdominal	NT	–	–	I	150
Q	Line	17	229, 390, 928	Line	18	NOVEL-02	–	U	75
R	Unknown	19	NOVEL-03	Unknown	19	NOVEL-03	–	R	26
S	Line	20	27, 294, 334, 1208, 1324, 1402	Urinary	20	27, 294, 334, 1208, 1324, 1402	R	R	26
T[e]	Unknown	21	147	Unknown	21	147	–	R	21
U	Unknown	22	277, 364, 1128, 1390	Unknown	22	277, 364, 1128, 1390	R	R	40
V	Line	23	298, 446, 691, 1133, 1185, 1581, 1603	Line	23	298, 446, 691, 1133, 1185, 1581, 1603	R	R	21
W	Line	24	NOVEL-04	Line	24	NOVEL-04	–	R	26

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Table 1 (*cont*.)

Subject (*n* = 28)	Primary BSI			Secondary BSI			rep-PCR relatedness of isolates[b]	Overall relatedness[b]	Time to recurrence (days)
	Infection source[a]	20-SNP type	Predicted ST	Infection source[a]	20-SNP type	Predicted ST			
X	Line	25	267, 440	Line	26	103, 244, 441, 462, 464, 594, 766, 986, 1038, 1181, 1227, 1338, 1412, 1423, 1530, 1661,	–	U	91
Y	Line	27	428, 1196	Line	27	428, 1196	R	R	48
Z	Line	28	360, 861, 862, 864, 1561	Line	29	885	–	U	80
AA	Line	30	232, 241, 247, 379, 471, 577, 1442	Cardiovas.	30	232, 241, 247, 379, 471, 577, 1442	R	R	20
AB	Line	11	65, 107, 109, 253, 297, 338, 342, 377, 532, 773, 815, 923, 1110, 1363, 1570, 1607, 1619	Line	11	65, 107, 109, 253, 297, 338, 342, 377, 532, 773, 815, 923, 1110, 1363, 1570, 1607, 1619	R	R	328

20-SNP, Sequenom iPLEX 20-single nucleotide polymorphism assay genotype; ST, multilocus sequence type; rep-PCR, DiversiLab repetitive element palindromic PCR genotype; NT, non-typeable.

[a] Line, line-related BSI; Skin/ST, skin or soft tissue focus of BSI; Urinary, BSI arising from a urinary tract infection; Unknown, a source of infection could not be identified; Cardiovas, BSI arising from endocarditis; Abdominal, BSI arising from diverticulitis.

[b] R, related genotypes; U, unrelated genotypes; I, indeterminant genotypic relationship.

[c]Subject G experienced two relapsing episodes of *P. aeruginosa* BSI. The second episode involved a line related infection comprising 20-SNP type CGTCGGCTCCTCCCCGGGCG (Predicted ST: 910, 1689; rep-PCR type: 13) at 70 days after the primary relapse BSI.

[d]Subject J experienced two relapsing episodes of *P. aeruginosa* BSI. The second episode involved a line related infection comprising 20-SNP type CGCAAGCTACCTCCTGGGTA (Predicted ST: NT) at 322 days after the primary relapse BSI.

[e]Subject T experienced two relapsing episodes of *P. aeruginosa* BSI. The second episode involved a unknown source of BSI comprising 20-SNP type CGTCGGCTCTCCTTTGGGTA (Predicted ST: 147) at 56 days post the primary relapse BSI.

Table 2. *Relationship of clinical and laboratory variables to the genotypes of primary and relapsing P. aeruginosa BSI isolates*

	Genetically related recurrent BSI episodes ($n = 14$)	Genetically unrelated recurrent BSI episodes ($n = 9$)	Indeterminate relationship between BSI episodes ($n = 5$)
Neutropenic during both episodes of bacteremia	4	2	2
Both episodes from the same source	7	6	3
Both episodes line related source of Infection	7	4	3
Development of meropenem resistance in the second episode	2	0	0
Median time between episodes (days)	26	91	150

when both episodes were from the same source of infection; the latter were more likely to be due to genetically related isolates. Unfortunately line removal data were not available to determine if line retention contributed to the relapse of BSI. It is noteworthy that intravascular devices were found to be the most common cause of recurrent all-cause Gram-negative bacteraemia by Wendt *et al.* [16].

In two relapsing BSI episodes, the isolate had become meropenem resistant in comparison with the previously sensitive isolates in the primary BSI episode. The patient's antibiotic therapy preceding the secondary BSIs was unknown. It would seem likely that antibiotic selection pressure drove the development of antibiotic resistance.

This study has some limitations. First, the number of recurrent *P. aeruginosa* BSI episodes is relatively small due to a combination of the infrequency of relapse cases and dependency on external laboratories having stored the isolates after processing of blood cultures. Second, sampling bias may have occurred owing to the differing time periods individual laboratories had stored isolates available for study. Finally, only limited clinical data were available on all of the study patients.

In summary, we have shown that relapsing *P. aeruginosa* BSI due to the same genotype is more frequent, and occurs earlier in time than a subsequent infection from an unrelated genotype. Line related infections and gut related translocations of bacteria are likely important sources of relapsing infection. Development of meropenem resistance in two relapsing BSI episodes was seen.

ACKNOWLEDGEMENTS

We would like to thank the participating hospitals and laboratories for allowing us to conduct this study: Greenslopes Private Hospital, Mater Pathology, Mater Private Hospital, Mater Public Hospital, Pathology Queensland, The Prince Charles Hospital, Princess Alexandra Hospital, Royal Brisbane and Women's Hospital, St Andrew's Private Hospital, Sullivan Nicolaides Pathology. The Australian Genome Research Facility Ltd performed the *P. aeruginosa* iPLEX MassARRAY MALDI-TOF MS genotyping. Kyra Cottrel and Dr Hanna Sidjabat performed the DiversiLab genotyping. This research did not receive any specific grant from finding agencies in the public, commercial or not for profit sectors. Dr KL McCarthy is supported through an Australian Government Research Training Program Scholarship.

DECLARATION OF INTEREST

The authors have no conflicts of interest to declare.

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