Title: New genotyping method discovers sustained nosocomial Pseudomonas aeruginosa outbreak in an intensive care burn unit.
Authors: Tissot F, Blanc DS, Basset P, Zanetti G, Berger MM, Que YA, Eggimann P, Senn L
Journal: The Journal of hospital infection
Year: 2016 Sep
Volume: 94
Issue: 1
Pages: 2-7
DOI: 10.1016/j.jhin.2016.05.011

New genotyping method discovers sustained nosocomial Pseudomonas aeruginosa outbreak in an intensive care burn unit.

F. Tissot[1*], D. S. Blanc[1*], P. Basset[1], G. Zanetti[1], M. M. Berger[2], Y-A Que[2], P. Eggimann[2], L. Senn[1]

[1]Service of Hospital Preventive Medicine, [2]Intensive Care Service, Centre Hospitalier Universitaire Vaudois and Lausanne University Hospital, Lausanne, Switzerland

*equal contribution

Running title: *P. aeruginosa* outbreak in the ICU

Corresponding author: Frederic Tissot, MD, Infectious Diseases Service, Department of Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, rue du Bugnon 46, CH-1011 Lausanne, Switzerland. Tel: +41 79 556 82 01. Fax : +41 21 314 10 08. E-mail: frederic.tissot@chuv.ch

Preliminary results of the study were presented at the following meeting: Poster # P-973, 23th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Berlin, Germany, April 27–30, 2013.

Summary

Background: *P. aeruginosa* is a leading cause of healthcare-associated infections in the ICU.

Aim: this study investigated an unexplained increase in the incidence of *P. aeruginosa* recovered from clinical samples in the ICU over a two-year period.

Methods: after unsuccessful epidemiological investigation by conventional tools, *P. aeruginosa* clinical isolates of all patients hospitalized between January 2010 and July 2012 were typed by a novel double-locus sequence typing (DLST) method and compared to environmental isolates recovered during the investigation period.

Findings: in total, 509 clinical isolates from 218 patients and 91 environmental isolates were typed. Thirty-five different genotypic clusters were found among 154/218 patients (71%). The largest cluster, DLST 1-18, included 23 patients who were mostly hospitalized during overlapping periods in the burn unit. Genotype DLST 1-18 was also recovered from floor traps, shower trolleys and the shower mattress in the hydrotherapy rooms, suggesting environmental contamination of the burn unit as the source of the outbreak. After implementation of appropriate infection control measures, this genotype was recovered only once in a clinical sample from a burned patient and twice in the environment, but never thereafter during a 12-month follow-up period.

Conclusion: the use of a novel DLST method allowed the genotyping of a large number of clinical and environmental isolates, leading to the identification of the environmental source of a large unrecognized outbreak in the burn unit. Eradication of the outbreak was confirmed after implementation of a continuous epidemiological surveillance of *P. aeruginosa* clones in the ICU.

Key words: *Pseudomonas aeruginosa*, ICU, burns, outbreak, molecular typing

Introduction

Pseudomomas aeruginosa remains a leading cause of healthcare-associated infections in critically-ill patients, particularly ventilator-associated pneumonia and burn wound infection (1, 2). It is found in the digestive tract of 3-24% of hospitalized patients. The source of this opportunistic pathogen can be either endogenous or exogenous (3-6).

Since 1998, our infection control team performs regular epidemiological surveillance of *P. aeruginosa* in the intensive care unit (ICU), based on molecular typing (5, 7). All clinical strains are stored at -80°C in the microbiology laboratory. Pulsed-field gel electrophoresis (PFGE) is generally considered the gold standard for local epidemiological studies because of its high discriminatory power. However, this method is labor intensive and shows low inter-laboratory reproducibility (8, 9), especially when large numbers of isolates are analyzed. In this context, we developed the double-locus sequence typing (DLST) method based on the sequencing of two highly variable loci *ms*172 and *ms*217 (10). The high typability, discriminatory power, and ease of use of the proposed DLST scheme make it a method of choice for local epidemiological analyses of *P. aeruginosa* (10). Moreover, the possibility to give unambiguous definition of types allows standardization (http://www.dlst.org) and integration of results into hospital laboratory informative systems which can then be used for surveillance.

In our institution, incidence of *P. aeruginosa* in the ICU is prospectively monitored using an electronic alert system reporting any clinical sample growing *P. aeruginosa* in patients hospitalized in this unit. From 2009 onward, an unexplained 30% increase in the incidence of *P. aeruginosa* recovered from clinical samples was observed in

the ICU of our hospital, rising from 32.2 cases per 1000 admissions in 2009 to 41.5 in 2010 and 44.7 in 2011. Unusual early-onset *P. aeruginosa* infections in these patients were also reported. After unsuccessful investigation with conventional epidemiological tools, the DLST method was implemented to investigate this increase and identify potential outbreaks due to a chain of transmission or a common reservoir.

Methods

Study setting

Lausanne University Hospital is a 1000-bed tertiary-care centre with 32 adult ICU beds, including four burn ICU beds, one hydrotherapy room and one isolation room with full hydrotherapy and surgical equipment. Approximately 40 burned patients are hospitalized in this unit every year and 360 hydrotherapy treatments are performed. Hydrotherapy consists of showers with filtered tap water carried out on a trolley covered with a plastic mattress. According to burn extension, patients are showered one to three times a week. The hydrotherapy room is occasionally used for non-burned patients, such as Lyell syndrome. There are no automatic taps in the burn unit.

Clinical isolates

All consecutive patients hospitalized in the ICU with a clinical sample growing *P. aeruginosa* at any site between January 2010 and July 2012 were included. Based on colony morphology, one or several *P. aeruginosa* isolates per clinical sample were chosen for further typing analysis. For patients with prolonged ICU stays, multiple samples (one every two weeks until ICU discharge) were considered for isolates recovery. No routine screening of *P. aeruginosa* carriage was performed. The study was approved by local Ethics Committee: no consent was required.

Environmental isolates

Between March 2012 and July 2012, tap water samples and environmental swabs obtained from taps and sink traps of all ICU rooms, as well as from the environment of the hydrotherapy rooms, including shower trolleys and shower mattresses, were analyzed. Swabs were inoculated on a cetrimide agar plate. Water samples were filtered on a 0.45 μm membrane; which was deposited on a cetrimide agar plate. Plates were incubated 48h at 35°C.

Genotyping method

DLST was implemented in our institution for *P. aeruginosa* isolates genotyping in March 2012. The technique has been previously described (10). All environmental and clinical isolates from March 2012 onward were prospectively genotyped. Clinical strains before this date were unfrozen and analyzed retrospectively.

Epidemiological definitions

A case was defined as a patient hospitalized in the ICU and infected or colonized by a given genotype of *P. aeruginosa*. Cases sharing the same DLST genotype as other environmental or clinical isolates were defined as belonging to the same genotypic cluster. Epidemiological data (unit-s and room-s of hospitalization, dates of admission and discharge) and clinical data of patients belonging to the largest cluster were retrieved from the hospital information system and the medical charts.

Results

Clinical isolates genotyping

During the study period, 246 patients with at least one clinical sample growing *P. aeruginosa* were hospitalized in the ICUs. For 19 patients (17 from 2010), no isolate was available for typing. Overall, 525 clinical isolates from 227 patients were

analyzed (median 1 isolate per patient, range 1-23), of which 509 from 218 patients were successfully genotyped (16 isolates in 9 patients were untypable for technical reasons). For 12 patients, two different genotypes were recovered in the same individual and for one, three distinct genotypes were found. A unique genotype, not recovered from other patients or the environment, was found in 64/218 (29%) patients, while 35 genotypic clusters were isolated in the remaining 154/218 (71%) patients (median 3 patients per cluster, range 2-23). The largest cluster included 23 patients infected or colonized with the genotype DLST 1-18. This cluster was further investigated.

Environmental isolates genotyping

Between March 2012 and July 2012, 99 environmental isolates were recovered, mainly from sink traps. All water samples and swabs of taps were negative. Eight strains were untypable for technical reasons. Among the 91 isolates that could be analyzed, 24 different genotypes were found. DLST 1-18 was found in 14 isolates, of which 12 from samples collected in the hydrotherapy rooms, including floor traps, a plastic board under the shower mattress and a plastic rubber in a damaged corner of the mattress. One other DLST 1-18 isolate was recovered from the sink trap in the room of a burn patient and two from the sink trap of a single room in the neighboring ICU unit (Table 1).

Epidemiological investigation of cluster DLST 1-18

The 23 patients infected or colonized by *P. aeruginosa* DLST 1-18 were hospitalized between January 2010 and June 2012 mostly during overlapping periods. All patients but two were hospitalized either in the burn unit (18/23) or in the neighboring unit (3/23), of whom 19 were treated in the hydrotherapy room. The two other patients were a burned child hospitalized in the pediatric ICU who came frequently to the adult

hydrotherapy room and one patient (index case) hospitalized in a distant unit without geographical link with the rest of the cluster (Figure 1). The subjects contaminated with DLST 1-18 represented 19 % (18/95) of the total number of burned patients hospitalized in the burn unit between January 2010 and June 2012. Median time from ICU admission to recovery of first *P. aeruginosa* strain was 8.5 days (IQR: 4-15 days). Five of 23 patients (22%) died and for two, multiresistant *P. aeruginosa* infection was the direct cause of death. Clinical characteristics of these 23 patients are shown in Table 2.

Observations of practice standards and corrective measures

Following the identification of the DLST 1-18 cluster in the burn unit, audits of infection control practices by a nurse trained in infection control were carried out. Several failures in good practice standards were observed during the disinfection procedures of shower trolleys and mattresses of the hydrotherapy rooms. Chlorexhidin-based disinfectant liquid soap solution was used to disinfect shower mattresses, although this antiseptic agent is inappropriate for inert surface cleansing. Shower trolleys were disinfected with a glucoprotamin-based solution without leaving enough time for this agent to act efficiently. The plastic board under the shower mattress remained wet until reuse for the next patient, thus allowing growth of *P. aeruginosa* in this moist environment, as confirmed by environmental sampling. Finally, damaged areas of shower mattresses had been repaired with rubber patches, which were shown to contain *P. aeruginosa*. Following these observations, corrective infection control measures were implemented, including i) revision of the disinfection protocol of the shower trolley and mattress, ii) drying of wet surfaces on shower mattress after disinfection, iii) replacement of all damaged shower

mattresses, and iv) reinforcement of disinfection of sink traps of all rooms of the burn unit by pouring daily one liter of bleach down all sinks.

Follow-up screening

During a whole year following the implementation of the new infection control standards in the burn unit, clinical isolates of all patients hospitalized in the ICU were collected and genotyped. Three-monthly routine environmental samples were implemented in all ICU rooms and recovered *P. aeruginosa* isolates genotyped as well. DLST 1-18 was found in a single patient three months after the implementation of control measures. The only link with the outbreak was the hospitalization of this case in the burn unit in a room occupied six months earlier by one of the contaminated patients (room 725, Table 1). While DLST 1-18 had not been found in this room previously, it was recovered in October 2012 and then in January 2013 in the sink trap. Thereafter, this genotype was never recovered in this room or in any other location of the ICU during the following 12 months. The incidence of *P. aeruginosa* recovered from clinical samples in the ICU decreased from 44.7 per 1000 admissions in 2011 to 35.6 in 2012.

Discussion

We report an unrecognized two-year *P. aeruginosa* outbreak in a burn unit, uncovered after the implementation of a new DLST method. This fast and convenient technique, optimizing workflow by using 96-well plates, allowed retrospective and prospective genotyping of a large number of clinical and environmental isolates. This method gave unambiguous definitions of types facilitating comparison of strains and allowing the identification of this outbreak localized in the burn unit. In the follow-up period, it proved to be a useful tool to prospectively monitor all patients hospitalized in the ICU with clinical samples growing *P. aeruginosa*, thereby confirming the

complete eradication of the epidemic strain from hospitalized patients as well as from the environment of the burn unit. Next generation sequencing has emerged as the reference method and has been reported for epidemiological investigation of *P. aeruginosa* outbreaks, (11, 12). However, use of whole genome sequencing is currently limited to characterization of an outbreak strain and, unlike DLST, is not suitable for routine epidemiological surveillance.

P. aeruginosa is a well-recognized cause of nosocomial infections among burned patients, classically appearing more than 14 days after admission (1, 13). The remarkable ability of this organism to survive on wet surfaces allows widespread contamination of hospital environment in damp areas, such as sinks, traps and hosing (14). Once established in these environmental niches, *P. aeruginosa* contamination can persist for months within a unit, thereby allowing continuous transmission to patients exposed to these areas. Indeed, several *P. aeruginosa* outbreaks have been reported in burn units, mostly through contamination of hydrotherapy equipment, such as showers and connecting tubes, but also through contamination of disinfectant solutions (15-18). Likewise, contamination of the hydrotherapy equipment by DLST 1-18 was the confirmed source of the present outbreak, as this clone was not recovered from any other locations of other intensive care units, except for the sink trap of a single room of the neighboring unit. Contamination of burned patients hospitalized during overlapping periods most likely occurred in the hydrotherapy room, which served as a reservoir allowing the persistence of the clone during periods when no colonized or infected patients were hospitalized in the unit. A strong case in favor of this hypothesis is patient 19, in whom skin biopsies taken in the hydrotherapy room on the day of admission grew *P. aeruginosa*. On the other hand, three patients infected with DLST 1-18 had no direct

contact with the burn unit or the hydrotherapy room. One patient was hospitalized in the neighboring unit at the same time and in a bed next to patient 11, suggesting patient-to-patient transmission. For two patients, no epidemiological link could be found, suggesting another unrecognized way of transmission.

The persistent environmental transmission of DLST 1-18 could be successfully stopped after discovery of infection control failures and implementation of adequate corrective measures. Specifically, the avoidance of persistent wet surfaces, the appropriate use of disinfectants in the hydrotherapy room and the disinfection of sink traps yielded to the eradication of the epidemic strain DLST 1-18 from the environment. Indeed, except for a single case found in a patient hospitalized in a possibly contaminated room (positive sink trap), no further patient was contaminated with this strain after implementation of these corrective measures and follow-up environmental samplings showed complete disappearance of the strain during the following 12 months.

This study has several limitations. No routine screening of *P. aeruginosa* colonization was performed in patients hospitalized in our ICU. As skin biopsies for microbiological cultures were sampled on a regular basis as a standard of care in all burned patients, it is unlikely that cases infected or colonized with DLST 1-18 would have been missed in these patients during the investigation period. In non-burned patients, occult respiratory or digestive *P. aeruginosa* colonization cannot be ruled out. However, as most patients staying in the ICU have microbiological samples drawn from clinically relevant sites during their stay, we believe that potential missed cases among non-burned patients contributed little, if any, to the dissemination of the epidemic strain. Another potential limitation was the fact that systematic environmental samples were not available before 2012, raising the hypothesis of

another site of *P. aeruginosa* contamination within the ICU between 2010 and 2012. However, the heavy contamination of the hydrotherapy room in 2012 and the fact that DLST 1-18 was mostly recovered in burned patients support a persistent contamination of the hydrotherapy equipment as the main source of the outbreak.

Conclusions

DLST is a new and attractive genotyping method which can be implemented for the prospective epidemiological surveillance of *P. aeruginosa* strains in the ICU. This convenient and straightforward tool may play an important role in future years in the early detection of otherwise unrecognized outbreaks in the ICU.

Acknowledgments

Financial support: none reported.

Potential conflicts of interest: all authors report no conflicts of interest relevant to this article.

References

1. Morrison AJ, Jr., Wenzel RP. Epidemiology of infections due to *Pseudomonas aeruginosa*. Reviews of infectious diseases. 1984;6 Suppl 3:S627-42.

2. Gaynes R, Edwards JR, National Nosocomial Infections Surveillance S. Overview of nosocomial infections caused by gram-negative bacilli. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(6):848-54.

3. Blanc DS, Nahimana I, Petignat C, Wenger A, Bille J, Francioli P. Faucets as a reservoir of endemic *Pseudomonas aeruginosa* colonization/infections in intensive care units. Intensive care medicine. 2004;30(10):1964-8.

4. Bonten MJ, Bergmans DC, Speijer H, Stobberingh EE. Characteristics of polyclonal endemicity of *Pseudomonas aeruginosa* colonization in intensive care units. Implications for infection control. American journal of respiratory and critical care medicine. 1999;160(4):1212-9.

5. Cuttelod M, Senn L, Terletskiy V, Nahimana I, Petignat C, Eggimann P, et al. Molecular epidemiology of *Pseudomonas aeruginosa* in intensive care units over a 10-year period (1998-2007). Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011;17(1):57-62.

6. Berthelot P, Grattard F, Mahul P, Pain P, Jospe R, Venet C, et al. Prospective study of nosocomial colonization and infection due to *Pseudomonas aeruginosa* in mechanically ventilated patients. Intensive care medicine. 2001;27(3):503-12.

7. Petignat C, Francioli P, Nahimana I, Wenger A, Bille J, Schaller MD, et al. Exogenous sources of *Pseudomonas aeruginosa* in intensive care unit patients: implementation of infection control measures and follow-up with molecular typing.

Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America. 2006;27(9):953-7.

8. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of clinical microbiology. 1995;33(9):2233-9.

9. Botes J, Williamson G, Sinickas V, Gurtler V. Genomic typing of *Pseudomonas aeruginosa* isolates by comparison of Riboprinting and PFGE: correlation of experimental results with those predicted from the complete genome sequence of isolate PAO1. Journal of microbiological methods. 2003;55(1):231-40.

10. Basset P, Blanc DS. Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2014;33(6):927-32.

11. Witney AA, Gould KA, Pope CF, Bolt F, Stoker NG, Cubbon MD, et al. Genome sequencing and characterization of an extensively drug-resistant sequence type 111 serotype O12 hospital outbreak strain of *Pseudomonas aeruginosa*. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014.

12. Snyder LA, Loman NJ, Faraj LA, Levi K, Weinstock G, Boswell TC, et al. Epidemiological investigation of *Pseudomonas aeruginosa* isolates from a six-year-long hospital outbreak using high-throughput whole genome sequencing. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2013;18(42).

13. Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. Burns : journal of the International Society for Burn Injuries. 2004;30(4):357-61.

14. Douglas MW, Mulholland K, Denyer V, Gottlieb T. Multi-drug resistant *Pseudomonas aeruginosa* outbreak in a burns unit--an infection control study. Burns : journal of the International Society for Burn Injuries. 2001;27(2):131-5.

15. Pednekar SN, Dohe VB, Deshpande SM, Kamble SS, Bharadwaj RS, Shouche YS. An outbreak of *Pseudomonas aeruginosa* in a burn unit. Burns : journal of the International Society for Burn Injuries. 2010;36(7):e130-1.

16. Kolmos HJ, Thuesen B, Nielsen SV, Lohmann M, Kristoffersen K, Rosdahl VT. Outbreak of infection in a burns unit due to *Pseudomonas aeruginosa* originating from contaminated tubing used for irrigation of patients. The Journal of hospital infection. 1993;24(1):11-21.

17. De Vos D, Lim A, Jr., Pirnay JP, Duinslaeger L, Revets H, Vanderkelen A, et al. Analysis of epidemic *Pseudomonas aeruginosa* isolates by isoelectric focusing of pyoverdine and RAPD-PCR: modern tools for an integrated anti-nosocomial infection strategy in burn wound centres. Burns : journal of the International Society for Burn Injuries. 1997;23(5):379-86.

18. Tredget EE, Shankowsky HA, Joffe AM, Inkson TI, Volpel K, Paranchych W, et al. Epidemiology of infections with *Pseudomonas aeruginosa* in burn patients: the role of hydrotherapy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1992;15(6):941-9.

Table 1. Molecular typing of environmental strains from rooms of unit 3 (burn unit) and 4 (neighboring unit) during initial investigation (2012) and follow-up period (2013).

	March 2012	July 2012	November 2012	January 2013	March 2013
Rooms Unit 3					
722	25-11	25-11	-		-
723	-	6-7 / 8-37	-		-
724	-	-	-		-
725	1-21	-	1-18	1-18 / 1-21	25-11
726*	1-18	6-7	26-46		-
727	-	-	-		-
728*	1-18	1-18	-		-
Rooms Unit 4					
775	1-18	1-18	-		0-14
780	18-34	18-34	0-14		0-14 / 48-40
781	8-33	48-40	0-14		-
782	20-30 / 48-40	20-30 / 48-40	20-30		18-34
783	-	-	-		19-93

* hydrotherapy rooms

338 **Table 2.** Clinical characteristics and outcome of patients colonized or infected with *P.aeruginosa* genotype DLST 1-18

Patient	Sex, age	Underlying condition	Treated in the hydrotherapy room	Time between ICU admission and first *P. aeruginosa* recovery (days)	Site of *P. aeruginosa* infection or colonization	Outcome	Cause of death
1	F, 80	Heart failure	No	15	urine	survived	-
2	M, 81	Burn (13% BSA)	Yes	2	wound	survived	-
3	M, 50	Burn (38% BSA)	Yes	9	wound , sputum	survived	-
4	M, 27	Burn (22% BSA)	Yes	10	blood, wound	survived	-
5	F, 59	Necrotizing fasciitis	Yes	15	wound	survived	-
6	F, 20	Cystic fibrosis	No	2*	sputum, blood	survived	-
7	F, 82	Burn (18% BSA)	Yes	2	wound	survived	-
8	M, 60	Burn (90% BSA)	Yes	7	wound, sputum, blood	died	*P. aeruginosa* bacteremia
9	M, 28	Burn (72% BSA)	Yes	15	wound, sputum, blood	survived	-
10	F, 38	Burn (15% BSA)	Yes	14	wound	survived	-
11	M, 81	Mediastinitis	No	41	wound	survived	-
12	F, 67	Pneumectomy	No	34	sputum	survived	-
13	M, 70	Burn (80% BSA)	Yes	1	wound, sputum	died	*C.albicans* candidemia
14	F, 3	Burn (20% BSA)	Yes	5	urine, wound	survived	-
15	M, 61	Burn (15% BSA)	Yes	4	wound	survived	-
16	M, 53	Burn (88% BSA)	Yes	4	wound, sputum, blood	died	therapeutic withdrawal
17	F, 58	Burn (60% BSA)	Yes	34	wound, sputum, blood	died	*P. aeruginosa* bacteremia
18	F, 44	Burn (40% BSA)	Yes	15	wound, sputum, urine	survived	-
19	F, 51	Burn (97% BSA)	Yes	1	wound	died	refractory shock
20	F, 51	Burn (25% BSA)	Yes	21	wound	survived	-
21	M, 33	Burn (30% BSA)	Yes	7	wound, sputum, blood	survived	-
22	M, 57	Sacral pressure ulcer	Yes	8	wound, sputum	survived	-
23	M, 38	Burn (60% BSA)	Yes	7	wound, sputum	survived	-

339 BSA : body surface area.

340 *Already colonized with *P. aeruginosa* during previous hospital stays (no typisation of those strains were performed

Figure 1. Hospital stay of patients colonized or infected with the *P. aeruginosa* genotype DLST 1-18. Patients are numbered chronologically according to the time of first DLST 1-18 isolation.