International Journal of Hygiene and Environmental Health 220 (2017) 880–885

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

High prevalence and moderate diversity of *Pseudomonas aeruginosa* in the U-bends of high-risk units in hospital

Amélie Varin[a], Benoît Valot[b], Pascal Cholley[a,b], Camille Morel[a,b], Michelle Thouverez[a,b], Didier Hocquet[a,b,c], Xavier Bertrand[a,b,*]

[a] Hygiène Hospitalière, Centre Hospitalier Régional Universitaire, Besançon, France
[b] UMR 6249 Chrono-environnement, Université de Bourgogne-Franche-Comté, Besançon, France
[c] Centre de Ressources Biologiques – Filière Microbiologique de Besançon, Centre Hospitalier Régional Universitaire, Besançon, France

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form 2 March 2017
Accepted 9 April 2017

Keywords:
Pseudomonas aeruginosa
Plumbing
Water
Hospital
Population structure

ABSTRACT

The presence of *P. aeruginosa* in water supply is clearly identified as a risk factor for *P. aeruginosa* infection in critical care units, even if routes of transmission are often unclear and remain a matter of debate. We determined here the frequency of U-bends contaminated with *P. aeruginosa* in high-risk units and described the population structure of this opportunistic pathogen in a non-outbreak situation. Eighty-seven U-bends from sinks of rooms in five wards were sampled 3 times and *P. aeruginosa* was detected in 121 of the 261 (46.4%) U-bend samples. We genotyped 123 *P. aeruginosa* isolates with pulsed-field gel electrophoresis and multilocus sequence typing and found 41 pulsotypes distributed in 21 Sequence Types (STs). Seven major ST (ST111, CC235, CC253, ST520, ST539, ST1216, and ST1725) were overrepresented in the collection, including the high-risk clones ST111, CC253, and CC235. The distribution of the 21 STs in the cladogram of the species was uneven with most major STs clustering into 2 clades. The major STs were found in different units and buildings and could be represented by a high diversity of pulsotypes. Altogether, this suggests a long term presence of *P. aeruginosa* in the hospital water network, possibly contaminated by the distribution water or by plumbing fittings before putting into service. Analysis of resistance rates showed that the deficiency of porin OprD was very frequent in U-bends isolates that may benefit from this resistance mechanism in hospital water fittings. In conclusion, our study demonstrates that U-bends of high-risk units are very frequently contaminated with *P. aeruginosa* with a moderate genomic diversity and with an over-representation of adapted clones.

1. Introduction

P. aeruginosa is a common hospital-acquired pathogen in all hospital wards but particularly in intensive care units, where 10–15% of healthcare-associated infections are attributed to this pathogen (Kerr and Snelling, 2009). It is also ubiquitous in hospital water networks and water points (Venier et al., 2014). Indeed, in large buildings such as hospitals, this pathogen is present as a biofilm in many locations within the network of water supply disposal. *P. aeruginosa* thrives best in the distal parts of the water distribution system, such as taps, sinks, U-bends or toilets (Bedard et al., 2016). Although a consensus has not been reached regarding the role of hospital water supplies in *P. aeruginosa* acquisition, its presence in water supply of the rooms of intensive care units (ICUs) was identified as a risk factor for *P. aeruginosa* acquisition (Venier et al., 2014). Moreover, investigations of hospital outbreaks frequently retrieved epidemic clones in the water supply system (Witney et al., 2014). Water systems can act as a source of *P. aeruginosa* infection and patients' contamination by a *P. aeruginosa* water supply strain has been estimated at 15–50% in the literature, with many differences in the design of the studies (Blanc et al., 2004; Cholley et al., 2008; Rogues et al., 2007; Trautmann et al., 2006). However, few studies have explored the population structure of *P. aeruginosa* colonizing hospital water supplies of high-risk units in a non-outbreak setting. Here, we describe the population structure of a collection of isolates of *P. aeruginosa* retrieved from the U-bends of the rooms of ICUs and hematology units of a hospital, outside a period of outbreak with this pathogen.

* Corresponding author at: Hygiène Hospitalière, Centre Hospitalier Régional Universitaire, 3 boulevard Fleming, Besançon, Cedex 25030, France.
E-mail address: xbertrand@chu-besancon.fr (X. Bertrand).

http://dx.doi.org/10.1016/j.ijheh.2017.04.003
1438-4639/

2. Material and methods

2.1. Setting

This study was carried out over 8 weeks (January and February 2015) in two adult ICUs (one surgical ICU and one medical ICU) and three hematology units (two adult and one pediatric) at the 1200-bed Besançon University Hospital (Eastern France). The wards were located in two different buildings of the hospital. The two ICUs and the two adult hematology units were located in a building constructed in 1982. The pediatric hematology unit was in a building dating from 2012. In ICUs, the sinks were cleaned daily before pouring of the detergent-disinfectant Aniosurf® containing didecyldimethylammonium chloride, chlorexidine digluconate and polyhexamethylene biguanide hydrochloride (Anios, France) in the U-bends. Plumbing fittings were descaled weekly. Hematology units used bleach instead of Aniosurf® for the decontamination of U-bends. The study was approved by the hospital's review board. Of note, no outbreak of *P. aeruginosa* was detected in the hospital during the time of the study.

2.2. Environmental sampling and microbiological analysis

All U-bends from sinks of rooms in the five units were sampled 3 times two weeks apart. Eighteen sinks in medical ICU (MICU), 20 in surgical ICU (SICU), 18 in adult hematology unit 1 (AHU1), 19 in adult hematology unit 2 (AHU2) and 12 in pediatric hematology unit (PHU) were sampled for a total of 261 samples. For each of them, 50 ml of U-bend content were collected using a suction catheter and a syringe and centrifuged 5 min at 5000 *g*. The pellet was streaked on *Pseudomonas* selective agar plates containing cetrimide which were incubated for 48 h at 35 °C. *P. aeruginosa* colonies were first detected by standard microbiology methods (*i.e.* colony morphology, positive oxidase reaction, pigment production) and further identified by MALDI-TOF MS with a log value ≥ 2 according to the manufacturer's recommendations (Bruker Daltonik GmbH, Bremen, Germany).

We assessed the activity of 10 antibiotics from four different classes: non-carbapenem β-lactams (cefepime, piperacillin-tazobactam, ticarcillin, ceftazidime), carbapenems (meropenem, imipenem), aminoglycosides (gentamicin, tobramycin, amikacin), and fluoroquinolones (ciprofloxacin) against *P. aeruginosa* isolates by the disk diffusion method, as recommended by EUCAST 2015 (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf). Three resistance phenotypes were defined: 'wild-type' (susceptible to all the tested antibiotics), 'resistant' (non-susceptible to antibiotics from ≤ 2 classes), and 'multidrug-resistant' (non-susceptible to antibiotics from ≥ 3 classes). We also identified ESBLs and MBLs in isolates resistant to third-generation cephalosporins by the phenotypic method described elsewhere. Briefly, ESBL and MBL activities were inhibited by clavulanate and EDTA as a cation chelating agent, respectively (Hocquet et al., 2011). For isolates considered positive by this approach, the β-lactamases involved were identified by PCR and sequencing with primers targeting ESBL- and MBL-encoding genes (Hocquet et al., 2010).

2.3. Genotyping

The clonality of strains was investigated by PFGE with *Dra*I digestion as previously described (Slekovec et al., 2012). The banding patterns were analyzed by scanning photographic negatives. GelCompar software was used for analysis of PFGE patterns (Applied Maths, Kortrijk, Belgium). Pulsotypes (PTs) were defined according to international recommendations (Tenover et al., 1995). We determined the sequence type (ST) of one isolate representative of each pulsotype. Multilocus sequence typing (MLST) was performed according to the protocol of Curran et al. (Curran et al., 2004). *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/) was used for the assignment of allele numbers and ST.

2.4. Analysis of MLST data

In order to build a dendrogram with the 2335 STs available at the time of the study (including the new ST described in this collection), we concatenated the sequences of 7 MLST genes to form a 2882-bp sequences alignment, defining 1190 polymorphic positions. The best-fit, as determined with jModelTest 0.1.1 (Posada, 2008). We used the *Pseudomonas fluorescens* SBW25 (GenBank NC_012660.1) as the outgroup. Maximum likelihood tree was constructed with RAxML 7.2.8 using GTR + G + I nucleotide substitution model and partition model corresponding to the 7 MLST genes (Stamatakis, 2006). The results were visualized with Dendroscope 3.2.10 (Huson et al., 2007).

3. Results

In total, *P. aeruginosa* was detected in 121 of the 261 (46.4%) U-bend samples. The 87 water supplies were sampled three times: 18 were constantly negative for *P. aeruginosa*, 10 were constantly positive, the 59 remaining were positive once or twice. In other words, 69 out of 87 of the U-bends (79.3%) contained *P. aeruginosa* at least once. The proportion of U-bends samples containing *P. aeruginosa* varied according to the unit: 35% (21/60) in surgical ICU, 37% (20/54) in hematology unit 1, 52.6% (30/57) in hematology unit 2, 55.6% (20/36) in paediatric hematology unit and 55.6% (30/54) in medical ICU.

In 12 samples, we identified 2 phenotypically different *P. aeruginosa* isolates. PFGE genotyping revealed that 7 of these samples contained 2 phenotypically different isolates sharing the same pulsotype. In these cases, one isolate of the pair was discarded and not further analysed. In contrast, 5 U-bend samples contained 2 isolates with different pulsotypes that were kept for analysis. Finally, 123 isolates were genotyped by PFGE and MLST. PFGE analysis showed that the 123 isolates clustered in 41 pulsotypes (PT1 to PT41) which belonged to 21 different STs. Table 1 shows the distribution of the seven major STs identified: ST1725 (including 26 isolates), ST539 (14 isolates), ST540 (12 isolates), ST1216 (12 isolates), ST111 (11 isolates), ST622 (8 isolates), and ST520 (7 isolates). Others minor STs (ST27, ST179, ST244, ST277, ST308, ST313, ST395, ST481, ST1123, ST1158, ST1185, ST2263, ST2267, and ST2295) were represented by 1–4 isolates.

Antimicrobial resistance rates were higher in ICUs than in hematology units with the exception of imipenem (Fig. 1) for which resistance rates were higher than 60% in the two types of wards. In the hematology unit 1, 2 MDR isolates belonging to ST520 and ST622 and producing the class B carbapenemase IMP-29 were identified in the same U-bend 2 weeks apart.

The distribution of the 21 STs found in the U-bends in the cladogram of the entire species *P. aeruginosa* was uneven (Fig. 2). Hence, the 4 major ST520, ST539, ST540, and ST1216 and the minor ST1185 clustered in the clade I (colored in red in Fig. 2). Also, the 2 major ST622 and ST1725, and the 2 minor ST313 and ST2263 were found in the clade II (colored in green in Fig. 2). Of note, 6 out of the 7 major STs (*i.e.* frequently retrieved in the U-bends) clustered in either clade I or II.