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Hand soap contamination by *Pseudomonas aeruginosa* in a tertiary care hospital: whole genome sequencing ruled out any impact on patients

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Keywords: *Pseudomonas aeruginosa*, soap, contamination, molecular typing, whole genome sequencing, epidemiological investigation, ICU

Running title: Impact of contaminated soap on ICU patients

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Summary

Background

During an environmental investigation of *Pseudomonas aeruginosa* in ICUs, the liquid hand soap was found highly contaminated (up to $8x10^5$ cfu/g) with this pathogen. It had been used over the previous 5 months and was probably contaminated during manufacturing.

Aim

To evaluate the burden of this contamination on patients by conducting an epidemiological investigation using molecular typing combined with whole genome sequencing (WGS).

Methods

P. aeruginosa isolates from clinical specimens were analysed by double locus sequence typing (DLST) and compared to isolates recovered from the soap. Medical charts of patients infected with a genotype identical to those found in the soap were reviewed. WGS was performed on soap and patient isolates sharing the same genotype.

Findings

P. aeruginosa isolates (N=776) were available in 358/382 patients (93.7%). Only 3 patients (0.8%) were infected with a genotype found in the soap. Epidemiological investigations showed that the first patient was not exposed to the soap, the second could have been exposed, and the third was indeed exposed. WGS showed a high number of core SNPs differences between patients and soap isolates. No close genetic relation was observed between soap and patient isolates, ruling out the hypothesis of transmission.

Conclusions

Despite a highly contaminated soap, the combined investigation with DLST & WGS ruled out any impact on patients. Hand hygiene carried out with alcoholic solution for over 15 years

48 was probably the main reason. However, such contamination represents a putative reservoir of
49 pathogens which should be avoided in the hospital setting.
50
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52 **Keywords**: *Pseudomonas aeruginosa*, soap, contamination, molecular typing, whole genome
53 sequencing, epidemiological investigation, ICU
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Introduction

Pseudomonas aeruginosa is a ubiquitous environmental bacterium with minimal requirements for survival and a remarkable ability to adapt to a variety of environmental challenges. It is an opportunistic pathogen that can colonize and cause infection in patients who are immunocompromised or whose defences have been breached, and is thus a major cause of nosocomial infections in ICU patients [1]. The main reservoir of *P. aeruginosa* is humid environment. In the hospital setting, sinks, tap water, siphons, nutrition solutions, ultrasound gel, etc. were associated to patient contamination. Some of these reservoirs are difficult to avoid (taps, siphons), whereas others are preventable (solutions, gels, soaps, equipment, etc.).
An increase of *P.aeruginosa* infections in ICU patients led the infection control team of our hospital to investigate potential sources of infection and routes of transmission. Hand soap was analysed and found to be highly contaminated with *P. aeruginosa*. Subsequently, decision was taken to retrieve all soap containers from the hospital and to replace them by another brand. In order to evaluate the burden of this contamination on patients, we undertook a retrospective molecular epidemiological investigation using classical molecular typing, followed by whole genome sequencing (WGS) to increase the discriminatory power between selected isolates.

Material and Methods

Setting. The university hospital of Lausanne is a 1000-bed tertiary care hospital accounting for 36'000 admissions per year. Recommendation for hand disinfection with alcoholic solution was introduced in 1998. Hand washing with soap and water is only used when hands

are soiled. Liquid hand soap is available for patients and health care workers by all washbasins of the hospital and outpatient clinics. During the last years, three different types of soaps manufactured by the same company were used (Table I). Soap B was identical to soap A, except the addition of colouring. The composition of soap C was different in order to meet eco-label criteria. Methylchloroisothiazolinone and methylisothiazolinone (3:1) were used as preservative compounds in soap A and B at a final concentration of 0.0226%, whereas their final concentration was 0.0148% in soap C.

Bacterial isolates. *Soap isolates:* soap containers of batches still in use and other cosmetic products were analysed. Soap inoculums of 0.1 g and 10^{-1} serial dilutions were inoculated onto cetrimide agar and incubated at 37°C for 48 hours.

Clinical isolates: Routinely, clinical *P. aeruginosa* isolates for which an antibiogram is performed (clinical relevance) are stored at -20°C for one year. In general, one isolate per patient is collected every two weeks. All available *P. aeruginosa* isolates retrieved from clinical specimens during a 7-month period overlapping the period of exposure to the contaminated soap were selected for molecular analysis.

Molecular typing. Double locus sequence typing (DLST) was used to analyse *P. aeruginosa* isolates as previously described [2]. The method is based on partial sequencing of the *ms*172 (400 bp) and *ms*217 (350 bp) loci (www.dslt.org).

Whole Genome sequencing. Genomic DNA libraries were prepared using the Illumina Nextera XT DNA sample kit (Illumina, San Diego, USA). Subsequently, isolates were sequenced using an Illumina MiSeq platform generating paired-end reads with lengths of 150 bases. The isolates' sequence type (ST) was assigned from the short reads data using the SRST software [3]

Snippy (https://github.com/tseemann/snippy) was used to call the core SNPs in the sequence reads from the sequenced genomes as previously described [4]. The sequence reads were

aligned against the *P. aeruginosa* reference genome PAO1 (accession number NC_002516) [5] using Burrows-Wheeler Aligner. Afterwards, SAMtools and FreeBayes were used for variant calling under the following default settings: a minimum number of reads covering the variant position of 10, and a 0.9 minimum proportion of those reads that must differ from the reference. A "core site" was considered as a genomic position present in all samples, and with this program an alignment of the core genome was acquired. A maximum likelihood tree was generated from the core SNPs alignment enabled by Genealogies Unbiased by Recombination in Nucleotide Substitutions (GUBINNS) [6], which predicts and removes regions of high SNPs density suggestive of recombination.

Results

Hand soap contamination. In total, 83 soap containers of 17 different batches were analyzed (Table I). All containers (20/20) of the two most recent batches were highly contaminated with *P. aeruginosa*, whereas all others were negative. By crossing these data with the delivery date of each batch, we concluded that the contaminated soap had been used in the hospital between mid-August 2012 and the 11th of January 2013, date of the withdrawal of the soap. Unopened containers of the last delivered batch were also found positive for the presence of *P. aeruginosa*. The quantity of *P. aeruginosa* in the soap varied between $2x10^4$ and $8x10^5$ cfu/g. Thirty-six isolates retrieved from the contaminated soap containers were analysed by DLST. Two genotypes were observed, DLST 13-31 and 0-118, the former being more frequently observed in containers (found in 17/20 versus 3/20).

Hands contamination experiment. In order to evaluate if the use of the contaminated soap would contaminate the hands, two laboratory staff members washed their hands with the soap, rinsed them with tap water and dried them with disposable paper wraps. Then, fingerprints on cetrimide agar were performed. No *P. aeruginosa* was found on the hands of the person who

abundantly rinsed her hands, while many colonies were found on those of the person who only briefly rinsed her hands.

Patient analysis. As the exposition to the contaminated soap occurred from mid-August 2012 to mid-January 2013, the period of investigation was set-up from July 2012 to January 2013 (7 months). During this period, 382 patients had at least one clinical sample positive for *P. aeruginosa* (N=1730). A total of 776 isolates from 358/382 patients (93.7%) were recovered for typing (449 [58%] from respiratory tract samples, 143 [18%] from urines, 83 [11%] from wounds, 17 [2%] from blood cultures, and 84 [11%] from others sources). Classical molecular typing revealed that only 3 patients (0.8%) had clinical samples containing the DLST genotype 13-31 found in the soap, and no patient was infected with DLST 0-118. The first patient had community acquired *P. aeruginosa* mastoiditis and was not exposed to the soap. The second patient was hospitalized for a *P. aeruginosa* pyelonephritis. Transmission of *P. aeruginosa* from the contaminated soap was deemed possible during previous ambulatory visits. The third patient was admitted in the ICU and *P. aeruginosa* was recovered in respiratory secretions on day 3. *P. aeruginosa* transmission could have occurred during body washing.

Whole genome sequencing. WGS was performed to have a definitive answer on the possible transmission between the contaminated soap and the three patients. Two isolates from the soap (batches no. 05282 and 08184) and the three isolates from the patients were further analyzed by WGS. Five others clinical DLST 13-31 isolates obtained during our surveillance of *P. aeruginosa* in the ICUs outside the investigation period were also analyzed.

Sequence type (ST) assigned by SRST revealed that all 10 sequenced isolates belong to ST-155, which was highly concordant with DLST results since all 10 isolates were also from the same DLST type 13-31. Given that no ST 155 complete *P. aeruginosa* reference genome was

published so far, we proceeded with the analysis using the *P. aeruginosa* PAO1 reference genome.

The resulting phylogenetic tree obtained with all 10 sequenced isolates showed the occurrence of three major clades (A, B and C; Figure 1). Clade A comprised 5 clinical isolates recovered between 2003 and 2014. One subclade of this clade A contained isolates of patients 1 and 2, which appeared to be genetically closely related (49 SNPs differences). Patient 3 clustered with an isolate retrieved in January 2003, both constituting clade B. Clade C was further divided into a subclade composed of soap isolates 1 and 2. A high number of core SNPs differences was observed between patient 1, 2 and 3, and soap isolate 1 (219, 211, 259; respectively), as well as soap isolate 2 (219, 215, 267; respectively). Therefore, no close relation could be assumed between the contaminated soap isolates and the three patients deemed possibly contaminated.

Discussion

We report the added value of combining classical molecular typing with WGS to investigate the impact of highly contaminated hand soap with *P. aeruginosa* on patients of a tertiary care hospital. A large molecular investigation was first performed by a classical sequence-based typing method (DLST) followed by a deeper analysis with WGS on a few selected isolates. The workflow of DLST was optimized using 96-well plates and the analysis of data was simplified as unambiguous definitions of types were obtained. A large number of isolates could thus be analyzed in a relatively short period of time (2-3 days for 96 isolates). From this first molecular investigation, the hypothesis of a possible transmission from the contaminated soap was reduced to only 3 patients, targeting the use of WGS on a small number of selected isolates.

The phylogeny of the core SNPs alignment of 10 DLST 13-31 *P. aeruginosa* isolates contributed to a definitive conclusion. The three patients suspected to be contaminated by the

178 soap clustered in different clades from the one harboring the soap isolates. Additionally, a
179 high number of SNPs differences was observed between patients and soap isolates. Therefore,
180 it was possible to exclude a nosocomial acquisition of *P. aeruginosa* linked to the
181 contamination of the soap. Interestingly, isolates from patients 1 and 2 were closely related,
182 suggesting a possible transmission of the strain from one patient to the other through other
183 sources, such as environment or staff members.

184 Several nosocomial outbreaks have been attributed to contaminated liquid soaps [7-17]. All
185 reported outbreaks occurred in settings where hand hygiene was promoted by washing hands
186 with soap. In hospitals where hand hygiene is performed with an alcoholic solution, the
187 burden of such soap contamination might be lesser important. This is probably the main
188 reason why we did not find any impact of the contaminated soap on patients.

189 The fact that unopened soap containers were found contaminated with *P. aeruginosa* proved
190 that the contamination occurred during product manufacturing. According to the Swiss
191 regulation [18], no bacteriological quality is required for cosmetics, unless it is used on babies or
192 around the eyes, which is not the case for hand soap. Despite the fact that the contaminated
193 soap had no impact on patients, such additional reservoir of *Pseudomonas* should not be
194 tolerated in hospitals where high risk patients are present.

195 In the European Community, the microbial quality of cosmetics is regulated since July 2013
196 [19, 20]. Among microbiological criteria, the absence of *P. aeruginosa* must be demonstrated.
197 These new regulations should lead to safer cosmetic products, in particular those used in
198 hospitals.

199 In conclusion, the use of a high throughput molecular typing method (DLST) allowed us to
200 investigate a large number of isolates and select only those that were possibly linked to the
201 contaminated soap. The use of WGS on these few selected isolates allowed us to conclude
202 that the highly contaminated soap had no impact on the patients. This was probably due to the

fact that hand disinfection by soap was replaced by rubbing with an alcoholic solution. Nevertheless, we considered that such a contaminated product represented an unacceptable reservoir of a nosocomial pathogen in the hospital setting and the new European regulation on cosmetic safety should be a sufficient quality criterion for such products.

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Legend to figure

Figure 1. Phylogeny of *P. aeruginosa* sequenced isolates. Maximum-likelihood phylogenetic tree based on the core SNPs alignment (25173 SNPs in total) of 10 *P. aeruginosa* isolates. The tree was rooted by using *P. aeruginosa* PAO1 reference strain as an outgroup. The scale bar represents the mean number of substitutions per site. Patients 1 to 3 correspond to isolates of the three patients included in the study; soap isolates 1 and 2 indicate the isolates retrieved from two contaminated soap batches; and surveillance 1 to 5 represent clinical isolates recovered during routine epidemiological surveillance outside the study period. Phylogenetic clades A, B, and C are indicated.