Pseudo-outbreak of extremely drug-resistant *Pseudomonas aeruginosa* urinary tract infections due to contamination of the automated urine analyzer

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**Abstract**

**Background:** By the end of May 2010, an increase in the number of urines culture-positive for extremely drug-resistant (XDR) *Pseudomonas aeruginosa* was observed in our 800-bed university hospital. This led to an infection control alert. No epidemiological link between the patients and no increase in the frequency of XDR *P. aeruginosa* in non-urine samples were observed. Therefore, a pseudo-outbreak due to analytical contamination in the laboratory was rapidly suspected.

**Methods:** A prospective and retrospective search of cases was initiated and sampling of the automated urine analyzers used in the laboratory was performed. Antibiotypes were determined by disc-diffusion and genotypes by pulsed-field gel electrophoresis (PFGE).

**Results:** From February to July 2010, 17 patients admitted to 12 different departments and six outpatients were included. The mixing device of the cytometric analyser used for numeration of urinary particles (Sysmex UF1000i) was proved to be heavily contaminated. Isolates recovered from 12 patients belonged to the same antibio- and PFGE type as the isolate recovered from the analyzer. Extensive disinfection with a broad spectrum disinfectant and replacement of the entire tubing was necessary to achieve complete negativity of culture samples taken from the analyzer.

**Conclusion:** A pseudo-outbreak caused by a XDR *P. aeruginosa* clone was proved to be due to the contamination of the cytometric analyzer for urinary sediment. Users of such analyzers should be aware that contamination can occur and should always perform culture either before the processing of the urine sample on the analyzer or on a distinct sample tube.
**Introduction**

_Pseudomonas aeruginosa_ is a Gram negative aerobic rod that inhabits a wide range of bio-environments such as water, soil, rizosphere and animals. It is also known as a frequent opportunistic pathogen in both animals and humans. Due to its potential to develop resistance against multiple antibiotic and antiseptic compounds, _P. aeruginosa_ has also emerged as a major, difficult to treat, nosocomial pathogen. _P. aeruginosa_ is an important cause of morbidity and mortality in high risk patients such as immunosuppressed, cystic fibrosis, burn, cancer and ventilated intensive care patients. To date, countless _P. aeruginosa_ nosocomial outbreaks have been described either due to patient to patient transmission, environmental source or contaminated medical devices. _P. aeruginosa_ has also been reported as a cause of pseudo-outbreaks due to the contamination of media used for clinical specimen collection, transport or analysis.

The Sysmex UF1000i is an automated fluorescence flow cytometer used to count and classify cells and particle in urine samples. This automated system, which has been used in our 800 beds university hospital-serving laboratory since January 2010, is positioned as a rapid and sensitive screening method with high negative predictive value which avoids unnecessary culture of negative urine samples and presents large beneficial impact on both results turnaround time and laboratory economics. The present report describes a pseudo-outbreak that took place in our hospital from May to July 2010 and was due to the contamination of this urinary sediment analyzer by an extremely drug-resistant (XDR) _P. aeruginosa_.

**Material and Methods**

**Patients**
Cases, defined as “patients with a culture positive for *P. aeruginosa* resistant to ceftazidime, meropenem and ciprofloxacin”, were searched in the LIS database from January 2010 onwards.

**Routine clinical samples process**

Urine samples are collected in sterile containers. The urine is then transferred with vacuum system in sterile tubes without additive (Vacutainer, Becton Dickinson) and transported to the laboratory. The analytical procedure starts with plating 10 µL of urine on cystine lactose electrolyte deficient (CLED) medium (BioMérieux). The sediment analysis is then performed on the same tube by the UF1000i Urine flow cytometry analyzer (Sysmex). Finally, the tube is placed on the Auton Max AX-4280 (Menarini) for a semi-quantitative dipstick test (if requested). The CLED plates are incubated aerobically overnight at 35°C and interpreted quantitatively.

**Sampling of the analysers**

Vacutainer sterile tubes (n=6) filled with liquid Schaedler broth (Becton Dickinson) were processed as urine samples both serially and in parallel on both analyzers (UF1000i and Auton max), preceded and followed by quantitative culture (10 µL) on CLED agar. Ten µL of each reagent used by the analyzer were cultured on CLED agar as well. Plates were incubated aerobically 48h at 35°C and interpreted daily.

**Identification and antimicrobial susceptibility profiles**

Identification of *P. aeruginosa* isolates was based on positive arginine dihydrolase and cytochrome oxydase tests, a non-fermentative Kliegler and growth at 42°C. Antimicrobial susceptibility testing (AST) was performed by disc-diffusion on Mueller-Hinton agar incubated 24h at 35°C and interpreted according to CLSI criteria. AST profiles were considered as different if presenting 1 major (susceptible vs. resistant) or 2 minor (intermediate vs. susceptible or vs. resistant) discrepancies among 11 antibiotics: ceftazidime,
Pulsed-field gel electrophoresis (PFGE) profiles obtained after speI macrorestriction were analyzed with BioNumerics (Applied Maths) as previously described. The presence of VIM and IMP metallo-betalactamases genes was searched by PCR. Additionally, multilocus sequence typing (MLST), serotyping and multiplex PCRs targeting \( \text{bla}_{\text{SHV}}, \text{bla}_{\text{TEM}}, \text{bla}_{\text{HEL}}, \text{bla}_{\text{PER}}, \text{bla}_{\text{VEB}} \) and \( \text{bla}_{\text{GES}} \) alleles were performed on the isolate recovered from the analyzer as described by Glupczynski et al.

### Results

#### Background and description of the cluster

During the third and the fourth weeks of May 2010, six urine samples processed in the laboratory serving our 800 beds teaching hospital were culture-positive for 75,000 CFU/ml or more of XDR \( P. \text{aeruginosa} \). This led to an infection control alert.

#### Clinical and laboratory investigation

The medical records of the six patients implicated were reviewed to identify an epidemiological link between them while patients with positive culture for \( P. \text{aeruginosa} \)
resistant to ceftazidime, meropenem and ciprofloxacin were retrospectively searched from January 2010. No epidemiological link between the six patients (four patients were hospitalized in four distinct wards, two samples were from outpatients – Supplemental data, Figure 1) and no increase in the frequency of *P. aeruginosa* resistant to ceftazidime, meropenem and ciprofloxacin in non-urine samples were observed. Therefore, a pseudo-outbreak due to analytical contamination of the urine samples in the laboratory was rapidly suspected.

All the analyzer’s reagents that were in-use at that time were replaced and cultured. A sampling of the analyzers was conducted. Samples that underwent the complete routine analytical flow (UF1000i and Aution max, n=2) and samples from the UF1000i (n=2) were positive for *P. aeruginosa* (7.5x10^4 to >10^5 CFU/ml) while samples from the Aution Max (n=2) remained negative after 48h of incubation. All reagents were culture-negative.

The case definition was changed to “patient with an urine sample culture-positive for *P. aeruginosa* resistant to ceftazidime, meropenem and ciprofloxacin”. Using this second case definition, a total of 23 cases were included from January 1 to August 31: the six previously mentioned cases, three retrospective and 14 prospective cases (last case detected, July 26, – Supplemental data - Figure 1): seventeen patients admitted to 12 different departments and six outpatients. In four cases, the *P. aeruginosa* was co-cultured with another bacterial strain and four patients had another clinical site colonized or infected with an *P. aeruginosa* presenting the same AST profile. Of note, in 18 out of the 23 incriminated urine samples, the bacterial count executed by the UF1000i was below the inferior threshold of pathologic bacteriuria (set to 3500 bacteria/µL - Figure 1).

**Intervention and corrective measures**
A reminder was made to the technical laboratory team to respect (1) the standard routine sample process (the culture has to be performed before processing the sample on the analyzers) and (2) the standard protection procedure (laboratory coat and gloves). Physicians in charge of the patients implicated were informed by phone of the analytical contamination problem and were asked to send a control urine sample.

The first disinfection procedure advised by the supplier of the UF1000i (Sysmex Belgium), using 99% methanol processed as routine urine samples 10 times twice a day, failed to reach the negativity of the control samples taken from the analyzer. An extensive disinfection of the UF1000i analyzer with a broad spectrum disinfectant (Virkon® - potassium peroxymonosulphate) failed as well. Finally, the replacement of the entire tubing of the analyzer (including the mixing device) combined to a second extensive disinfection with Virkon was necessary to achieve culture-negative control samples. Meanwhile, and despite several reminders, few non-observances to our standard routine sample process continued to happen as new cases occurred until the disinfection of the analyzer was achieved.

A weekly disinfection procedure of the UF1000i using 99% methanol followed by control sampling was implemented.

**AST profiles and molecular analysis**

*P. aeruginosa* isolates of 17 out of 23 cases displayed the same XDR AST profile as the isolate recovered from the UF1000i: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, gentamycin, tobramycin imipenem, meropenem, and ciprofloxacin resistant, amikacin and colistin susceptible. Thirteen of these 17 XDR *P. aeruginosa* belonged to the same PFGE type (ZT4) as the isolate recovered from the analyser while four isolates were unavailable for molecular analysis (Figure 1). All the 13 PFGE ZT4 isolates but one (belonging to a patient with other body sites positive for XDR *P. aeruginosa*) were VIM-positive, as was the isolate recovered from the analyzer. The remaining six isolates harbored
unrelated antibio- and PFGE types. The extensive characterization of the isolate recovered from the analyzer revealed a MLST type 235, a serotype O15 and the production of a VIM-2 metallo-carbapenemase and a PER-1 extended-spectrum-beta-lactamase.

With a final definition of “patient with an urine sample positive for *P. aeruginosa* resistant to all antimicrobial tested except amikacin and colistin, PFGE type ZT4 and VIM positive”, 12 patients out of 23 were classified as definite cases, and the four patients presenting a *P. aeruginosa* with compatible AST profiles but unavailable for molecular analysis were classified as possible cases. Among these 16 patients, no other body sites or control urine sample was found positive for XDR *P. aeruginosa* as opposed to six out of the seven excluded cases (Figure 1). One of these 16 patient received one day of treatment by colistin, before his control urine sample was confirmed negative.

**Discussion**

Clinical, microbiological and molecular evidences were all consistent with our hypothesis of a pseudo-outbreak, involving 12 definite and four possible cases, due to the contamination of the UF1000i cytometric analyzer for urinary sediment by an XDR *P. aeruginosa* strain. This XDR *P. aeruginosa* strain was found to produce both a VIM metallo-carbapenemase and a PER extended-spectrum-beta-lactamase. It belongs to the MLST235 international clone, known to be widely disseminated in Belgium and in Europe. However, this strain belongs to serotype O15, which is uncommon in Belgium where the serotype O11 is predominant among ST235 multidrug resistant *P. aeruginosa*. This VIM-positive ZT4 O15 XDR *P. aeruginosa* clone was first detected in our hospital in 2008, as the cause of an outbreak in our hematology unit. Concerning the present pseudo-outbreak, however, we failed to identify a potential « index patient », whom urine sample could have contaminated the analyzer.
This pseudo-outbreak happened with the help of a few non-observances to our standard routine sample process (culture performed before processing on the analyzers). These non-observances, although proportionally minimal (the monthly activity is of 3000 urines), continued to happen despite the reminder made to the team to respect the standard routine flow. We finally found out that some members of the team had the habit to process samples on the analyzers before performing the culture when dealing with urgent samples coming from the emergency room to shorten the turnaround time of sediment analysis results.

During the present pseudo-outbreak, cultures were significantly contaminated. Conversely, bacterial counts executed by the analyzer were “not influenced” by the contamination as they remained, in most cases, under the threshold of pathologic bacteriuria, leading to discordant results between the sediment analysis and the culture. This, together with the unusual XDR AST profile of the contaminating strain, led to the early recognition of the pseudo-outbreak which ended with very limited consequences regarding the patient care.

The UF1000i analyzer uses a single analysis needle equipped with a rinsing system designed to prevent sample to sample carry-over in the bacterial count. It consists in two automatic rinsing cycles with buffer in-between samples. However, the mixing device which mixes the samples prior to analysis by pumping it up and down is connected to a second needle which is not involved in the rinsing circuit and thus never properly rinsed. This is probably the reason why this contamination happened, together with the ability of the \textit{P. aeruginosa} species to form biofilm on plastic devices. Consequently, a weekly disinfection procedure was implemented that consists in methanol processed and mixed as routine urine samples. The present contamination did not influence the bacterial counts executed by the analyzer. Contamination of the analyzer is thus not interfering with the final result as long as culture is performed either before the processing on the analyzer (as in our laboratory) or on a distinct sample tube. However, in both case scenarios, the economic benefit of the UF1000i,
that lies in the simplification of the workflow and the prevention of unnecessary cultures,\textsuperscript{9,10} is lower than expected; notwithstanding the biohazard incurred by the laboratory technicians working with the analyzer.

In conclusion, we faced a pseudo-outbreak caused by an XDR \textit{P. aeruginosa} clone due to the contamination of the UF1000i cytometric analyzer for urinary sediment. Users of such analyzers should be aware that contamination can occur and should consider to perform regular sterility controls of their analyzer. Furthermore, culture should always be performed either before the processing of the urine sample on the analyzer or on a distinct sample tube.


