Pseudo-Outbreak of Extremely Drug-Resistant
\textit{Pseudomonas aeruginosa} Urinary Tract Infections
Due to Contamination of an Automated Urine Analyzer

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By the end of May 2010, an increase in the number of urine specimens that were culture positive for extremely drug-resistant (XDR) \textit{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 \textit{P. aeruginosa} in non-urine samples was observed. Therefore, a pseudo-outbreak due to analytical contamination in the laboratory was rapidly suspected. A prospective and retrospective search of cases was initiated, and the sampling of the automated urine analyzers used in the laboratory was performed. Antibiotypes were determined by disc diffusion, and genotypes were determined by pulsed-field gel electrophoresis (PFGE). From February to July 2010, 17 patients admitted to 12 different departments and 6 outpatients were included. The mixing device of the cytometric analyzer used for the enumeration of urinary particles (Sysmex UF1000i) proved to be heavily contaminated. Isolates recovered from 12 patients belonged to the same antibiotypetype and PFGE type as the isolate recovered from the analyzer. Extensive disinfection with a broad-spectrum disinfectant and the replacement of the entire tubing was necessary to achieve the complete negativity of culture samples taken from the analyzer. A pseudo-outbreak caused by an XDR \textit{P. aeruginosa} clone was proven 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 tubec.

\textit{Pseudomonas aeruginosa} is a Gram-negative aerobic rod-shaped bacterium that inhabits a wide range of environments, such as water, soil, the rhizosphere, and animals (11). It also is known as a frequent opportunistic pathogen in both animals and humans. Due to its potential for developing resistance against multiple antibiotic and antiseptic compounds, \textit{P. aeruginosa} also has emerged as a major, difficult-to-treat nosocomial pathogen. \textit{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 (11). To date, countless \textit{P. aeruginosa} nosocomial outbreaks have been described that were caused by patient-to-patient transmission, environmental sources, or contaminated medical devices (5, 8–10). \textit{P. aeruginosa} also has been reported as a cause of pseudo-outbreaks due to the contamination of media used for clinical specimen collection, transport, or analysis (6, 12, 13).

The Sysmex UF1000i is an automated fluorescence flow cytometer used to count and classify cells and particles in urine samples. This automated system, which has been used in our 800-bed university hospital laboratory since January 2010, is established as a rapid and sensitive screening method with high negative predictive value which avoids the unnecessary culture of negative urine samples and presents large beneficial effects for both result turnaround time and laboratory economics (2, 4). The present report describes a pseudo-outbreak that took place in our hospital from May to July 2010 that was caused by the contamination of this urinary sediment analyzer by an extremely drug-resistant (XDR) isolate of \textit{P. aeruginosa}.

\textbf{MATERIALS AND METHODS}

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

\textbf{Routine clinical sample process.} Urine samples are collected in sterile containers. The urine then is transferred in a 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 then is performed on the same tube by the UF1000i urine flow cytometry analyzer (Sysmex). Finally, the tube is placed on the Aution Max AX–4280 (Menarini) for a semiquantitative dipstick test (if requested). The CLED plates are incubated aerobically overnight at 35°C and interpreted quantitatively.

\textbf{Sampling of the analyzers.} 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 Aution Max), preceded and followed by quantitative culture (10 µl) on CLED agar. Ten µl of each reagent used by the analyzer was cultured on CLED agar as well. Plates were incubated aerobically for 48 h at 35°C and interpreted daily.
Identification and antimicrobial susceptibility profiles. The identification of *P. aeruginosa* isolates was based on positive arginine dihydrolase and cytochrome oxidase tests, a nonfermentative Klügler, and growth at 42°C.

Antimicrobial susceptibility testing (AST) was performed by disk diffusion on Mueller-Hinton agar incubated for 24 h at 35°C and was interpreted according to CLSI criteria. AST profiles were considered different if presenting 1 major (susceptible versus resistant) or 2 minor (intermediate versus susceptible or resistant) discrepancies among 11 antibiotics: ceftazidime, cefepime, piperacillin-tazobactam, aztreonam, imipenem, meropenem, gentamicin, amikacin, tobramycin, ciprofloxacin, and colistin. XDR *P. aeruginosa* was defined as resistance to all antipseudomonal antimicrobial classes except colistin.

Molecular characterization. Pulsed-field gel electrophoresis (PFGE) profiles obtained after SpeI macrorestriction were analyzed with BioNumerics (Applied Maths) as previously described (3). The presence of VIM and IMP metallo-beta-lactamase genes was determined by PCR (1).

Additionally, multilocus sequence typing (MLST), serotyping, and multiplex PCRs targeting *bla*$_{GES}$, *bla*$_{GES}$, *bla*$_{ZET}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{GES}$, and *bla*$_{MIR}$ alleles were performed on the isolate recovered from the analyzer as described by Głąpczyński et al. (7).

Medical record review. For each case, the medical record was reviewed for infection or colonization by XDR *P. aeruginosa* at any body site within the year preceding the incriminating urine sample and for results of control urine culture and antimicrobial treatment received within the week following the incriminating urine sample.

RESULTS

Background and description of the cluster. During the third and fourth weeks of May 2010, six urine samples processed in the laboratory serving our 800-bed teaching hospital were culture positive for 75,000 CFU/ml or more of XDR *P. 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 cultures positive for *P. aeruginosa* resistant to ceftazidime, meropenem, and ciprofloxacin were searched retrospectively from January 2010 onwards. No epidemiological link between the six patients (four patients were hospitalized in four distinct wards, and two samples were from outpatients; see Fig. S1 in the supplemental material) 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 the analytical contamination of the urine samples in the laboratory was rapidly suspected.

All of 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 complete routine analytical flow (UF1000i and Aution Max; n = 2) and samples from the UF1000i (n = 2) were positive for *P. aeruginosa* 

\[ 7.5 \times 10^{4} \text{ to } 10^{5} \text{ CFU/ml}, \]

while samples from the Aution Max (n = 2) remained negative after 48 h of incubation. All reagents were culture negative.

The case definition was changed to “patient with a 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 6 previously mentioned cases as well as 3 retrospective and 14 prospective cases (the last case was detected on July 26; see Fig. S1 in the supplemental material). Seventeen patients were admitted to 12 different departments, and there were 6 outpatients. In four cases, *P. aeruginosa* was cocultured with another bacterial strain and four patients had another clinical site colonized or infected with *P. aeruginosa* presenting the same AST profile. Of note, in 18 of the 23 incriminated urine samples, the bacterial count determined by the UF1000i was below the inferior threshold of pathobacteriuria (set to 3,500 bacteria/µl; see Fig. S1).

Intervention and corrective measures. A reminder was made to the technical laboratory team to respect (i) the standard routine sample process (i.e., culture has to be performed before processing the sample on the analyzers) and (ii) the standard protection procedure (i.e., the use of laboratory coats 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 samples processed as routine urine samples 10 times twice a day, failed to reach the level of 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 with a second extensive disinfection with Virkon was necessary to achieve culture-negative control samples. Meanwhile, and despite several reminders, a few nonobservances of 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, gentamicin, tobramycin imipenem, meropenem, and ciprofloxacin resistant but amikacin and colistin susceptible. Thirteen of these 17 XDR *P. aeruginosa* samples belonged to the same PFGE type (ZT4) as the isolate recovered from the analyzer, while four isolates were unavailable for molecular analysis (see Fig. S1 in the supplemental material). All of the 13 PFGE ZT4 isolates except 1 (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 antibiograms and PFGE types. The extensive characterization of the isolate recovered from the analyzer revealed MLST type 235, 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 antimicrobials tested except amikacin and colistin, PFGE type ZT4, and VIM positive,” 12 patients out of 23 were classified as definite cases, and the 4 patients presenting *P. aeruginosa* with compatible AST profiles but unavailable for molecular analysis were classified as possible cases. Among these 16 patients (12 definite and 4 possible), no other body sites or control urine sample was positive for XDR *P. aeruginosa*, as opposed to six out of the seven excluded cases (see Fig. S1 in the supplemental material). One of these 16 patients received 1 day of treatment by colistin before his control urine sample was confirmed to be negative.
DISCUSSION
Clinical, microbiological, and molecular evidences all were consistent with our hypothesis of a pseudo-outbreak, involving 12 definite and 4 possible cases, due to the contamination of the UF1000i cytometric analyzer 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, which is known to be widely disseminated in Belgium and in Europe (7). However, this strain belongs to serotype O15, which is uncommon in Belgium, where serotype O11 is predominant among ST235 multidrug-resistant *P. aeruginosa* isolates (7). This VIM-positive ZT4 O15 XDR *P. aeruginosa* clone was first detected in our hospital in 2008 (7) as the cause of an outbreak in our hematology unit. Concerning the present pseudo-outbreak, however, we failed to identify a potential index patient from whom a urine sample contaminated the analyzer.

This pseudo-outbreak happened with the help of a few nonob-servances of our standard routine sample process (i.e., culture performed before processing samples on the analyzers). These nonob-servances, although proportionally minimal (the monthly activity is 3,000 urine specimens), continued to happen despite the reminder made to the team to respect the standard routine operations. We finally learned that some members of the team had the habit of processing 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 significan-tly contaminated. Conversely, bacterial counts executed by the analyzer were not influenced by the contamination, as they remained, in most cases, under the threshold of pathological bacte-riuria, 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 patient care.

The UF1000i analyzer uses a single analysis needle equipped with a rinsing system, which is designed to prevent sample-to-sample carryover in the bacterial count. It consists of two automatic rinsing cycles with buffer between samples. However, the device which mixes the sample 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 is never properly rinsed. This, together with the ability of the *P. aeruginosa* species to form biofilms on plastic devices, is probably the reason why this contamination happened (8). Consequently, a weekly disinfection procedure was implemented that consists of methanol samples processed and mixed as routine urine samples. The present contamination did not influence the bacterial counts executed by the analyzer. The contamination of the analyzer thus does not interfere with the final result as long as the 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, which lies in the simplification of the workflow and the prevention of unnecessary cultures (2, 4), is lower than expected, not to mention the biohazard to the laboratory technicians working with the analyzer.

In conclusion, we faced a pseudo-outbreak caused by an XDR *P. aeruginosa* clone due to the contamination of the UF1000i cytometric analyzer. Users of such analyzers should be aware that contamination can occur and should consider performing 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.

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