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Pseudo-outbreak of *Pseudomonas putida* Respiratory Infection Caused by Laboratory Contamination

To the Editor—Pseudomonas putida is a gram-negative, aerobic bacterium that is a common inhabitant of soil, plants, and water. It has been found to cause septicemia in immunocompromised patients,[1,2] and nosocomial transmission has been reported, associated with contaminated heparin or antiseptic solutions.[3-5] Nevertheless, its isolation from clinical specimens is rare, and it is usually considered an environmental contaminant. *P. putida* has also been reported as a cause of pseudo-outbreaks in contaminated urine collection kits and contaminated commercial antifog solutions.[6,7] We now report a pseudo-outbreak of *P. putida* respiratory infections, involving 5 patients, caused by an automated spiral plater.

The infection control unit was notified on October 2, 2009, of a cluster of *P. putida* isolations from blind distal bronchial samples from 3 patients undergoing ventilation in the intensive care unit (ICU). The first positive sample had been recorded 5 days before, and the two others the day of notification. In each sample, *P. putida* was isolated among other bacteria at a significant level (at least 10^3 cfu/mL). Review of the previous 3 months of microbiology laboratory records found 2 other instances of *P. putida* isolation, one from a bronchial sample of another ICU patient and the other from sputum of a patient in the pulmonology department. All isolates presented an identical antibiotic susceptibility pattern. During the same period, *P. putida* was not isolated from other clinical specimens. A thorough ward-based investigation revealed no epidemiological link to suggest cross-infection between the patients. In particular, the pulmonology department patient had never been hospitalized in the ICU and did not share any device with the ICU patients. Therefore, the investigation focused on the microbiology laboratory, where the 5 samples were processed by the same device (a Whitley Automated Spiral Plater WASP 2; Don Whitley Scientific) dedicated to the clinical respiratory samples.

The WASP 2 was used for many years without any problem. It is a fully automated spiral plater, able to load a sample with a stylus, inoculate a plate, clean the stylus in a sanitizing solution (70% alcohol), and finally rinse the stylus with sterile water loaded from a 110-mL reusable container (Figure 1). The recommendation of the WASP 2 user manual[8] is to sterilize the containers filled with sterile water by autoclaving.

Laboratory procedures for handling specimens and cleaning processes were reviewed with laboratory personnel. Aseptically collected samples of domestic water, demineralized rinse water, stylus, sanitizing solution, and each of the 12 reusable containers were obtained for bacterial culture. For the stylus, a 100-μL aliquot of sterile water was loaded by the stylus and directly deposited on a plate. For domestic water, demineralized water, sanitizing solution, and reusable containers containing 110 mL of sterile water, 100 mL of liquid were filtered and inoculated on plates. Cultures of all specimens were obtained using conventional microbiologic methods. Restriction endonuclease DNA profiles were determined by pulsed-field gel electrophoresis for all available isolates, using the restriction enzyme *Spe*I.

There was no change in personnel, microbiological technique, or culture medium. The review of the procedures with the microbiology laboratory personnel revealed a change in the process of reusable-container disinfection a few weeks before the first case occurred. The autoclave usually used to sterilize the containers filled with sterile water had broken down, and an alternate procedure consisting of a chemical disinfection was performed until the autoclave was repaired. However, as the written procedure requested a rinse with sterile water after immersion in a bactericidal solution (DDN 250, Franklab Laboratory) for 60 minutes, the employee responsible for container disinfection had immersed the con-

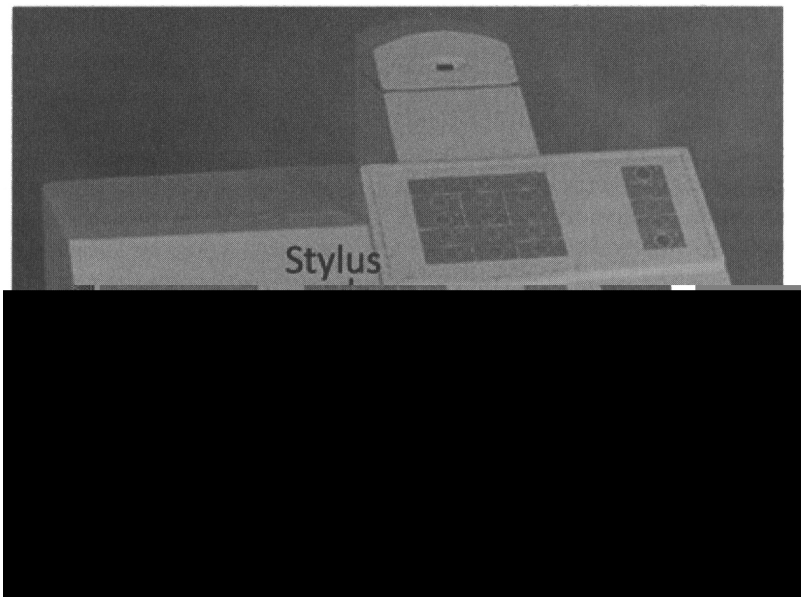

FIGURE 1. Whitley automated spiral plater (WASP 2). *1*, The stylus loads the clinical sample. *2*, The plate is inoculated. *3*, The stylus is disinfected in the sanitizing solution. *4*, The stylus is rinsed with sterile water in reusable containers and become available for the next clinical sample.

tainer in a bactericidal solution, then rinsed it with demineralized water, and finally filled it with sterile water. Isolates of *P. putida* were obtained from 4 of the 12 reusable containers. No other bacteria were found. No other environmental sample was contaminated. Environmental and clinical strains isolated from respiratory samples had identical pulsotypes.

Although we did not find the source of the contamination of the reusable containers, the most likely explanation is an unrecognized transitory contamination of the demineralized water network of the laboratory by *P. putida*, which is a common inhabitant of water. As containers were rinsed with demineralized water after chemical disinfection was performed, introduction of a few strains of *P. putida* could lead to container colonization. Therefore, when the contaminated container was used on the WASP 2, it could contaminate a clinical sample, because rinsing of the stylus with sterile water occurs after decontamination of the stylus in the sanitizing solution and just before the next sampling and the inoculation on the plate.

The microbiology laboratory is a well-recognized source of pseudo-outbreaks, which usually follow a change in personnel, technique, or culture medium. Among 20 reported pseudo-outbreaks, Weinstein and Stamm[9] identified 7 that originated within the laboratory. In our report, the cause of the pseudo-outbreak was clearly related to the replacement of the recommended autoclaving by a new process, misunderstood by the employee in charge of container disinfection.

This report illustrates the potential consequences of inappropriate disinfection practices in a microbiology laboratory. Pseudo-outbreaks are time-consuming and sometimes expensive and, more importantly, could lead to unnecessary treatment of patients with a false positive diagnosis. As *P. putida* is not a common cause of infection outbreaks, infection control units should suspect a pseudo-outbreak if a cluster of isolations of such an uncommon pathogen is observed.

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