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Spread of *Pseudomonas fluorescens* Due to Contaminated Drinking Water in a Bone Marrow Transplant Unit[▽]

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***Pseudomonas* infections are an important cause of morbidity and mortality in immunocompromised patients. We present here data for the spread of *Pseudomonas fluorescens* caused by a contaminated drinking water dispenser in a bone marrow transplant unit. Over a 1-month period we observed a sharp increase in the isolation of *P. fluorescens* from weekly pharyngeal surveillance swabs. Environmental samples were taken from a variety of water sources throughout the unit. These samples were cultured on cetrimide agar medium, and isolates were epidemiologically characterized by antibiotic susceptibility patterns and molecular typing methods. Nine patients became colonized with *P. fluorescens*, and six out of the nine developed febrile neutropenia. *P. fluorescens* was cultured after the filtration of 100 ml of drinking water from one of two stand-alone chiller units supplying cooled bottled water to the bone marrow transplant unit. All other environmental samples were negative. There were no further cases of *P. fluorescens* colonization after the contaminated dispenser was removed. Molecular typing showed that all *P. fluorescens* isolates were identical by both random amplification of polymorphic DNA PCR and pulsed-field gel electrophoresis. We recommend that such bottled water supplies not be used in high-risk areas or be subject to regular microbiological monitoring.**

Pseudomonas infections are an important cause of morbidity and mortality in immunocompromised patients (16), with *Pseudomonas aeruginosa* being the most common species isolated from clinical specimens (11). *Pseudomonas fluorescens* is a member of the fluorescent pseudomonad group and (unlike *P. aeruginosa*) has generally been regarded to be of low virulence and an infrequent cause of human infection (7). However, it has been reported to cause infections such as blood transfusion-related septicemia (8, 13), catheter-related bacteremia (7), and peritonitis in peritoneal dialysis patients (15).

Pseudomonas fluorescens is a rod-shaped aerobic, non-lactose-fermenting, Gram-negative bacterium (2). It can survive and replicate in moist reservoirs, and as a result, nosocomial outbreaks often lead to the investigation of water sources (1). Optimal growth generally occurs at lower temperatures than those for *P. aeruginosa*, which can make identification difficult at the standard microbiology laboratory incubation temperature of 37°C (11). It can grow at temperatures as low as 4°C, temperatures at which blood products, distilled water, and disinfectants provide the ideal environment for proliferation.

To date, most clinical reports of *P. fluorescens* outbreaks have involved immunocompromised patients. The Centers for Disease Control and Prevention (CDC) recently described an outbreak of *P. fluorescens* bacteremia in cancer outpatients for whom syringes with an intravenous heparin catheter flush were being used on implantable venous ports (5). There was also a previous report of an outbreak of catheter-related bacteremia in four oncology patients, the source of which was not identified (7). Furthermore, the organism has been linked to outbreaks of pseudobacteremia from the contamination of disinfectants (14) and blood collection tubes (10).

Over a recent 1-month period, we observed a sudden increase in rates of *P. fluorescens* isolation from weekly surveillance pharyngeal swabs from hematology patients in the bone marrow transplant unit of our teaching hospital. This unusual phenomenon provoked us to investigate the cause, and here we report the findings of our epidemiological and microbiological investigation.

MATERIALS AND METHODS

Background. All hematology patients at our center are routinely screened for *Pseudomonas* species in their stool and pharynx. From 31 January to 13 March 2010, 9 out of 41 (22%) hematology inpatients were identified as being colonized with a meropenem-resistant strain of *P. fluorescens* from weekly surveillance pharyngeal swabs. Their screening stool samples from this period were negative for this organism. These patients were all inpatients of the bone marrow transplant unit of our 1,663-bed teaching hospital in the United Kingdom, which was comprised of 18 isolation rooms with en suite facilities. Upon review of the microbiology database, no *Pseudomonas fluorescens* isolates had been isolated from pharyngeal swabs or stool samples from hematology patients 16 months prior to this period. Therefore, these findings raised the possibility of a common source of *P. fluorescens* and prompted us to investigate further.

Epidemiological investigation. An environmental sampling program was instigated on 16 March 2010 following the identification of five of the nine cases from pharyngeal specimens taken on 7 March 2010.

Volumes of 100 ml of water were sampled in 250-ml sterile bottles (Sterilin Ltd., London, United Kingdom) from the commercially supplied drinking water delivered by two dispensers and from hand washbasins and showers throughout the unit. Each sample was filtered through a Microfil V filtration device that consisted of a 47-mm-diameter, 0.45-μm-pore-size membrane (Millipore, MA). The filtrate was then immediately seeded onto *Pseudomonas*-selective cetrimide agar (Oxoid Ltd., Basingstoke, United Kingdom). Other environmental samples were taken with cotton swabs (premoistened with sterile water) (Bunzi Healthcare UK, London, United Kingdom) from a variety of sites, including the cleaning equipment, nozzles of the two drinking water dispensers, and taps and drains of the washbasins and showers. The surfaces were rubbed four times with the

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same swab, and the swabs were then plated directly onto cetrimide agar. Afterwards, the samples were transported to the microbiology laboratory for incubation.

The commercial water dispenser consisted of a polycarbonate 18.4-liter bottle of natural still spring water bottled at the source and placed on top of a refrigerated (chiller) unit supplied from the electrical mains system. The dispenser can provide 10 liters per hour of cooled water at 10°C, an estimate based on an ambient temperature of 32°C. Patients filled plastic cups, dispensed from the side of the machine, by pressing a button on the unit to pour the water. Water was sampled via the nozzle of the chiller unit and not directly from the bottle before or after installation.

The medical records of all patients for whom a positive culture of *P. fluorescens* had been recovered were reviewed. Data collected included demographic characteristics, underlying diagnoses, symptoms, white blood cell counts, microbiology results, antibiotic prophylaxis and therapy, and outcome.

Microbiology. The pharyngeal swabs of the patients and environmental samples were cultured on cetrimide agar medium at 37°C for 24 h under aerobic conditions. Identification was done on the basis of the colony morphology, oxidase reaction, and biochemical profiles obtained by the Vitek Gram-negative identification (GNI) card (bioMérieux-Vitek, Basingstoke, United Kingdom). In addition, CFU of bacterial growth in the environmental samples was assessed. All isolates underwent genotypic analysis by using random amplification of polymorphic DNA (RAPD) PCR and pulsed-field gel electrophoresis (PFGE). RAPD PCR was performed with primer D10514, as described previously (17). The bacterial isolates were also analyzed by using PFGE of SpeI-digested genomic DNA according to standard methodologies (12).

MICs for the isolates were then determined by the Etest (PDM Epsilometer test; AB Biodisk, Solna, Sweden) with Mueller-Hinton agar (Oxoid Ltd., Basingstoke, United Kingdom) after 24 h of incubation at 30°C. This low temperature was used because there was a problem with a poor growth of *P. fluorescens* at the standard temperature of 35°C ± 2°C. The antibiotics tested were ciprofloxacin, gentamicin, meropenem, piperacillin-tazobactam, ceftazidime, tobramycin, aztreonam, and colistin. The MIC breakpoints for defining susceptibility were in accordance with Clinical and Laboratory Standards Institute (CLSI) standards (3).

RESULTS

Patient characteristics. The clinical features of nine patients with *P. fluorescens* isolated in their pharyngeal swabs are shown in Table 1. The mean age was 47 years (range, 21 to 64 years). The mean duration of stay at the bone marrow transplant unit to the first isolation of *P. fluorescens* was 18 days (range, 4 to 36 days) All patients colonized with *P. fluorescens* had hematological malignancies and were receiving treatment that rendered them neutropenic during their hospital stay.

Each patient had only one pharyngeal swab that was positive for *P. fluorescens*, except for patient 4, who had two positive swabs 21 days apart. All stool samples and other microbiological specimens were negative for *P. fluorescens*. Of note was that all nine patients were on prophylactic oral colistin prior to the isolation of the organism, and no antibiotics were changed or added in response to the culture-positive result because the patients were asymptomatic. Subsequently, six out of nine patients developed febrile neutropenia and were started on empirical antibiotics (piperacillin-tazobactam and gentamicin) within 1 week of their first culture-positive pharyngeal swab. The mean length of time that antibiotics were started after the first isolation of *P. fluorescens* was 3.5 days (range, 0 to 7 days). One patient (patient 8) was found to be colonized with *P. fluorescens* on the same day that he developed febrile neutropenia, and thus, antibiotics were started that day. Another patient (patient 9) died during her hospital stay from a cause unrelated to her *P. fluorescens* infection.

Epidemiological investigation. A semiconfluent growth of >100 CFU per ml of *P. fluorescens* with the same antibiotic

TABLE 1. Characteristics of the patients with *P. fluorescens* recovered from pharyngeal swabs[a]

Patient	Age (yr)/ sex	Underlying disease	No. of days from admission to first isolation	White blood cell count on date of first isolation (10^9 cells/liter)	Antibiotic prophylaxis (oral) prior to isolation	Type (duration ([no. of days]) of antibiotic treatment commenced for febrile neutropenia	No. of days after first positive swab when antibiotics were started for febrile neutropenia	Date(s) of isolation from pharyngeal swab (day/mo/yr)	Patient outcome
1	61/M	Myeloma	6	9.7	COL, TMP	TZP, VAN, GEN (6)	3	31/01/10	Survived
2	41/F	AML	36	0.1	COL, TMP	None	NA	28/03/10	Survived
3	64/M	AML	18	0.5	COL, TMP, CLR	None	NA	28/03/10	Survived
4	21/F	ALL	35	0.3	COL, TMP	TZP, GEN (26)	4	07/03/10, 28/03/10	Survived
5	54/F	HL	11	0.3	COL	TZP, GEN, VAN (6)	1	07/03/10	Survived
6	47/M	AML	5	1.2	COL	TZP, GEN (6)	7	07/03/10	Survived
7	46/M	CMML	11	0.7	COL, PEN, CIP	None	NA	07/03/10	Survived
8	47/M	ALL	36	0.8	COL, TMP, PEN	TZP, GEN (10)	0	07/03/10	Survived
9	43/F	MDS	4	0.0	COL, TMP	TZP, GEN (3)	6	14/03/10	Died

[a] F, female; M, male; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; HL, Hodgkin lymphoma; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; COL, colistin; TMP, trimethoprim-sulfamethoxazole; CLR, clarithromycin; TZP, piperacillin-tazobactam; PEN, penicillin V; CIP, ciprofloxacin; GEN, gentamicin; VAN, vancomycin; NA, not applicable.

susceptibility pattern (meropenem resistant) as that of the patient isolates was cultured from one of the two water dispensers in the unit. All other environmental samples were negative.

Identification of bacteria. The Vitek automated identification system identified all nine patient isolates and the one environmental isolate as being *Pseudomonas fluorescens* isolates. The isolate from the water dispenser was found to be genotypically identical to the patients' isolates: all isolates of *P. fluorescens* produced identical RAPD patterns (type b pattern), and typing by PFGE revealed that all isolates recovered were indistinguishable, with a designated profile of NOTT PF1.

Antibiotic susceptibilities. Antibiotic susceptibility testing using the Etest showed that the strain was resistant to aztreonam (MIC, 16.0 mg/liter), intermediate resistant to meropenem (MIC, 4.0 mg/liter), and susceptible to ciprofloxacin (MIC, 0.064 mg/liter), ceftazidime (MIC, 0.25 mg/liter), piperacillin-tazobactam (MIC, 0.5 mg/liter), gentamicin (MIC $\leq$ 0.016 mg/liter), tobramycin (MIC $\leq$ 0.016 mg/liter), and colistin (MIC, 0.125 mg/liter).

DISCUSSION

Here we report the spread of *P. fluorescens* involving nine bone marrow transplant unit patients who became colonized with the organism from a contaminated water dispenser that supplied bottled natural spring water. *Pseudomonas fluorescens* was isolated in bottled water previously (18); however, to the best of our knowledge, this is the first description of the nosocomial spread of *P. fluorescens* in immunocompromised patients associated with a contaminated water dispenser.

Our results indicate that indistinguishable isolates of *P. fluorescens* were obtained from the nine colonized patients. Following an examination of multiple environmental samples from the bone marrow transplant unit, the water from one of two dispensers yielded *P. fluorescens* that was genetically identical to the patients' strain. All nine patients had reported drinking from this dispenser; the fact that *P. fluorescens* was isolated in their pharyngeal screening swabs further supported our hypothesis that this was the likely source.

Clinical opinion was that the strain most likely originated from the surroundings of the chiller unit rather than from the bottled water itself, because no *P. fluorescens* was cultured from samples from the second dispenser (which provided bottled water from the same order batch). Moreover, there were no further cases of *P. fluorescens* colonization or infection after the contaminated dispenser was removed, and the patients continued to drink bottled water from the remaining dispenser. However, we did not sample the water directly from the bottle as well as via the chiller unit, which would have helped us discriminate between these two possible sources of contamination.

If the source of the contamination was indeed the chiller unit, it remains unclear how exactly the *P. fluorescens* strain managed to enter the unit. Possibilities include contamination during the installation of the bottle onto the chiller unit by hospital staff or, perhaps, entrance into the nozzle via the hands of a member of the staff or a patient using the dispenser.

Establishment of the optimal treatment of an immunocompromised patient with *P. fluorescens* infection is difficult because there are limited antibiotic susceptibility data for the organism. The *in vitro* antibiotic susceptibility profiles of 13 *P. fluorescens* strains isolated from cancer patients demonstrated that the strains showed susceptibility to gentamicin, neomycin, tetracyclines, polymyxin B, and colistin and resistance to chloramphenicol, ampicillin, and narrow-spectrum cephalosporins (9). Some reports have shown susceptibility to carbapenems and ceftazidime but reduced susceptibility to other cephalosporins, including cefuroxime, cefotaxime, cefmenoxime, and cefsulodin (7). Data for our *P. fluorescens* strain were partly in agreement with those findings, although the strain differed in its resistance to aztreonam and intermediate resistance to meropenem. However, as the antibiotic susceptibility criteria set by the CLSI are for *P. fluorescens* isolates grown at 35°C $\pm$ 2°C and not for isolates grown at the temperature of 30°C used to test our isolates, these limited observations may not apply to the organism *in vivo*.

Carbapenem resistance is not widely reported for this organism, although one previous study recognized an imipenem-resistant strain of *P. fluorescens* (18). This is of some concern, because this class of antibiotics is commonly recommended for the management of neutropenic sepsis in hematology patients.

Although the *P. fluorescens* strain cultured from pharyngeal swabs was not quantified, it appears that our patients were not heavily colonized, as the organism was never isolated from their stool samples. The antibiotics of the patients found to be colonized were not changed, as they were asymptomatic at the time of the result. However, when six out of the nine patients developed febrile neutropenia, we started a treatment that took into account the antibiotic susceptibility profile of the *P. fluorescens* strain isolated. A combination of piperacillin-tazobactam and gentamicin was commenced, and meropenem was not used for these patients.

It is not clear whether the febrile neutropenia seen for two-thirds of the cases was a result of *P. fluorescens* or another pathogen that could have been transmitted by the contaminated water dispenser. Although *P. fluorescens* is not known to be particularly virulent, it cannot be discounted as the source of the febrile neutropenia, as this organism was reported previously to cause sepsis in both immunocompetent and immunocompromised patients (5, 7, 8, 13).

In practice, bottled water is still often used in hospitals, with one survey reporting a marked disparity in the methods of providing potable water to immunocompromised patients across the United Kingdom (6). Our long-term plan for the bone marrow transplant unit is to install filtered plumbed-in main water dispensers and to implement regular qualitative and quantitative water assessments. Another study of an outbreak of *P. aeruginosa* infection associated with contaminated bottled water in six intensive care units in Germany also recommended the removal of such sources of drinking water and the introduction of water filters (4).

This report not only highlights the potential risk of the transmission of pathogenic organisms via bottled water supplies but also reinforces the value of high-level surveillance programs in detecting the nosocomial spread of pathogens. We recommend that such bottled water supplies not be used in high-risk areas or that they be subjected to regular microbiological monitoring.

REFERENCES

1. **Anaissie, E. J., S. R. Penzak, and M. C. Dignani.** 2002. The hospital water supply as a source of nosocomial infections: a plea for action. Arch. Intern. Med. **162:**1483–1492.
2. **Blondel-Hill, E., D. A. Henry, and D. P. Speert.** 2007. *Pseudomonas*, p. 734–748. *In* P. R Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
3. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing: 20th informational supplement, M100-S20, vol. 30, no. 3. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Eckmanns, T., et al.** 2008. An outbreak of hospital-acquired *Pseudomonas aeruginosa* infection caused by contaminated bottled water in intensive care units. Clin. Microbiol. Infect. **14:**454–458.
5. **Gershman, M. D., et al.** 2008. Multistate outbreak of *Pseudomonas fluorescens* bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy. Clin. Infect. Dis. **47:** 1372–1379.
6. **Hall, J., G. Hodgson, and K. G. Kerr.** 2004. Provision of safe potable water for immunocompromised patients in hospital. J. Hosp. Infect. **58:**155–158.
7. **Hsueh, P. R., et al.** 1998. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. J. Clin. Microbiol. **36:**2914–2917.
8. **Khabbaz, R. F., et al.** 1984. *Pseudomonas fluorescens* bacteremia from blood transfusion. Am. J. Med. **76:**62–68.
9. **Moody, M. R., V. M. Young, and D. M. Kenton.** 1972. In vitro antibiotic susceptibility of pseudomonads other than *Pseudomonas aeruginosa* recovered from cancer patients. Antimicrob. Agents Chemother. **2:**344–349.
10. **Namnyak, S., S. Hussain, J. Davalle, K. Roker, and M. Strickland.** 1999. Contaminated lithium heparin bottles as a source of pseudobacteraemia due to *Pseudomonas fluorescens*. J. Hosp. Infect. **41:**23–28.
11. **Pier, G. B., and R. Ramphal.** 2004. *Pseudomonas aeruginosa*, p. 2587–2615. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 6th ed. Churchill Livingstone, New York, NY.
12. **Scott, F. W., and T. L. Pitt.** 2004. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. J. Med. Microbiol. **53:**609–615.
13. **Scott, J., et al.** 1988. A fatal transfusion reaction associated with blood contaminated with *Pseudomonas fluorescens*. Vox Sang. **54:**201–204.
14. **Siebor, E., et al.** 2007. Presumed pseudobacteremia outbreak resulting from contamination of proportional disinfectant dispenser. Eur. J. Clin. Microbiol. Infect. Dis. **26:**195–198.
15. **Taber, T. E., T. F. Hegeman, S. M. York, R. A. Kinney, and D. H. Webb.** 1991. Treatment of Pseudomonas infections in peritoneal dialysis patients. Perit. Dial. Int. **11:**213–216.
16. **Verbist, L.** 1993. Epidemiology and sensitivity of 8625 ICU and hematology/oncology bacterial isolates in Europe. International Study Group. Scand. J. Infect. Dis. Suppl. **91:**14–24.
17. **Vogel, L., G. Jones, S. Triep, A. Koek, and L. Dijkshoorn.** 1999. RAPD typing of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens* and *Pseudomonas aeruginosa* isolates using standardized reagents. Clin. Microbiol. Infect. **5:**270–276.
18. **Wilkinson, F. H., and K. G. Kerr.** 1998. Bottled water as a source of multiresistant *Stenotrophomonas* and *Pseudomonas* species for neutropenic patients. Eur. J. Cancer Care (Engl.) **7:**12–14.