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Concise Communication

Large hospital-wide outbreak of *Paenibacillus* spp pseudobacteremia associated with contaminated nonsterile gloves

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Abstract

We report a large, hospital-wide outbreak of pseudobacteremia by *Paenibacillus* spp. In total, 139 patients presented at least 1 positive blood culture during a 13-month period. Microbiological experiments indicated that contaminated nonsterile gloves were associated with the pseudobacteremia episodes. The outbreak was resolved by discontinuing the use of the involved brand.

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Originally included in the *Bacillus* genus, *Paenibacillus* spp were reclassified into a separate new genus in 1993.[1] These spore-forming, rod-shaped, gram-positive bacteria are mostly found in soil, especially in plant roots, and have rarely been associated with human infections, when they usually present as opportunistic pathogens.[1] Sporulation enables them to stay in dormant state in inhospitable conditions for extended periods.[1]

Although case reports and case series of infections by *Paenibacillus* spp exist,[2–6] these bacteria have not been associated with outbreaks in a hospital setting. In addition, only 1 report of a small pseudo-outbreak, related to the equipment contaminated with *Paenibacillus* spp, has been published.[7]

In 2020, a progressive increase in blood cultures with the growth of *Paenibacillus* spp was observed in our institution; the first case that could be tracked occurred in August. An outbreak of *Paenibacillus* spp bloodstream infections could not be ruled out initially. Still, the fact that the positive blood cultures were not restricted to one or a few related hospital units and were detected in patients from neonatal and pediatric wards as well as from adult wards led us to suspect the occurrence of an outbreak of pseudobacteremia. In this study, we investigated a large, hospital-wide outbreak of pseudobacteremia caused by *Paenibacillus* spp.

Methods

This study describes an outbreak of pseudobacteremia involving patients from whom a blood culture yielded the growth of *Paenibacillus* spp from March 2020 (when the first case was detected) to March 2021 (when the last case was detected) at Hospital Moinhos de Vento, Brazil.

Infections were defined according to National Healthcare Safety Network (NHSN) 2021 criteria.[8]

Blood cultures were collected in Bactec Aerobic Plus bottles (Becton Dickinson, Franklin Lakes, NJ) after rubber-septum disinfection with 70% isopropyl alcohol (Supplementary Methods). Bacterial identification was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker, Germany) after subculture on sheep blood agar (SBA).

The investigation of the possible pseudobacteremia outbreak consisted of an inspection of storage conditions of blood-culture bottles, assessing blood-sample collection by direct observation, and filming the procedures. The microbiological investigation of the materials used in blood-sample collections was performed as follows. First, 2 aerobic blood-culture bottles (Becton Dickinson, batch 70566) were inoculated with 10 mL sterile saline and incubated for 120 hours in a Bactec FX system. Next, culture of 10 randomly selected wipes containing 70% isopropyl alcohol (Labor Import, Osasco, Brazil, batch 20030238), used for blood-culture-bottle rubber-septum disinfection, was performed. The wipes were removed from the sachets using previously sterilized forceps (autoclaved). They were immersed entirely to sterile plastic tubes containing 2 mL tryptic soy broth (TSB). The tubes were spun in a vortexer for mixing and homogenization and were then incubated in air at 35±2°C for 10 days. Blind subcultures on sheep blood agar (SBA) were performed on days 7 and 10 of incubation.

We also used sterile swabs to sample the outer surface of the rubber septum from 10 blood-culture vials, and we cultured them in thioglycolate medium at 35±2°C for 10 days. Turbid broths were

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subcultured on SBA plates. Colonies grown on SBA were picked for smear preparation for identification using MALDI-TOF MS.

The experiments performed with nonsterile gloves were performed sequentially. In experiment 1, 48 gloves from a single box (brand A used by the hospital) were analyzed. One dry swab was swiped on the outer surfaces of 4 gloves, totaling 12 swabs. Those swabs were cultivated in tubes containing TSB (3 swabs in each tube). Blind subcultures on SBA were performed after 3 days of incubation at 35±2°C. Experiment 2 was similar to experiment 1, except that 24 gloves of a further batch of brand A were analyzed and swabs were incubated in 2 separate tubes. Experiment 3 was similar to experiment 1, except that 1 dry swab was used to sample 1 pair of gloves; 5 swabs were cultivated in a single tube containing TSB; and 10 gloves from each batch of 4 different brands used in the hospital were tested. In experiment 4, the analyst put on the glove and touched the surface of an SBA with the outer surface of the glove corresponding to the fingertips. Also, 2 gloves from each batch of 4 different brands were tested. The plates were incubated in air at 35±2°C for 48 hours. Colonies grown on SAB were picked for smear preparation for identification using MALDI-TOF MS.

Results

From March 15, 2020, to March 11, 2021, a total of 172 blood cultures from 139 patients yielded *Paenibacillus* spp, of which 27 were identified at the species level by MALDI-TOF MS (Table 1). The incidence rate of positive blood cultures in hospitalized patients and the proportion of positive blood cultures are displayed in Figure 1. In 15 patients, *Paenibacillus* spp were identified in 2 or more blood cultures collected on separate occasions, of whom 12 fulfilled NHSN Laboratory Confirmed Bloodstream Infection (LCBI) 2 criteria in a strict interpretation (Table 1).

No evidence of nonconformities was detected during the inspections of the lots, storage conditions of blood-culture bottles and blood-culture collection procedures, which were analyzed by the laboratory lead collector. Both blood-culture bottles with saline and isopropyl alcohol wipes cultures were negative. None of the cultures of rubber-septum surfaces of blood culture vials recovered *Paenibacillus* spp.

In experiment 1 with gloves, 2 of 4 TSB tubes revealed the growth of *Paenibacillus* spp. This growth was observed in both tubes in the experiment 2. The results of experiments 3 and 4 revealed the growth of *Paenibacillus* spp only in brand A gloves (Supplementary Tables 1 and 2).

On December 2020, after the results of experiment 1 became available, the infection control team recommended that the gloves of brand A should be avoided for blood-culture collection and catheter manipulation. On January 2021, the infection control team issued an order to discontinue the use of brand A gloves in the hospital (Fig. 1). The local municipal surveillance agency was notified of our results in January 2021.

Table 1. Characteristics of Patients and Blood Cultures With Growth of *Paenibacillus* spp

Characteristic	Total
Patients	(n = 139)
Demographics	
Age, median y (IQR)	69 (50–79)[a]
Sex, female	63 (45.3)
No. of positive blood cultures and positivity in distinct days	
1 positive bottle in a set of blood cultures[b]	124 (89.2)
2 positive bottles in a set of blood cultures[b,c,d]	14 (10.1)
3 or more positive bottles in a set of blood cultures[b,c,d]	1 (0.7)
Patients with positive blood cultures on distinct days	15 (10.8)
Blood cultures	(n = 172)
Characteristics of blood cultures	
Time to blood-culture positivity, median h (IQR)	54 (41–77)[e]
Site of blood-culture collection	
Peripheral venipuncture	66 (38.4)
Central venous catheter	85 (49.4)
Arterial puncture	4 (2.3)
Arterial catheter	17 (9.9)
Unit where the blood sample was collected	
Adult ICU	83 (48.3)
Pediatric ICU	4 (2.3)
Neonatal ICU	8 (4.7)
Medical wards	43 (25.0)
Emergency department	33 (19.2)
Hemodialysis unit	1 (0.6)
Isolates identified	
Paenibacillus spp	145 (84.3)
Paenibacillus phoenicis	14 (8.1)
Paenibacillus barengoltzii	8 (4.7)
Paenibacillus ginsengihumi	4 (2.3)
Paenibacillus urinalis	1 (0.6)

Note. Data are presented as no. (%) unless otherwise indicated. IQR, interquartile range; ICU, intensive care unit.
[a]Range, 0–97 y.
[b]1 set of blood cultures corresponds 2 or more bottles collected from distinct sites.
[c]Some patients also presented a single blood culture positive in an additional set of blood-culture collection on a distinct day. For this counting, they were only considered in this category.
[d]Of these 15 patients, two cases had their blood cultures collected at the emergency department and were classified as "present on admission". Of the 13 remaining patients, 12 had fever (n = 9) or hypotension (n = 3), therefore fulfilling NHSN Laboratory Confirmed Bloodstream Infection 2 criteria. Of 9 patients, 8 had a microbiologically confirmed infection at another site, and 1 had ischemic colitis with intestinal perforation. All patients with hypotension presented other identified causes for this sign (eg, digestive hemorrhage, cardiogenic shock, and septic shock from other identified source).
[e]Including all 172 positive blood cultures. Range, 14–119 h.

Discussion

We described the first hospital-wide outbreak of pseudobacteremia caused by species of the genus *Paenibacillus*. Pseudo-outbreaks may be challenging for infection control and microbiology laboratory teams. Therefore, reporting this occurrence and investigation is critical to guide others facing similar epidemiologic situations.[9]

A previous pseudo-outbreak involving this genus was limited to 8 patients.[7] The extensive microbiological investigation carried out with all materials used in blood-culture collection strongly indicates that gloves contaminated with these bacteria were responsible for this pseudo-outbreak. In addition, the recommendation to avoid the use of gloves A was followed by a reduction in the incidence of cases. Furthermore, 3 additional cases (2 in the same unit) were noted after the use of gloves A was discontinued, but we believe that these late cases might have occurred via the inadvertent continued use of some gloves of this brand in some wards.

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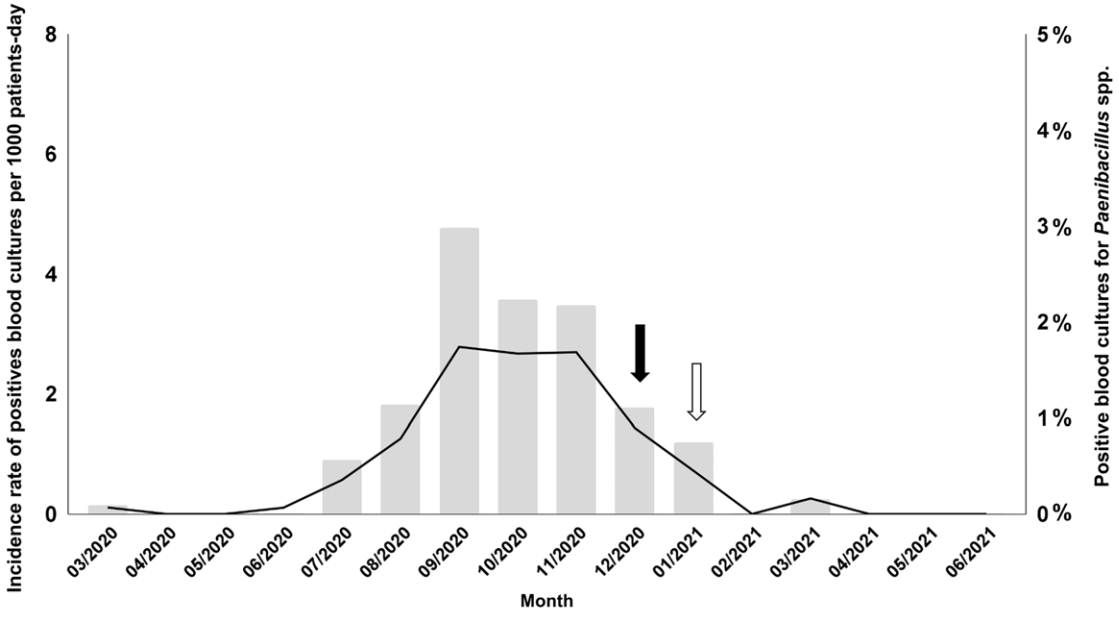

Positive blood cultures	1	0	0	1	8	16	46	36	34	17	10	0	3	0	0	0
Blood cultures collected	1161	1127	977	1220	1442	1422	1547	1616	1570	1553	1350	1344	2088	1685	1458	1570
Patients with positive blood cultures	1	0	0	1	6	14	31	31	30	15	7	0	3	0	0	0
Patients-day	9212	7563	8544	9550	10569	11143	11148	11618	11145	10472	9696	9381	11559	10241	11417	11454

Fig. 1. Incidence rate and proportion of blood cultures positive for *Paenibacullus* spp. Black line: incidence rate; if a patient presented >1 positive blood culture, only the first was considered for the incidence rate. Grey columns: proportion of positive blood cultures. Black arrow: first recommendation to avoid the use of gloves from brand A for blood-culture collection procedures. White arrow: discontinuation of gloves from brand A order in the hospital.

We first hypothesized that gloves A could be associated because previous reports from healthcare workers from our institution complained of an excessive amount of powder in the gloves of this brand compared to other brands. Although we cannot state that only the powder was contaminated because the experiments with the surface of the gloves also yielded the growth of *Paenibacillus* spp, we believe that the excess powder that remained in the surfaces touched by the gloves contributed to the contamination of skin, catheters, and/or blood-culture bottles through dispersion of the spores of *Paenibacillus* spp. A previous pseudo-outbreak caused by spore-forming *Bacillus* spp has also been associated with contamination of nonsterile gloves.[10] Notably, although 12 patients fulfilled NHSN LCBI 2 criteria, considering that all of these patients had other causes identified for fever and hypotension, no case represented a real bacteremia in our interpretation.

This study had several limitations. The lack of molecular typing limited our investigation. However, detection of different species indicates that the outbreak of pseudobacteremia has not been primarily driven by clonal dissemination. Additionally, MALDI-TOF MS could not identify all isolates at the species level, and we did not carry out molecular tests for species identification. Finally, we only evaluated materials related to blood-culture collection, and we did not expand the investigation to other potential sources of spore-forming bacteria, such as the hospital environment. Although it could improve the quality of our report, it is unlikely that the environment of all hospital wards would be contaminated by this organism.

In conclusion, this is the first report of a large, hospital-wide outbreak of pseudobacteremia caused by *Paenibacillus* spp. The possibility of a pseudo-outbreak should be considered whenever there is an increase in positive cultures for an unusual pathogen, particularly spore-forming bacteria. We emphasize the possibility that the high amount of powder observed in the involved gloves may have contributed to the dispersion of spores and contamination of blood cultures.

Supplementary material. For supplementary material accompanying this paper visit https://doi.org/10.1017/ice.2023.46

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Conflicts of interest. A.P.Z. is a research fellow of the National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology, Brazil. A.P.Z. received a research grant from Pfizer and was a member of advisory board for Spero Therapeutics and Eurofarma. All other authors have no conflicts to declare.

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