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Healthcare-associated respiratory tract infection and colonization in an intensive care unit caused by *Burkholderia cepacia* isolated in mouthwash

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SUMMARY

Objectives: *Burkholderia cepacia* has been linked to healthcare-associated infections and colonization caused by contamination of alcohol-free mouthwash used in patients undergoing mechanical ventilation. The purpose of our study was to establish the source of a clustering of healthcare-associated *B. cepacia* isolates in patients on mechanical ventilation in the intensive care unit (ICU).
Methods: During April 2012 the Infection Control Committee became concerned when *B. cepacia* was isolated from tracheal aspirate cultures of three ICU patients. The medical records for the years 2011 and 2012 were reviewed to identify further cases. Cultures of potential reservoirs were done. Isolates from patients and an alcohol-free mouthwash were submitted to multilocus sequence typing (MLST) analysis and antimicrobial resistance testing.
Results: Four patients with positive cultures for *B. cepacia* were identified before the review of the medical records for the years 2011 and 2012. Nine further cases were identified in the review, defined as a patient with pneumonia who had a culture of respiratory secretions that was positive for *B. cepacia*. Three were cases of infection and 10 were colonizations. All of the isolates from patients (J, K, L, and M) and mouthwash samples (B19, B20, and B21) were genetically identical by MLST analysis.
Conclusions: Our findings strongly suggest that alcohol-free mouthwash solution intrinsically contaminated with *B. cepacia* was the source of these colonizations and infections involving adults in the ICU.

1. Introduction

Burkholderia cepacia is a species of bacterium widely distributed in natural environments such as water, soil, and agricultural products. *B. cepacia* is not virulent in healthy people, but is a serious opportunistic pathogen in immunocompromised and cystic fibrosis patients. *B. cepacia* has been linked to healthcare-associated outbreaks caused by the contamination of medical devices,[1,2] antiseptic solutions, parenteral and nebulized medications,[3,4] and other environmental sources.[5] It has also been implicated in healthcare-associated outbreaks caused by the contamination of alcohol-free mouthwash solutions.[6,7] Mouthwashes are used widely in patients undergoing mechanical ventilation. Such patients are highly vulnerable to pathogens that typically cause upper respiratory tract infections because of their inability to maintain the mucociliary and cough mechanisms that normally protect the lower respiratory tract.[8] These bacteria have also contaminated many drug products, leading to public health concerns.

From March 2011 through May 2012 a cluster of healthcare-associated *B. cepacia* isolates was identified among ventilated patients in a general medical intensive care unit (ICU). This study reports the results of an investigation to determine the source of this *B. cepacia* and summarizes our experience with the management of these colonizations and infections.

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2. Methods

2.1. Hospital and patients

Vozandes Hospital is a 75-bed private, acute care teaching hospital in Quito, Ecuador; eight of these beds are in the ICU. The study was performed by the Infection Control Committee.

During the month of April 2012, *B. cepacia* was cultured from the tracheal aspirates of three patients. All of them were hospitalized in the ICU. The *B. cepacia* was isolated on April 16 in two cases, and on April 27 in another. A fourth case occurred on May 2. As part of routine surveillance for ventilator-associated pneumonia (VAP), this finding prompted an investigation of these cases. The bacterial strains were saved for further analysis.

With the results of these cultures, we proceeded to review the medical records of any patient who had been in the ICU and who had had a respiratory specimen culture positive for *B. cepacia*. We reviewed the medical records for the years 2011 and 2012. All results of routine cultures from patients admitted to the ICU and all medical records from patients whose respiratory sample was positive for *B. cepacia* were reviewed. A case was defined as a patient with pneumonia who had a respiratory sample culture that was positive for *B. cepacia* (sputum, or bronchial lavage, or tracheal aspirate), elevated procalcitonin and C-reactive protein, and had a pulmonary infiltration. All of the patients required mechanical ventilation. In order to identify the reservoir of *B. cepacia*, we cultured samples from solutions used commonly in patients undergoing mechanical ventilation, i.e. hydrating cream, povidone–iodine solution, water supplies, distilled water, and alcohol-free mouthwash.

2.2. Microbiological investigations

Samples from the patients and samples from opened (one per patient) and unopened 15-ml bottles of the mouthwash were cultured under laboratory standards. The samples were cultured in blood, chocolate, and MacConkey agars. Strain identification was carried out using the API 20 NE strip system (bioMérieux, Marcy l'Etoile, France) and Vitek 2 system (bioMérieux, Marcy l'Etoile, France). The antimicrobial susceptibility of the four isolates from the patients and three isolates from the mouthwash was determined by Etest (M.I.C Evaluator Strip, Oxoid, UK) in Mueller–Hinton agar and by Vitek 2 system, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

2.3. Multilocus sequence typing (MLST) analysis

Seven strains were sent to the Institute of Microbiology at San Francisco University in Quito to investigate their clonal relationship by MLST. Four of them were isolated from clinical samples obtained from patients in April through May 2012 (J, K, L, and M). Three strains were isolated from mouthwash samples used in the same hospital. An isolate from the bacterial collection belonging to another confirmed outbreak was used in the MLST analysis as an external strain. Genomic DNA was isolated with DNAzol Reagent following the manufacturer's instructions. Amplification of the seven most variable loci was performed as described previously.[9] Sequences were determined by Functional Biosciences Inc. (South Rosa, USA). The sequences of the seven loci were aligned using ClustalW method, except for the *trp*B gene which was aligned using the MUSCLE alignment method (MEGA version 5.05).

3. Results

From March 2011 to May 2012, a total of 13 patients had cultures positive for *B. cepacia*. All of the positive cultures came from tracheal aspirates. Four cases were identified over a short period of time (April 16 through May 2), with two of them occurring on the same day. There was concern about a possible outbreak, and we subsequently identified nine patients retrospectively (Figure 1). All 13 patients were in the ICU and were ventilated. Three patients developed VAP. This was caused by *B. cepacia* in two of them; the other patient developed VAP caused by another pathogen (see **Supplementary Material**). Ten patients were considered to be colonized. The dates of isolation coincided with the start date of the use of the same batch of mouthwash.

Patients ranged in age from 47 to 92 years (mean 72.5 years); four were female and nine were male. The patients had been admitted to hospital with various diagnoses. All but two were elderly. None had a medical condition associated with *B. cepacia* infection (e.g., cystic fibrosis or chronic granulomatous disease). After *B. cepacia* was identified, five patients were treated with oral trimethoprim–sulfamethoxazole alone and one patient was treated with oral trimethoprim–sulfamethoxazole and meropenem; two of the five patients who were treated with oral trimethoprim–sulfamethoxazole alone died and the one patient who received trimethoprim–sulfamethoxazole and meropenem died. These three patients apparently died of the disease process for which they had been hospitalized. It is impossible to determine whether the cause of death in these three patients was a *B. cepacia*

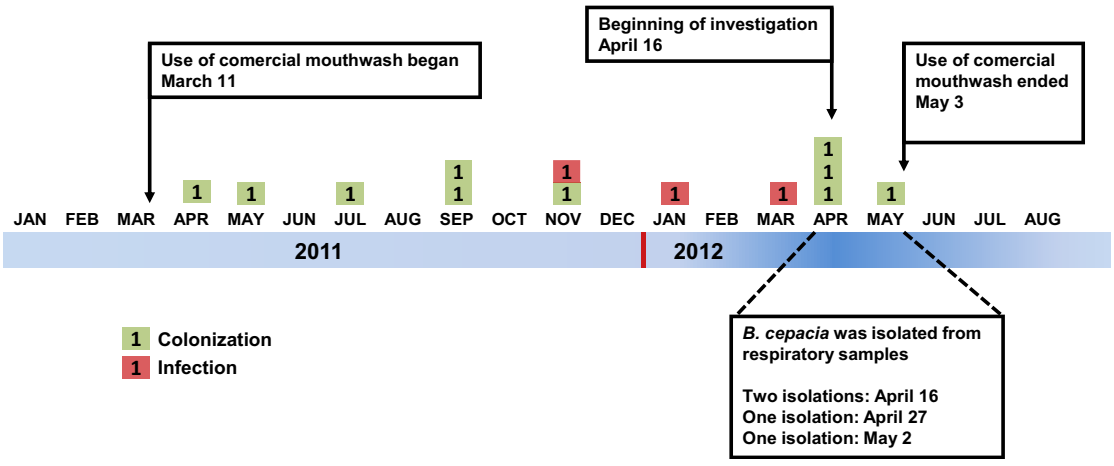

Figure 1. Temporal distribution of patient cases by month. *Burkholderia cepacia* was isolated from respiratory samples. All patients were admitted to the ICU and were ventilated.

infection. Two of the five patients who were treated with oral trimethoprim–sulfamethoxazole alone and the patient treated with trimethoprim–sulfamethoxazole plus meropenem developed VAP caused by another pathogen. One patient was treated with meropenem only and developed VAP. Three additional patients were treated with ceftazidime, and none of them died. Three patients were not treated.

We looked for a potential reservoir of *B. cepacia* and cultured samples from other solutions such as hydrating cream, povidone-iodine solution, water supplies, and distilled water, the latter because there had already been an outbreak caused by sterile distilled water contaminated with *B. cepacia*.[10] Eighteen samples were cultured. All of them were culture-negative for *B. cepacia*. We finally isolated *B. cepacia* from the alcohol-free mouthwash. A quantitative culture of this commercial product revealed 10^5 CFU/ml of *B. cepacia*. One batch of the mouthwash from the same manufacturer was found in the hospital supply department. Samples were cultured from unopened bottles of mouthwash belonging to the same batch as had been distributed in the ICU. Batches distributed in that period were highly contaminated. Three samples of this product were studied in molecular assays.

Samples of the mouthwash were uniformly positive for the same organism. The hospital supply department had been purchasing this product from this source for 13 months before the *B. cepacia* was identified. Four isolates recovered from patients in this investigation and three isolates recovered from samples of this commercial mouthwash were investigated using phylogenetic analysis. MEGA 5.05 software was used to construct a gene tree of the concatenated sequences (5018 bp) for each isolate by the neighbour-joining method. The significance of branching within the tree was evaluated by bootstrap analysis of 500 computer-generated trees. For MLST we used the same size in the seven genes for the alignment and no gaps were found. All of the isolates from patients (J, K, L, and M) and mouthwash samples (B19, B20, and B21) were shown by MLST analysis to be genetically identical (Figure 2). All of the isolates shared the same allelic profile (Table 1). Our study identified two new allele sequences (*gyr*B 546 and *pha*C 288) and a new sequence type (ST762) that corresponded to the outbreak strain. The data were submitted to the *B. cepacia* complex PubMLST database (http://pubmlst.org/bcc/). In addition, all isolates (four from the patients and three from the mouthwash) were susceptible to the antimicrobials tested: trimethoprim–sulfamethoxazole (<20 μg/ml), ceftazidime (4 μg/ml), and levofloxacin (2 μg/ml) by Vitek 2 cards; meropenem (1 μg/ml) and minocycline (2 μg/ml) by Etest.

On May 3, the use of the mouthwash product in the hospital was discontinued and the last isolate of *B. cepacia* was obtained on the same date. *B. cepacia* was recovered during the period when the implicated mouthwash was being used by the hospital. As soon as the commercial mouthwash was confirmed to be contaminated with *B. cepacia*, the product was removed from the hospital and a formal report was submitted to the Ecuador Ministry of Health. Intrinsic contamination of the mouthwash was confirmed by the Instituto Nacional de Investigación en Salud Pública (INSPI). The methods used to aliquot and distribute the product and the extent to which this mouthwash had been used in other hospitals were investigated by the Ministry of Health. There are no official figures yet, but another private hospital that used the same product has also reported patients infected with *B. cepacia*.

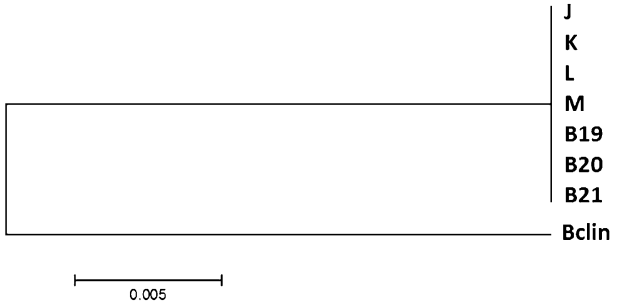

Figure 2. Neighbour-joining tree of the concatenated sequences of the seven genes. J, K, L, and M are patient isolates; B19, B20, and B21 are mouthwash isolates; Bclin is the *Burkholderia cepacia* clinical isolate used as control.

Table 1
Allelic profiles, corresponding sequence type (ST), and number of polymorphic sites in the seven loci of the Bclin and hospital isolates.

Gene	Allelic profile of hospital isolates	Allelic profile of Bclin strain[a]	Number of polymorphic sites	Sequence length analyzed (bp)
*atp*D	64	16	8	443
*glt*B	80	108	7	400
*gyr*B	546	326	25	454
*rec*A	217	49	29	393
*lep*A	122	68	11	397
*pha*C	288	185	12	385
*trp*B	214	226	18	301
ST	762	395	-	-

[a] The strain Bclin was isolated from a urine sample obtained using a Foley catheter from a 66-year-old patient in a local hospital, May 24, 2011. It was identified by API 20 NE strip system (bioMérieux, Marcy l'Etoile, France). According to the PubMLST database, ST395 was previously characterized in 2012 and corresponds to a *Burkholderia cenocepacia* strain isolated from a urine sample in 2006 in the Institut Fédératif de Biologie, Hôpital Purpan, France.

4. Discussion

B. cepacia is a common organism that typically causes respiratory tract infections in ICU patients. The severity of an infection or colonization by *B. cepacia* may be different for individual patients. However, overall, pulmonary colonization reduces survival by 50%; about a third to a half of patients develop *B. cepacia* syndrome, a rapidly fatal necrotizing pneumonia.[11]

Initially a few *B. cepacia* isolates were recovered and the organism was not consistently isolated each month. A full investigation was not initiated until after *B. cepacia* was documented in a patient and after several patients had the same organism recovered from tracheal aspirates in the same month (two on the same day). A review of the medical records for 2011 and 2012 identified nine previous isolates that were consistent with the timing of use of the mouthwash batch. These infections and colonizations were caused by the alcohol-free mouthwash contaminated by *B. cepacia.* Mouthwash has been implicated in outbreaks of pneumonia due to *B. cepacia* in other countries.[12,13]

All ICU patients in this study had undergone intubation and mechanical ventilation during their ICU stay. As a part of routine oral care for mechanically ventilated patients, they had had their mouths swabbed with an alcohol-free mouthwash. The active ingredient in this product is chlorhexidine 0.12%. *B. cepacia* is a well-known healthcare-associated pathogen that is intrinsically resistant to aminoglycosides and first- and second-generation cephalosporins. This multiple antibiotic resistance of *B. cepacia* has been attributed to an impermeable selective outer membrane, an efflux pump mechanism, biofilms, and/or the production of an inducible chromosomal beta-lactamase.[14–16] However in this study, all of the isolates were sensitive to the five antimicrobial agents studied. All of the isolates showed the same antimicrobial susceptibility pattern.

Although *B. cepacia* does not appear to survive on completely dry surfaces for more than 1 week, it can survive for many months

in water.[17] *B. cepacia* can use other routes of transmission including contact with hard surfaces. Perhaps most important to note is this organism's ability to remain viable under harsh conditions (e.g., organic solvents, antiseptics, low nutrients, etc.) for many months. Given the nature of this microorganism, its ability to grow in low-nutrient conditions, and resistance to chemical preservatives, it is important to consider the relatively high patient risk when this microorganism is present in manufacturing equipment, components, raw materials, or the potable water used in manufacturing pharmaceutical products.

It sounds paradoxical, but no antiseptic products are free from the possibility of bacterial contamination. Sometimes manufacturing practices are substandard. The literature talks about good manufacturing practices, which have to be scrupulously respected. Another possibility is that *B. cepacia* is becoming increasingly resistant to commonly used antiseptics, resulting in its multiplying to unacceptable levels. However, this last remark brings us back to the issue of good manufacturing practices, which should be supervised by competent authorities. In some quality control situations, the review is limited to how the product is identified and sealed and if it follows established standards.

More thorough surveillance of microbiological contamination of alcohol-free products used in adults predisposed to infection should be mandatory. These findings highlight the importance of hospital surveillance and the investigation of unusual clusters of infection and colonization to promptly identify unexpected sources of pathogens and to protect patients at risk. According to Torbeck et al.,[18] the drug manufacturing process should include the microbiological screening of materials, equipment, and environments, as well as the final product, and the drugs should be subjected to microbiological tests before use.

In conclusion, our findings strongly suggest that alcohol-free mouthwash solution intrinsically contaminated with *B. cepacia* was the source of these colonizations and infections involving adults in the ICU. *B. cepacia* organisms are a clear and present danger to patient health and safety. The challenge is undeniable: there have been many outbreaks caused by *B. cepacia* reported in the medical literature. Removing it from pharmaceutical manufacturing areas and products will not be easy, but it must be done.

Acknowledgements

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Ethical approval: Because the Control of Hospital Infections Committee of Vozandes Hospital determined the presence of the *B. cepacia* outbreak, the normal course of an outbreak investigation was followed; hence in these cases no informed consent was required. Authorization to review the medical records was obtained from the Department of Medical Auditing. Furthermore, this is the report of an established process which did not include any intervention in the course of the disease or in the patient's progress. All this was discussed with the Ethics Committee of Vozandes Hospital.

Conflict of interest: All authors report no conflicts of interest relevant to this article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2014.07.016.

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