

## Full Length Research Paper

# Outbreak of *Burkholderia cepacia* complex associated with contaminated liquid soap for hospital use: A case study

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***Burkholderia cepacia* complex (BCC) is gram negative bacteria which causes different types of human infections including catheter-associated infections and respiratory tract infections especially in immunocompromised patients. This study was conducted to decide the source, to prevent the possible hospital outbreak of BCC and to determine the epidemiological source. Eleven cartridge of liquid soaps were purchased by Near East University Hospital, Nicosia. Liquid soaps were cultured on 5% sheep blood agar and Eosine Methylene Blue Agar at 35°C in 24 h. Colonies which suspected were confirmed by Phoenix 100. Patient blood was cultured in BD Bactec Plus Aerobic culture bottles. BCC strains and patient blood sample were identified by using two different polymerase chain reaction (PCR) methods. The liquid soaps were contaminated with BCC. A few days later, in the blood culture of the 35-year-old Turkish Cypriot male patient, BCC was identified. The molecular results indicated that the BCC strain isolated from the patient blood culture was identical with the strain isolated from the liquid soaps. The molecular epidemiological studies showed that the patient blood culture strain and the liquid soap strain was in the same cluster. It was suggested that the liquid soaps should always be analyzed and then distributed to the hospital departments.**

**Key words:** *Burkholderia cepacia* complex (BCC), liquid soap, prevent.

## INTRODUCTION

*Pseudomonas cepacia* was first described by the William Burkholder as a plant pathogen which caused the onion rot in 1950 (Mahenthiralingam, 2008; Dolan, 2011). However, when the molecular analysis was executed, this

species were categorised to a new genus, *Burkholderia* (Mahenthiralingam, 2008). After 16S rRNA analysis, results indicated that, *B. cepacia* could be associated with *Burkholderia cepacia* complex (BCC) as they were related

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related species (Torbeck, 2011). BCC is a non-fermenting gram negative bacteria which causes different types of human infections including catheter-associated infections and respiratory tract infections, especially in immunocompromised patients (Lee, 2013). BCC also causes endocarditis, wound infections, catheter-related urinary infections, intravenous bacteremia and foot infections (Torbeck, 2011). BCC is an avirulent bacterium for many healthy people and is usually related with pneumonia in cystic fibrosis patients (Hua, 2013). However, if BCC infection is seen in healthy people, physician should give an attention. BCC can be potential microorganisms that cause hospital outbreaks as it generally dissipates at hospital environment (Lee, 2013). Hospital outbreaks have commonly been caused by the contaminated sources such as disinfectant products, intravenous solutions or medical devices (Kuzumoto, 2011). Studies reported that BCC has been usually found in contaminated nebulizer solutions, intravenous solutions, mouthwash, hospital sinks, antiseptics (Lee, 2013), pharmaceuticals cosmetics, preservative products (Torbeck, 2011), tap water, and reusable temperature probes (Dolan, 2011).

The outbreak of BCC among 250 bed hospital in Nicosia, Cyprus of North in November 2013 was described and prevented. The epidemiological investigation was started by the Infection Control Unit. At the end of the investigation, the source of this contamination was located. Molecular typing of the samples obtained from the patient and environmental isolates were performed by DiversiLab and Pulsed Field Gel Electrophoresis (PFGE).

## MATERIALS AND METHODS

### Identification of the problem

At the Near East University Hospital, as a routine process before the liquid soaps are distributed to the hospital departments, samples are analyzed and report of the results are discussed by the Infection Control Unit.

In November 2013, 11 cartridge of liquid soaps were purchased by Near East University Hospital, Nicosia. Among 4 liquid soaps, samples were taken randomly for culture analysis by the infection control nurse before distributed to the hospital departments. BCC were identified in the liquid soap at the end of the culture analysis. After one week, BCC bacteremia was identified from the blood culture of one non-cystic fibrosis patient. Three experts including two infection control doctors and an infection control nurse made an investigation about the outbreak.

### Microbiological testing

Clinical specimens that were sent to the Near East University Hospital Microbiology Laboratory, were then cultured according to their types of material. The BCC was cultured according to standard microbiological methods. Patient blood (3 to 6 ml) was cultured in BD Bactec Plus Aerobic culture bottles (Becton Dickinson, USA) and incubated for 7 days at 35°C in Bactec 9120 (Becton Dickinson, USA) with daily visual inspection of the chromogenic indicator for growth. When the blood culture system was alarmed, patient blood

samples were cultured using 5% sheep blood agar (Oxoid) and Eosine Methylene Blue Agar (Oxoid). Samples were plated and incubated at 35°C in 24 h. Colonies were counted and microorganisms were identified by Phoenix 100 automated system (Becton Dickinson, USA). Liquid soaps were tested by filtration with a 0.45 µm cellulose membrane filter and cultured on 5% sheep blood agar and Eosine Methylene Blue Agar at 35°C in 24 h. Colonies suspected were confirmed by Phoenix 100 automated system. For further analysis, 12 BCC isolates were stored at -80°C on porous beads (Microbank, Pro-Lab Diagnostic, Richmond Hill, ON, Canada).

### Species determination

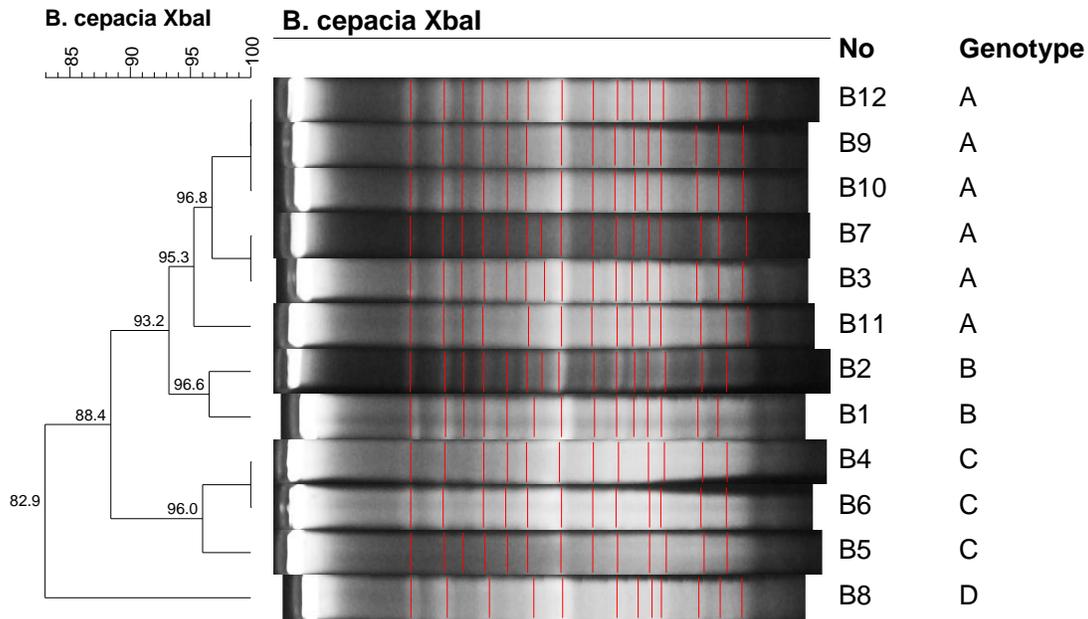
BCC strains isolated from liquid soaps as well as one patient blood sample were identified by using two different genotyping methods; DiversiLab and PFGE.

### Strain genotyping

BCC strains isolated from liquid soaps products and the patient were genotyping by using PFGE with a modification of the protocol by Shueh et al. (2013). Isolates were digested with *Xba* restriction endonucleases. PFGE was performed using the Bio-Rad Gene Path system (Bio-Rad, Hercules, USA) and run on a 1% agarose gel in 0.5x TBE Buffer at 14°C with a linear ramp time of 5.3 to 52.4 s over a period of 24 h. Afterwards, gels were stained with ethidium bromide and photographed. DiversiLab protocol was used as described by Fluid et al. (2010). Isolates were cultured on blood agar for 24 h at 35°C. Extraction of *B. cepacia* DNA was made with the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) following the manufacturer instructions. Extraction samples were diluted until the samples were 35 ng/µl. Repetitive sequence-based PCR (REP-PCR) of extracted DNA was made using the DiversiLab Bacterial Kit (bioMérieux, Marcy l'Etoile, France). Briefly, 35 ng genomic DNA, 2.5 U AmpliTaq polymerase, 2.5 µl 10x PCR buffer (Applied Biosystems) and 2 µl primer mix were added to the rep-PCR master mix in a total volume of 25 µl per reaction. Thermal cycling parameters were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 92°C for 30 s, annealing at 50°C for 30 s and extension at 70°C for 90 s, with a final extension at 70°C for 3 min. Amplified fragments of various sizes and intensities were separated and detected with DNA chip (bioMérieux, Marcy l'Etoile, France) on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California). Results were demonstrated as dendrogram (with a Pearson correlation similarity matrix) including a virtual gel image of the fingerprint for each DNA sample. Strain-level discrimination is indicated between samples of the same species with >95% similarity and minor band differences.

## RESULTS

In November 2013, 11 cartridge of liquid soaps were purchased by Near East University Hospital, Nicosia. The liquid soaps were at pH 5.5 and the firm accreditation with the ISO 9001-140001-22000, OHSAS 18001 and GMP 22716. 4 liquid soaps randomly selected by Infection Control Nurse. The culture analysis results showed that these liquid soaps were contaminated with BCC. As a result of this report, all 11 liquid soaps were then analyzed. The culture results showed that all liquid soaps



**Figure 1.** Dendrogram of PFGE pattern showing similarities of 12 *B. cepacia* strains. Dice coefficient (threshold  $\geq 95$ ) and the “unweighted pair-group method with arithmetic mean” (UPGMA) cluster method were used for the dendrogram analysis (B3. environmental sample) Genotyping Pulsed Field Gel Electrophoresis (PFGE).

were contaminated with BCC. The group of these liquid soaps were not distributed before the culture analysis. All BCC strains were sensitive to ceftazidime, cotrimoxazole and meropenem, intermediate to levofloxacin and resistant to ticarcillin/clavulanic acid according to the Phoenix 100 results.

A few days later, in the blood culture of the 35-year-old Turkish Cypriot male patient, BCC was identified. The patient is sickle cell anemia and periodically needs hospital caring. He was treated with cotrimoxazole immediately to eradicate the infection.

After it was found that the source of the contamination was the liquid soaps, the Infection Control Committee showed special attention for the handling of disinfectant equipments. They collected all of the disinfectant bottles from all departments for inhibiting the dissemination of the outbreak.

Molecular and epidemiological studies were performed. Strains were analysed to be genotyping by both DiversiLab and PFGE. Molecular results showed that BCC has 4 different clusters (A, B, C and D). The patient strain was in the A cluster which was larger than the other clusters. DiversiLab result were confirmed with the PFGE result. PFGE (Figures 1 and 2) and DiversiLab (Figure 3) analysis results were shown.

After the source of the contamination was identified, in the discussion with the hospital authorities, infection control unit decided to eliminate all previously distributed liquid soaps and also abolish the agreement with the firm. As the precautions were taken, no other BCC contamination was detected through the hospital.

## DISCUSSION

BCC is one of the main microorganism that can be found in environment in diverse ranges (Mahenthalingam, 2008). BCC is important for the healthcare-associated infectious agents in especially hospitalized and immunocompromised patients (Kuzumoto, 2011). 16S rRNA results indicated that BCC strains are a group at least 17 species and 15 genospecies with high similarity ( $>97.5\%$ ) (Kuzumoto, 2011). Most of the members of this genus are useful in many biotechnological applications such as bioremediation, biological control of the plant diseases, water management and nitrogen fixations (Choh, 2013). BCC is also a bio-pesticide for inhibiting fungal diseases (Kuzumoto, 2011). When BCC is located in manufacturing equipment, components or water used in manufacturing pharmaceutical products, this can be associated with high patient risk (Torbeck, 2011). Although BCC does not live on completely dry surfaces more than one week, it can stay alive in water for months (Torbeck, 2011). BCC were transmitted by contact or aerosol and by environmental acquisition (Peeters, 2008).

Infection Control Unit decided that hand washing is important before contact with patient as hands are the main transmission way of the microorganism (Caetano, 2011). It was indicated that low-level disinfectants like benzalkonium chloride, chlorhexidine and alkyldiaminohydroxyglycine hydrochloride are not appropriate as the contamination has occurred by microorganisms (Oie, 2012). It has been reported that BCC had been alive in commercially manufactured providone iodine.



Figure 2. PFGE typing results of 12 *B. cepacia* strains.

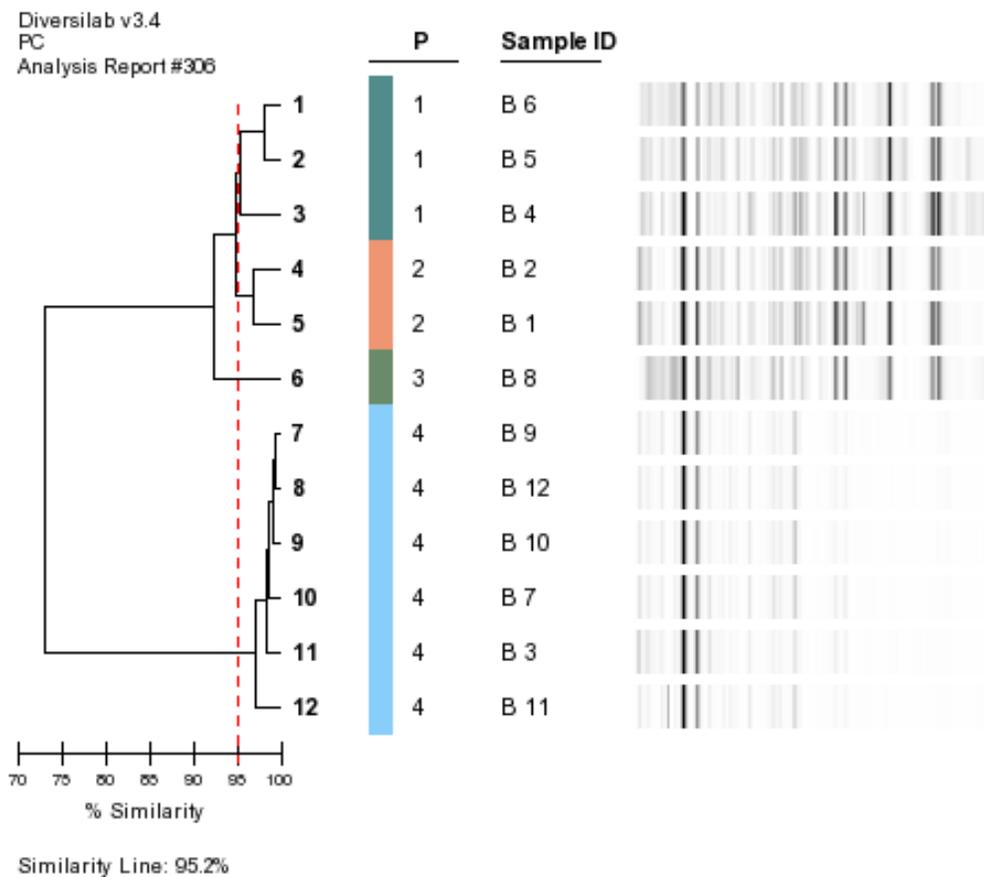


Figure 3. rep-PCR-based dendrogram and virtual gel image fingerprints obtained from 12 *B. cepacia* strains using the DiversiLab system with the bacterial kit. Pearson's correlation coefficient was used to create a pairwise percentage similarity matrix and the dendrogram was derived using UPGMA. An 95 % similarity threshold (vertical line) was chosen for *B. cepacia*. B3; patient, B1-2, B4-12; enviromental samples. Rep-PCR-based DNA fingerprinting by the DiversiLab System.

This contaminations cause both infections and pseudo-infections at hospital environment (Weber, 2007). Also, other studies indicated that contaminated quaternary ammonium compound used for the disinfection of the rubber stoppers of blood culture bottles caused pseudo-outbreak of BCC (Weber, 2007). These studies showed that the sources of BCC contamination can vary from medical instruments and disinfectants to alcohol-free mouthwash and body milk (Martin, 2011).

FDA reported that between years 2004-2011, BCC alone was mentioned in 34% of the non-steril recalls including mouthwashes, moist wipes, soaps, sanitizers, nasal products and hair dyes. The BCC contamination is an important situation for the FDA (Sutton, 2012). Lucero et al. (2011) reported that the most mechanism for transmission, BCC, was the hospital tap water for using the oral and tracheostomy care. Also, Smet et al. (2013) described the first blood-stream infection from a resource-limited setting from the BCC. Gel-alcohol based products are used by many hospitals for hand washing because of the antimicrobial activity, rapid action, good cutaneous tolerance and easy applications (Caetano, 2011). In Near East University Hospital, Infections Control Units recommends the gel-alcohol based and liquid products for the hand washing. In November 2013, it was shown that all the liquid soaps were contaminated with BCC. The group of these liquid soaps were not distributed before the culture analysis. Although the previously distributed liquid soaps were collected immediately, a few days later, blood culture of a patient was characterized by BCC. This situation implied that previous purchases of liquid soaps might be contaminated with BCC. As the patient is a sickle cell anemia patient, he is an immunocompromised person. Therefore, he is more convenient to the bacteremia than healthy people.

BCC is multiple resistant bacteria to many antibiotics such as aminoglycosides, quinolones, polymyxins and  $\beta$ -lactams so that the treatment of BCC is difficult (Choh, 2013). The strain obtained from blood culture was sensitive to cotrimoxazole. The clinician immediately started the treatment with cotrimoxazole. Both strains were analysed with DiversiLab and PFGE for their molecular epidemiology. The DiversiLab was based on REP-PCR. Our DiversiLab results were confirmed by the PFGE which is a gold standard. The advantage of the DiversiLab is that the result in the same day and property of high repeatability. Also, analysis of the results by DiversiLab was easy to perform in routine laboratory.

Therefore, DiversiLab may be used for the bacterial epidemiological analysis to begin early precaution. The molecular epidemiological studies showed that the patient blood culture strain and the liquid soap strain was in the same cluster. These results indicated that the outbreak of the BCC at the Near East University Hospital was prevented. It was suggested that the liquid soaps should always be analyzed and then distributed to the hospital departments.

## Conflict of Interests

The authors have not declared any conflict of interests.

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