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Epidemiological investigation and successful management of a *Burkholderia cepacia* outbreak in a neurotrauma intensive care unit

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ABSTRACT

Objective: The detailed epidemiological and molecular characterization of an outbreak of *Burkholderia cepacia* at a neurotrauma intensive care unit of a level 1 trauma centre is described. The stringent infection control interventions taken to successfully curb this outbreak are emphasized.
Methods: The clinical and microbiological data for those patients who had more than one blood culture that grew *B. cepacia* were reviewed. Bacterial identification and antimicrobial susceptibility testing was done using automated Vitek 2 systems. Prospective surveillance, environmental sampling, and multilocus sequence typing (MLST) were performed for extensive source tracking. Intensive infection control measures were taken to further control the hospital spread.
Results: Out of a total 48 patients with *B. cepacia* bacteraemia, 15 (31%) had central line-associated blood stream infections. Two hundred and thirty-one environmental samples were collected and screened, and only two water samples grew *B. cepacia* with similar phenotypic characteristics. The clinical strains characterized by MLST typing were clonal. However, isolates from the water represented a novel strain type (ST-1289). Intensive terminal cleaning, disinfection of the water supply, and the augmentation of infection control activities were done to curb the outbreak. A subsequent reduction in bacteraemia cases was observed.
Conclusion: Early diagnosis and appropriate therapy, along with the rigorous implementation of essential hospital infection control practices is required for successful containment of this pathogen and to curb such an outbreak.

Introduction

Bacteria of the *Burkholderia cepacia* complex (BCC) are known opportunistic pathogens causing a plethora of infections and clinical complications, resulting in significant morbidity and mortality, especially in the hospitalized immunocompromised population and outbreak and intensive care settings (Sousa et al., 2017; Gautam et al., 2011; Mangram and Jarvis, 1996; Mahenthiralingam et al., 2008).

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Members of BCC are ubiquitous Gram-negative bacilli that are found in natural habitats such as soil and water. They have the potential to survive and multiply in the presence of disinfectants and indwelling invasive medical devices, thus acting as a potential reservoir for infections in the hospital setting (Donlan and Costerton, 2002). Various host and environmental risk factors such as immune suppression, clinical co-morbidities, prolonged hospital stay, use of a central venous access, and exposure to medical devices, further predispose the patient to infections by BCC. This group of pathogens is emerging and is of increasing concern in trauma patients with multiple injuries (Kim et al., 2016; de Oliveira and Lisboa Lde, 2010). Various medical and surgical interventions, a prolonged hospital stay, and the use of multiple invasive medical devices make trauma patients susceptible to infections by BCC. The organisms have a unique and challenging antimicrobial profile. Increasing multidrug resistance and inherent resistance to polymyxin further complicate the clinical management of these infections (Rhodes and Schweizer, 2016). Only a few outbreaks due to this organism, involving contaminated water, contaminated medication, nebulization solution, antiseptic solution, and intravenous (IV) fluids, have been reported (Memish et al., 2009; Lucero et al., 2011; Martins et al., 2010).

In August 2014, an outbreak of 48 cases of invasive blood stream infection (BSI) due to BCC occurred in a neurotrauma critical care unit of a level 1 trauma care centre in India. This initiated an intensive surveillance and search process to identify additional cases/carriers of the pathogen and institute prompt treatment in order to control the outbreak. This appears to be one of the first reported massive outbreaks of BSI due to BCC especially in trauma patients. This report describes the detailed epidemiological and molecular characterization of the causative pathogen, along with the aggressive infection control measures taken to contain the outbreak.

Material and methods

The Jai Prakash Narayan Apex Trauma Centre is a 170-bed, level 1 hospital of the All India Institute of Medical Sciences in New Delhi. The cluster of BCC infections was observed in a 20-bed neurotrauma intensive care unit (ICU). This ICU has five cubicles and each cubicle contains four beds (Figure 1). Critical patients with head and spinal trauma are admitted for variable numbers of days until they are clinically stable. These cubicles are adjacent but independent, with each having a laminar airflow system. The nurse to patient ratio in the unit is usually 1:2 and the doctor to patient ratio is usually 1:3. There are also other ICU members of staff and counter nurses, and there is free range of movement of the staff inside the ICU premises. One physiotherapist and one radiology technician are available round the clock for the entire unit. All patients have separate disposable invasive devices such as endotracheal tubing, central line devices, ventilator machines, and bedding.

There is a common water supply source for the entire hospital, with different outflow storage chambers for individual ICUs. However, all water supplies are disinfected by chlorination at regular intervals. Chlorination is done twice a year. Also, water supplies are treated by reverse osmosis (RO water) on a daily basis and filtered water is used for various purposes including nebulizers, disinfection of body surfaces, humidifiers, and haemodialyser and analyser equipment. Nebulization machines and flushing solutions are usually commonly shared among patients; however, nebulization solutions and syringes used for flushing the lines are disposable and for one-time use only. There are three ultrasound machines with transducers and ports and these are commonly shared among patients for interventions such as central line insertion, intercostal drain, and haemodynamic monitoring.

The outbreak was suspected in August 2014 and an investigation was triggered when four subsequent cases of bacteraemia caused by *B. cepacia* in two cubicles of this ICU occurred over a period of 7 days. An unusually high incidence of BCC in blood cultures from the neurotrauma ICU was observed, which prompted a detailed epidemiological and microbiological investigation (Mathur et al., 2015). The strains had similar colony morphology and unique pigmentation on culture plates (Figure 2). The relevant demographic characteristics and risk factors were assessed and analysed: age, sex, type and mode of trauma, duration of hospitalization, presence of prior antibiotic treatment, clinical co-morbidities, exposure and duration of exposure to central venous access, device days and history of indwelling devices, and clinical outcome.

Outbreak cases were defined as patients with a clinical suspicion of sepsis (fever, tachycardia, tachypnoea, leukocytosis or leukopenia, with or without hypotension) who had one or more BCC-positive blood cultures. Hospital-acquired infections were defined in accordance with the US Centers for Disease Control and Prevention (CDC) guidelines ((CDC) CfDC, 2017). 'Outbreak' was

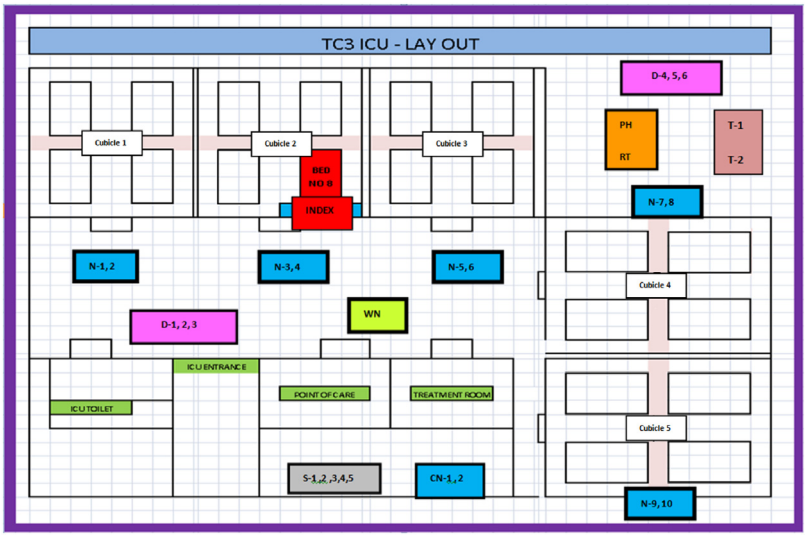

Figure 1. Layout of the neurotrauma ICU where the outbreak occurred. N = nurse (n = 10, two for each cubicle); CN = counter nursing staff (n = 2), D = doctors (n = 6; in shifts, with three for 10 patients); WN = wound nurse; PH = physiotherapist; RT = radiology technician; S = staff (n = 5); T = technician (n = 2, for ABG analyser, ventilator assistant).

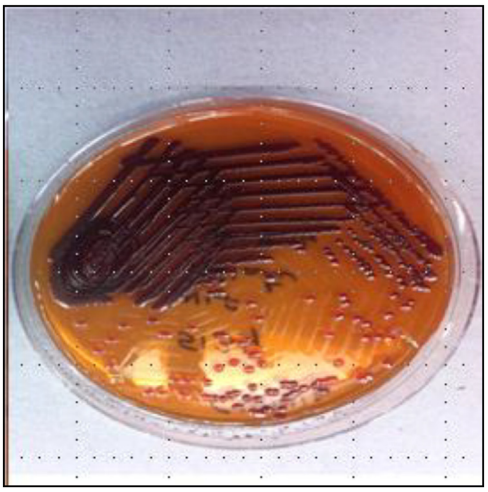

Figure 2. Unusual pigmented colonies of the outbreak *Burkholderia cepacia* complex strain on MacConkey agar.

defined as the simultaneous presence of more than two patients with positive cultures for BCC. The outbreak period was defined as the time between August 2014 and November 2014 during which more than two patients were concurrently infected until the last case of BCC infection with similar phenotypic and genotypic characteristics was reported.

Laboratory diagnosis of sepsis was confirmed by collecting a set of two blood samples. Blood was collected in BacT/ALERT aerobic blood culture bottles (bioMérieux, India), incubated, and monitored regularly using the BacT/ALERT system (bioMérieux, India). All bottles with positive signals were removed from the instrument, Gram-stained, and sub-cultured on blood agar and MacConkey agar. Phenotypic identification was confirmed with a Vitek 2 ID-GNB card (bioMérieux, India). Antimicrobial susceptibility of the clinical isolates was determined both by disc diffusion method in accordance with the US Clinical and Laboratory Standards Institute (CLSI) recommendations and the Vitek 2 AST card (bioMérieux, India) (Institute CaLS, 2015).

The charts of all case patients were also reviewed for potential risk factors and the clinical course. Considering all cases had similar clinical presentation, potential exposures and likely mechanisms of acquisition were sought to track the source and dynamics of transmission. It was hypothesized that the common sources mentioned above would be the possible routes for acquisition of BCC, such as intravenous medications, total parenteral nutrition (TPN), flushing solutions, antiseptics, the water supply, or cross-transmission through staff nurses, doctors, and assistants, as observed in previous BCC outbreaks reported in the literature (Memish et al., 2009; Lucero et al., 2011; Martins et al., 2010).

Combined prospective surveillance and environmental sampling was performed according to this hypothesis, for source tracking. Microbiological cultures were obtained from environmental surfaces, water, air, laminar air flow, the floor, water (tank, drinking water (RO water)), dialysate water, TPN solution, Patients' meals, intravenous (IV) solutions, medications, disinfectants, antiseptics, stethoscopes, and the hands of the healthcare staff/doctors. A total of 231 environmental specimens were collected (Table 1). Phenotypic and genotypic identification was done using standard microbiological methods, as described above for the clinical samples.

Genetic and clonal relatedness among clinical and environmental isolates was assessed by multilocus sequence typing (MLST). MLST was performed by PCR amplification of seven housekeeping genes (ATP synthase beta chain (*atpD*), glutamate synthase large subunit (*gltB*), DNA gyrase subunit B (*gyrB*), recombinase A (*recA*), GTP binding protein (*lepA*), acetoacetyl-CoA reductase (*phaC*), and tryptophan synthase subunit B (*trpB*)), followed by sequencing (Table 2). Total genomic DNA was extracted using an automated instrument (QIAsymphony SP, Qiagen, Germany). The primers and reaction conditions for amplification of the housekeeping genes were obtained from the *B. cepacia* complex PubMLST database (Jolley, 2010). The amplified products were resolved on 2% agarose gel (Millipore Sigma, Saint Louis, MO, USA) and purified using the QIAquick PCR purification kit (Qiagen).

The sequencing of PCR amplicons was performed with the Big Dye Terminator (v3.1) cycle sequencing kit (Applied Biosystems), according to the manufacturer's protocol. Post cycle sequencing products were purified by ethanol/EDTA/sodium acetate precipitation method, as recommended by the manufacturer. The purified products were sequenced on an ABI 3500 Genetic Analyser (Applied Biosystems) using the fast sequencing module with performance-optimized polymer 7 (POP-7) and 50-cm capillary array.

The allele numbers for the seven housekeeping genes and the sequence types based on the seven allelic combinations were predicted from the *B. cepacia* complex PubMLST database (Jolley, 2010). The new allele variants and sequence types (STs) were submitted to the database. In order to establish the clonal association of the predicted sequence types, GeoBurst analysis was performed using PhyloWiz software, version 2 (Francisco et al., 2009).

Table 1
Environmental sampling sites and sample numbers.

S. No.	Environmental sample	Number
1	Water – main tank	5
2	Water – ICU supply	12
3	Humidifier (ventilator), Bain's circuit, Ventilator tubing	20
4	Ultrasound machine ports and transducers	6
5	Air sampling (settle plate method)	26
6	Kitchen feed	14
7	Dialysate water	10
8	IV normal saline, IV heparin solution	25
9	Disinfectants – alcohol solution, Korsolex (peracetic acid), glutaraldehyde solution	20
10	TPN feed	6
11	Nebulization medication – albuterol, tiotropium bromide	12
12	Swabs from hands of healthcare workers	27
13	Swabs from stethoscopes	12
14	Bed rails – head end and foot end, linen	36

ICU, intensive care unit; IV, intravenous; TPN, total parenteral nutrition.

Table 2
Characteristics of primers used in the MLST scheme for *Burkholderia cepacia* complex.

Gene	Direction	Primer sequence for PCR amplification (5′–3′)	Primer sequence for sequencing reaction (5′–3′)	Allele size (bp)
atpD	F	GATCGTACAGTGCATCGG	GTTCATCTGGCCGTACAC	443
	R	ATCGTGCCGACCATGTAG	AACTGACGCTCGAAGTCC	
gltB	F	CGCTCGAAGATCAAGCAG	CTTCTTCTTCGTCGCCGA	400
	R	GGGAACACCTTCACGAAC	TTGCCGACGTAGTCGTTG	
gyrB	F	CGACAACTCGATCGACGA	ATCGTGATGACCGAGCTG	454
	R	GACAGCAGCTTGTCGTAG	CGTTGTAGCTGTCGTTCC	
recA	F	GATAGCAAGAAGGGCTCC	TGACCGCCGAGAAGAGCAA	393
	R	CTCTTCTTCGTCCATCGCCTC	GACCGAGTCGATGACGAT	
lepA	F	CGACGGCAAGGTCTACAA	GGCATCAAGGAACTGACG	397
	R	AGCATGTCGACCTTCACG	CTGCGGCATGTACAGGTT	
phaC	F	CTCAGCGAATTGCGTACG	AGACGGCTTCAAGGTGGT	385
	R	CCGTTCAGCGAGAAGTCG	ACACGGTGTTGACCGTCA	
trpB	F	GATCTACCTGAAGCGCGA	CTGGGTCACGAACATGGA	301
	R	GTGTGCATGTCCTTGTCG	CCGAATGCGTCTCGATGA	

MLST, multilocus sequence typing; F, forward; R, reverse.

After the fourth case of BCC infection, intensive and aggressive infection control measures were taken, including cleaning of the ICU, fumigation of the ICU using vapour phase hydrogen peroxide, and water disinfection. The use of sterile gloves and barrier nursing were ensured; closed monitoring of the reconstitution and preparation of IV medications was done. Infection prevention and control activities, i.e., hand hygiene and a central line preventive bundle strategy, were observed. Compliance to these infection control protocols was assessed thoroughly and infection control policies were made more stringent to avoid cross-transmission and to curb further spread. All infection control steps and interventions are described in the Results section below.

Results

During the outbreak period from August 2014 to November 2014, a total of 48 cases of invasive BCC BSI were reported. Since the incidence of BCC bacteraemia over the last year in the neurotrauma ICU was 0–1.5 cases/month, this sudden increase in BCC infections was defined as an outbreak. All of the case patients were in the neurotrauma ICU over this period. The index case was a 62-year-old female patient with a history of fall from a staircase who had suffered a subarachnoid haemorrhage. The median age of the infected patients was 38.2 ± 2 years (range 16–71 years) and there was a greater proportion of male patients ($n = 31$; 64.5%). The mean length of hospital stay was 8 ± 1 days (range 3–62 days) and the mean length of ICU stay was 6.4 ± 2 days (range 3–31 days). The predominant patient injury was a head trauma ($n = 29$; 60.4%), followed by a spinal trauma ($n = 22$; 45.8%).

Various risk factors were observed in the BCC-positive cases during the study period, including a prolonged length of hospital stay >7 days (71%; $n = 34$) and central venous access (87.5%; $n = 42$), among others, in comparison to the non-infected cases ($n = 131$). Risk factors for cases and matched controls are listed in Table 3.

Of the total 179 patients admitted during the outbreak period, 48 patients with suspected clinical sepsis developed a laboratory-confirmed BSI on one or more occasions. Of these patients, 15 had a central line-associated BSI (CLABSI) and 14 also had ventilator-associated pneumonia (VAP), with 11 patients having positive bronchoalveolar lavage (BAL) culture and three patients having positive tracheal aspirate culture. Among the patients infected, the observed in-hospital mortality rate was 18.7% ($n = 9$), with mortality due to various causes: arrhythmias ($n = 1$), hyperkalemia causing cardiac arrest ($n = 2$), aspiration and refractory bradycardia ($n = 1$), hypovolemic shock due to bleeding ($n = 1$), and septic shock due to BCC ($n = 4$). Thus, the true attributable mortality rate due to BCC bacteraemia was 8.33%.

Microbiological workup

A total of 62 clinical isolates were obtained from the neurotrauma ICU (48 from blood, 11 from BAL, and three from tracheal aspirate). The mean time from admission to culture positivity was 8.1 days. Microbial culture demonstrated growth of unusually highly pigmented violet-coloured colonies of 0.5–1 mm, with an entire edge and regular margins, in contrast to typical non-lactose-fermenting colonies of BCC (Figure 2). All clinical isolates obtained had a similar morphological appearance and growth characteristics on microbial culture. All clinical isolates obtained from blood were identified as *B. cepacia* complex by Vitek 2 identification systems.

Sequence analysis of the *recA* gene revealed isolates to be 99% similar to those of the *B. cepacia* type strain, thus identifying them as *B. cepacia*. Antimicrobial susceptibility testing revealed that all outbreak-associated BCC isolates exhibited a similar antibiogram.

Table 3
Risk factors among cases and controls during outbreak.

Risk variables	Cases ($n = 48$)	Controls ($n = 131$)
Prolonged ICU stay >7 days	34 (71%)	78 (60%)
Central venous access	42 (87.5%)	91 (69.4%)
Exposure to mechanical ventilation >5 days	37 (77.5%)	87 (66.5%)
CSF shunts	15 (31.2%)	36 (27.5%)
Neutropenia	5 (10.4%)	11 (84%)
Age >50 years	16 (33.3%)	28 (21.4%)
Diabetes mellitus	11 (23%)	29 (22.1%)
Use of steroids	3 (6.3%)	12 (9.2%)
Multiple surgical interventions >2	9 (18.8%)	20 (15.3%)

ICU, intensive care unit; CSF, cerebrospinal fluid.

Table 4
Antibiogram of all the outbreak-associated *Burkholderia cepacia* complex isolates.

S. No	Antimicrobials	Antimicrobial sensitivity ($n = 56$ isolates)
1	Ceftriaxone	Resistant
2	Cefepime	Sensitive
3	Ciprofloxacin	Resistant
4	Levofloxacin	Resistant
5	Piperacillin and tazobactam	Resistant
6	Cefoperazone sulbactam	Sensitive
7	Imipenem	Sensitive
8	Meropenem	Sensitive
9	Tigecycline	Sensitive
10	Gentamicin	Resistant

All were sensitive to carbapenems and resistant to third-generation cephalosporins and piperacillin–tazobactam (Table 4).

Of the total 231 environmental samples screened at periodic intervals as part of the intensive source tracking and control, two samples of water (main supply from the tank and RO water) grew isolates that had similar growth characteristics and shared a similar antibiogram. The Vitek 2 identification systems and *recA* gene sequencing confirmed both isolates as BCC.

Due to limited resources, MLST analysis could be performed for only six of the BCC isolates (three blood isolates, one central venous catheter tip (CVC) isolate, and two environmental isolates). All of the blood isolates were selected randomly, considering that all of the blood isolates were from the neurotrauma ICU and all had similar phenotypic characteristics and antibiogram profile. However, the one isolate from a CVC tip was chosen to corroborate central line as the source of bacteraemia, because the index patient had CLABSI. Only two environmental samples (water samples) were culture-positive for BCC with similar phenotypic characteristics and antibiogram as the clinical strains, and these were subjected to MLST typing. MLST by eBURST identified all clinical strains as belonging to a single sequence type profile, ST-922, which remained as a singleton in the eBURST group (Figure 3). Furthermore, the environmental strains showed different allelic profiles and STs, indicating that the environmental isolates were unrelated to the outbreak strain. The isolate from the tank water (1573) belonged to ST-6 and was found to be associated with the minor clonal complex of two STs by eBURST analysis. On the other hand, the isolate from the RO water sample (2608) belonged to ST-1289, which belonged to the largest clonal complex consisting of 73 STs, with ST-234 as the founder ST. Interestingly, this is a novel and previously unreported sequence type among BCC isolates obtained from an environmental source (Table 5).

After four consecutive cases, detailed systematic and rigorous hospital infection control practices were undertaken to prevent further spread of the outbreak. Initially, the infected patients were isolated and cohorted by means of physical barrier/curtain stands within the cubicle. Every cubicle was disinfected by fogging using hydrogen peroxide vapour. Intensive terminal cleaning of cubicles, bed rails, and bedside objects was done with quaternary ammonium compounds. Thorough cleaning of the curtains, bedding, and linen was done using appropriate heat disinfection.

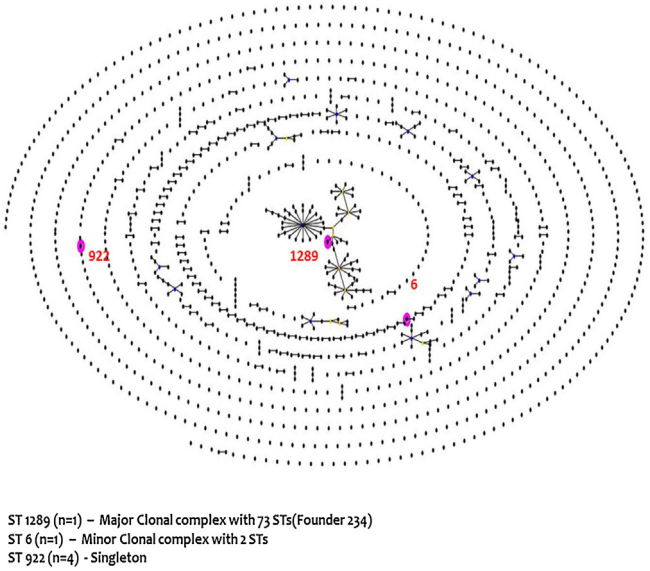

Figure 3. eBURST analysis of the *Burkholderia cepacia* isolates. The sequence types identified in this study are marked.

Hand hygiene with plain or antimicrobial soap and water or alcohol-based hand rub was intensively reinforced, and the use of personal protection equipment was encouraged if contact with patients or objects in the patient rooms was anticipated. Visitors were also asked to follow the same set of instructions and precautions. There is an intensive, automated on-going hospital-acquired infection surveillance network and hand hygiene monitoring system at the trauma centre (Mathur et al., 2015). The hand hygiene compliance rate was measured and followed up stringently. An increase in compliance from 58.5% to 61% was shown. All IV fluids, TPN, and medications were scrupulously checked and administered with significant caution. Feed preparation was strictly regulated and monitored. Exhaustive and meticulous cleaning of water tanks and chlorination was immediately done and then regularly monitored at frequent intervals. Environmental sampling, including sampling of healthcare workers, was done at regular intervals. Central line insertion was closely and meticulously monitored, and maintenance was regularly and strictly assessed and recorded. Central line bundle compliance was assessed and recorded on a daily basis and special training was given to healthcare workers to increase compliance to aseptic procedures and techniques. There was an increase in central line bundle compliance from the baseline rate of 70% to 74%, and this was maintained throughout the outbreak period (Figure 4). All staff and healthcare workers concerned participated in meetings held regularly to discuss measures and recommendations to control the outbreak.

After diligent, exhaustive, and timely efforts along with efficient source tracking and control activities, a drastic reduction in cases of BCC bacteraemia was observed over the next 4 months, and cases with isolation of this characteristic strain reduced to zero after December 2014.

Discussion

BCC are opportunistic Gram-negative nosocomial pathogens that can cause life-threatening disease in patients and have the propensity to produce serious outbreaks within the clinical setting (Sousa et al., 2011). BCC are emerging and challenging pathogens in trauma victims in whom several injuries and various medical and surgical interventions lead to a prolonged hospital stay, along with exposure and dependence on various invasive medical devices and the use of multiple broad-spectrum antibiotics as prophylaxis or therapeutic regimen (Bressler et al., 2007; Boszczowski et al., 2014). Furthermore, the bacteria have innate potential to survive and proliferate in the hospital environment, growing on moist surfaces such as water tanks and others, making these very successful nosocomial pathogens causing serious life-threatening infections in young adult trauma patients. *B. cepacia* is a well-reported pathogen of a substantial number of hospital outbreaks, attributed to a plethora of sources: antiseptics, disinfectants, and nebulizer solution (Memish et al., 2009).

This appears to be one of the first studies to describe a large outbreak of invasive BSI due to BCC in which a detailed clinical–genetic analysis has been performed. This study describes the epidemiological investigation and highlights how sustained infection control interventions successfully controlled this massive outbreak.

BCC are virulent pathogens that can produce serious outbreaks within a clinical setting, possibly through horizontal transmission. The usual incidence of nosocomial *B. cepacia* BSI was low in the study hospital (0.5–1 per 100 admissions). These infections are more frequent in and restricted to polytrauma patients, owing to multiple trauma and various medical and surgical interventions. The upsurge and consecutive increase in cases of BCC one after another over a period of 2–4 weeks, surmounting to the large

Table 5
MLST results for *Burkholderia cepacia* isolates.

S. No.	MLST genes/isolates	Sample source	*atpD*	*gltB*	*gyrB*	*recA*	*lepA*	*phaC*	*trpB*	Sequence type
1	2608	RO water	131	11	847[a]	14	11	6	79	1289[b]
2	1573	Tank water	66	109	49	3	3	40	53	6
3	5134	CVC tip	361	325	640	382	431	329	330	922
4	6019	Blood	361	325	640	382	431	329	330	922
5	7781	Blood	361	325	640	382	431	329	330	922
6	5947	Blood	361	325	640	382	431	329	330	922

MLST, multilocus sequence typing; RO, reverse osmosis; CVC, central venous catheter.
[a] Indicates the new allele number identified for the *gyrB* gene.
[b] Indicates the new sequence type.

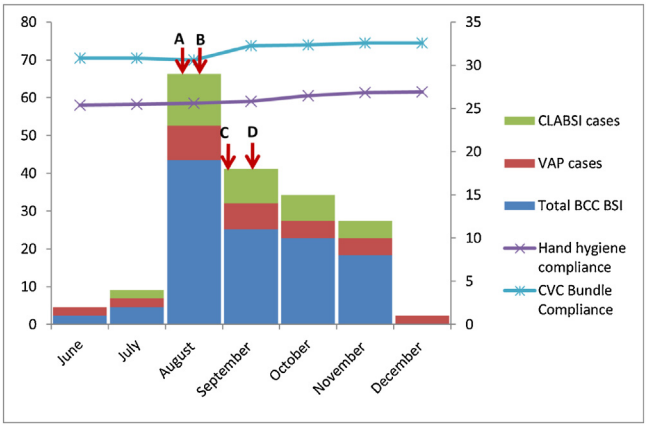

Figure 4. Trend of the outbreak cases and infection control practices over the outbreak period. 'A' indicates barrier precautions and cohorting of patients; 'B' indicates the onset of environmental surveillance and source tracking; 'C' indicates the start of water disinfection; 'D' indicates cleaning of the ICU and fumigation using hydrogen peroxide vapour.

number of 48 cases, particularly because it occurred in the ICU catering for head and/or spinal trauma patients who were otherwise healthy and immunocompetent, raised the suspicion of a common source aetiology. The upsurge in cases served as a warning to all competent clinical authorities and the hospital administration, indicating the need for an investigation and formulation of an outbreak control protocol to curb the outbreak.

In the study hospital, BSI, including catheter-related BSI, was the most common (27%) *B. cepacia* infection. *B. cepacia* bacteraemia, most often in association with polymicrobial catheter-related infection, has been reported in previous studies. Most of the infected patients had a central line access (87.5%), and prolonged exposure to central venous access of >7 days (mean of 11 device-days) was observed to be the major risk factor in this study. Two previous outbreaks of *B. cepacia* nosocomial BSI in a neonatal ICU were reported by Lee (2008). Another clinical presentation observed in the present study was *B. cepacia* pneumonia, which occurred in 7.8% of cases. In this outbreak, 37 (77.5%) of the patients were on mechanical ventilation. Mechanical ventilation was found to be a major risk factor for respiratory acquisition of BCC in this study, with 77% of patients on ventilators. Oropharyngeal bacterial colonization during intubation and poor cough reflex may contribute to the development of pneumonia (Gomes-Filho et al., 2010). The association with mechanical ventilation has been described in previous outbreaks (Guo et al., 2017; Zurita et al., 2014).

One of the major attributable causes in a large number of outbreaks due to BCC is the difficulty in appropriate and timely laboratory diagnosis. Furthermore, the constantly evolving and changing biological properties of BCC complicate and pose a challenge to the existing diagnostic armamentarium. With the aid of advanced and automated identification systems and rapid communication and critical care alert updates to the trauma centre clinicians, infected cases were managed in a timely manner. All clinical and environmental strains were identified phenotypically as BCC. One peculiar observation during the microbiological workup was the growth of highly pigmented strains of BCC on MacConkey agar, which is in contrast to the colonies typically described; it was thus hypothesized that a different strain type with altered virulence properties was responsible for this outbreak, thereby necessitating the use of genetic and advanced molecular studies.

BCC have a unique and challenging antimicrobial profile. They are resistant to multiple antibiotics, especially to the commonly used aminoglycosides, second-generation cephalosporins and intimidating polymyxin (Gautam et al., 2011). Different resistance patterns have been reported in the different outbreaks that have occurred worldwide (Rhodes and Schweizer, 2016; Dizbay et al., 2009; Tseng et al., 2014). Fortunately, in this outbreak, the strains were susceptible to carbapenems. Meropenem was administered in most of the cases with variable susceptibilities to other antimicrobials. All clinical and environmental strains had similar antimicrobial susceptibility patterns, in agreement with previous studies (Memish et al., 2009).

Clonal relatedness was established on the basis of MLST analysis. Although pulsed field gel electrophoresis (PFGE) has historically been used to define outbreaks and clusters (Dizbay et al., 2009; De Smet et al., 2013), the technique is cumbersome, requiring dedicated equipment that is not widely available (Machado et al., 2014). Many recent studies have explored the potential of MLST as a tool for detailed investigation of outbreaks (Mali et al., 2017; Singhal et al., 2015; Wang et al., 2015; Sails et al., 2003). Also, studies comparing the results of MLST schemes to PFGE have shown 100% agreement between the two techniques (Machado et al., 2014). MLST is also the method of choice for typing BCC due to its ability to differentiate the species in BCC complex (Mahenthiralingam et al., 2008; Vandamme and Dawyndt, 2011; Gautam et al., 2016).

All of the clinical isolates obtained in this study were clonal and were ST-922 on MLST analysis. The sequence type ST-922 identified in the study isolates was corroborative with a previously submitted clinical isolate (cystic fibrosis, Spain origin) recorded in the PubMLST database (Jolley, 2010). In the case of ST-6, the isolate source was of clinical origin in the PubMLST database (cystic fibrosis, North America) and it varied from the source of the isolate in the present study. The sequence type ST-1289, which was isolated from the tank water supply, had a clonal association with the sequence type ST-628, which was previously assigned to isolates from the blood and respiratory samples of cystic fibrosis patients in a previously reported study (Gautam et al., 2016).

ST-1289 is the single locus variant of ST-628 with allelic variation corresponding to the transition T > C at position 101 of the *gyrB* gene. However, ST-628 is linked to clonal complex 31,

comprising various ST types such as ST-839, 217, 826, 621, 822, and 841 (Gautam et al., 2016). ST-1289 was previously unreported and is a novel ST found in this study. The results obtained indicate the possible circulation of clonal complex I in India and transmission of the evolved ST-628 into the environment as ST-1289 (Gautam et al., 2016). However this needs further investigation in a study of a large set of isolates of clinical and environmental origin.

Inadequate cleaning of the water source or a breach in sanitation, environmental cleaning, and infection control policies could have been a conduit of transmission of the BCC. After the preliminary phenotypic association was obtained, the water tanks were immediately disinfected and monitored at regular intervals. After four cases of bacteraemia, the infection control team of the trauma centre responded promptly and methodically. The implementation of contact and isolation-based precautions, strict reinforcement of hand hygiene, adherence to device insertion, maintenance checklists, and other measures were conducted concurrently (Figure 4). There was an increase in hand hygiene compliance rate of 2.5% (from 58.5% to 61%) during the outbreak period. Central line compliance also increased from 70% to 74% after interventions and was maintained throughout the study duration. The increase in compliance rates in comparison to the pre-outbreak period was modest, due to the rotation of health-care staff every month. This necessitates repeated sets of training for the new staff and the reinforcement of infection control strategies. Although the compliance rates did not change dramatically, this increased compliance did contribute to a successful reduction of BCC bacteraemia cases, as supported by previous studies (De Smet et al., 2013; Kuzumoto et al., 2011; Wiener-Well et al., 2014).

The outbreak control measures were broad-based, and water was implicated as a major environmental source. Water samples grew BCC with similar phenotypic characteristics as those of the clinical isolates, including the morphological resemblance of colonies and similar antibiogram. The authors also believe that water acted as a point source of infection in this outbreak. As a result, water disinfection was implemented urgently to contain further transmission. In most hospital outbreaks, adherence to infection control activities is an all or none phenomenon required to control the outbreak. In agreement with other studies, coordinated infection control activities such as outbreak control measures based on sound infection control principles, implementing policies concerning hand hygiene, patient cohorting, barrier and contact precautions, visitor restrictions, and enhanced environmental cleaning and disinfection were taken in conjunction in order to terminate the outbreak.

This study illustrates the value of vigilant suspicion and careful epidemiological investigation in controlling the colonization or infections due to BCC. Complete elimination of the transmission among the trauma patients was achieved only after the stringent intervention and adherence to infection control strategies. The use of automated and rapid diagnostic modalities with timely management and increased compliance to hand hygiene and other measures actually led to the cessation of the outbreak. Although molecular typing is essential for exact source tracking, the preliminarily establishment of a link inferred by advanced and accurate phenotypic tests may help in the identification of the source and thus timely containment (Bittar and Rolain, 2010).

In conclusion, early, effective diagnosis and appropriate therapy, along with the implementation of effective hospital infection control practices, are required for the successful management of this pathogen and better clinical outcomes. Furthermore, detailed investigations are critical to detect the emergence of novel genetic variants, understand the colonization history of the pathogens, and assess potential sources and scenarios contributing to the emergence of infections.

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Ethics statement

This study did not require any ethical clearance as it dealt primarily with a hospital outbreak, investigation and containment. Clinical samples such as human blood and other relevant samples were received in the microbiology laboratory as a part of routine patient care and diagnostic microbiological workup of suspected hospital-acquired infections.

Conflict of interest

None of the authors have any conflict of interest.

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