Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 3 March 2025

Research Article

Characterization of *Burkholderia* Species from Various Clinical Specimens and their Antimicrobial Susceptibility Patterns

Nishtha Singh[1], Sheetal Verma[2]*, Amber Azim[3], Vimala Venkatesh[4], Raj Kumar Kalyan[4] and Prashant Gupta[4]

[1]*Department of Microbiology, Assistant Professor, T.S. Misra University, India*

[2]*Department of Microbiology, Additional Professor, King George Medical University, India*

[3]*Department of Microbiology, Senior Resident, King George Medical University, India*

[4]*Department of Microbiology, Professor, King George Medical University, India*

***Corresponding Author:** Sheetal Verma, Department of Microbiology, Additional Professor, King George Medical University, India.

DOI: 10.31080/ASMI.2025.08.1495

Received: January 10, 2025

Published: February 21, 2025

Abstract

Introduction: *Burkholderia* spp. causes infections including bacteraemia, urinary tract infection, septic arthritis, peritonitis and respiratory tract infection in immunocompromised and competent individuals. Due to high intrinsic resistance and being one of the most antimicrobial-resistant organism encountered in the clinical laboratory, is responsible for increased mortality and morbidity.

Methods: Clinical samples were collected from patients admitted in intensive care units of a university hospital between January 16, 2023 to March 16, 2023. The samples were sub-cultured on 5% sheep blood agar and MacConkey agar. Plates were incubated at 37°C for up to 72 hours. Isolates were identified using MALDI-TOF-MS. Antimicrobial susceptibility was evaluated as per latest CLSI guidelines.

Results: Total *Burkholderia* spp isolated were 154 from different clinical samples. Majority of isolates were *Burkholderia cenocepia* (74%), followed by *Burkholderia* cepacia (18.2%), *Burkholderia contaminans* (1.29%), *Burkholderia vietnamiensis* (1.29%), *Burkholderia metallica, Burkholderia ambifaria* and *Burkholderia multivorans* (0.64%) each. *Burkholderia* was mainly isolated from blood (87.6%) followed by pus (9.09%). Isolates resistant to ceftazidime and levofloxacin were 14% and to chloramphenicol 7.05% and minocycline 1.04%. Isolates demonstrated 100% sensitive to meropenem and sulfamethoxazole plus trimethoprim.

Conclusions: *Burkholderia* spp. appears to be an emerging cause of septicaemia in intensive care units. Meropenem, minocycline and sulfamethoxazole trimethoprim appear to promising therapeutic options. MALDI-TOF-MS is a reliable tool for prompt diagnosis and commencement of appropriate antibiotic therapy based on antimicrobial susceptibility testing.

Keywords: *Burkholderia cepacia* Complex (Bcc); Cystic Fibrosis (CF)

Introduction

Burkholderia acepacia complex (Bcc) is a Gram-negative, oxidase-positive, non-fermenting saprophytic bacilli, which belongs to the *Burkholderiaceae* family. It comprises of twenty taxonomically valid species [1]. *This* complex (BCC) is considered a group; in which some of them can be distinguished phenotypically, whereas few require genotype identification. This is a group of aerobic, non-spore forming, catalase producing, lactose non-fermenting bacteria. It belongs to the *Burkholderiaceae* family and consists of diverse species which include both friends and foe [2,3]. Bcc is a devastating pulmonary pathogen in cystic fibrosis patients and has also been reported with increasing frequency as a cause of bacteraemia, particularly in patients with indwelling catheters, urinary tract infection and peritonitis [4]. In particular, non-fermenting gram negative bacteria pose a significant problem in the clinical environment, as they are a common cause of nosocomial infections and are resistant to many antibiotics. Bcc species of the *Burkholderia* genus are diverse and can adapt to varying environmental conditions including nutrient scarcity, antibiotics, antimicrobial peptides and toxic substances [5]. These species have a complex genome with three chromosomes and a high capacity for rapid mutation and adaptation [6]. This organism particularly affects the lungs in patients with Cystic Fibrosis (CF) and is regarded as an important opportunistic pathogen in hospitalised and immunocompromised patients. Bcc has emerged as a serious nosocomial pathogen worldwide, due to its high intrinsic resistance to most antibiotics, acquired resistance to fluoroquinolones and antiseptics [7]. The intrinsic resistance is because of mechanisms of resistance include changes in lipopolysaccharide structure, the presence of several multi drug efflux pumps, altered penicillin binding proteins and also strongly associated with the development of biofilms. *B. cepacia* is a resilient organism, able to survive in environments devoid of significant nutritional resources. It can grow in aqueous environments and on surfaces commonly found in hospitals, such as polyvinyl chloride, a material frequently used in respiratory therapy equipment [8,9]. Many of these contamination episodes have been associated with the ability of Bcc bacteria to thrive in the presence of antimicrobials and disinfectants, particularly the biocides used in pharmaceutical products' formulations [10,11]. The speed and low cost of bacterial identification by MALDI-TOF MS make it an attractive technology in the clinical microbiology laboratory which allows the identification of a broad range of microorganisms [12]. There is lack of data on *Burkholderia* speciation and characterization to prevent hospital acquired infection, thus more exhaustive research is required in this field.

Method

The present hospital based cross-sectional study was carried out in the intensive care unit and Department of Microbiology, King George Medical College, Lucknow, from January to March 2023. All patients with nosocomial infections (NI) due to *Burkholderia* were included in this study. The data was obtained from infection control committee records. About 154 patients who were admitted in different Intensive care units and wards in the month of January-March 2023 having *Burkholderia* infections were included. Surveillance was performed actively, based on both laboratory and patients records, during the period. The diagnosis of nosocomial infections was made according to Centres for Disease Control and Prevention (CDC) criteria. The laboratory data of the patients were collected and analyzed: age, sex, ward, duration of hospitalization, microbiological data and outcome. As a part of routine investigations blood, urine, sputum or tracheal secretions sample were sent for culture and sensitivity to the Microbiology laboratory. By conventional method, all the samples were cultured (except blood) onto Blood agar, chocolate agar and Mac- Conkey' s agar; incubated for 18-24 hours at 37^0C. Blood cultures were performed in BACT/Alert 3D (Biomeriux), only positives were subculture by conventional method. Further analysis was done in culture positive samples only. In positive cases the isolates were identified to the species level by conventional biochemical tests. After 24 hour so aerobic incubation, typical large, circular, low convex, moist β-haemolytic colonies was observed on Blood agar and non-lactose fermenting colonies on Mac-Conkey's agar. On Gram staining, Gram negative bacilli were seen, they were motile, oxidase positive. Phenotypic identification was confirmed with a MALDI-TOF MS; Vitek 2 ID-gram negative bacteria (GNB card), (bioMérieux, India). Antimicrobial susceptibility of the clinical isolates was determined by disc diffusion method in accordance with the US Clinical and Laboratory Standards Institute (CLSI) recommendations and the Vitek 2 AST card (bioMérieux, India). Antibiogram of the isolate was performed in accordance with latest CLSI guidelines. Antibiotic susceptibility tests (MIC) were performed by Vitek 2 compact system on minocycline, meropenem, ceftazidime, levofloxacin and cotrimoxazole. The data was analyzed and interpreted.

Results

The data presents a distribution of individuals based on two key variables: age and sex. In terms of age, the population is divided into several age groups. The majority falls within the 20 to 39-year-

Table 1: Distribution of Subjects by Age and Sex.

Variable		No.	%
Age	10 - 19 yr	7	4.5%
	20 - 29 yr	35	22.7%
	30 - 39 yr	37	24.0%
	40 - 49 yr	28	18.2%
	50 - 59 yr	24	15.6%
	60 - 69 yr	18	11.7%
	>= 70 yr	5	3.2%
SEX	Male	115	74.7%
	Female	39	25.3%

Table 2: Distribution of Subjects by *Burkholderia* Species.

Species	No.	%
B. Cenocepacia	114	74.0%
B. Cepacia	26	16.9%
B. Species	5	3.2%
B. vietnamiensis	3	1.9%
B. contaminans	2	1.3%
B. Multivorans	1	.6%
B. Metallica	1	.6%
B. Glandeao	1	.6%
B. Ambifara	1	.6%

old range, with 24.0% in the 30 to 39-year-old category and 22.7% in the 20 to 29-year-old category. The 40 to 49-year-old group constitutes 18.2% of the population, while those aged 50 to 59 years make up 15.6%. A smaller but still noteworthy portion is in the 60 to 69-year-old category at 11.7%, and a smaller percentage, 3.2%, comprises individuals aged 70 years or older. Regarding sex, the data shows a distribution between males and females. The majority, 74.7%, are male, while the remaining 25.3% are female.

The data provides insights into the distribution of bacterial species within a specific population. The majority, accounting for 74.0% of the population, is composed of *B. cenocepacia*. Following closely is *B. cepacia*, representing 16.9% of the population. *B. species*, a broader categorization, constitutes 3.2%, while *B. vietnamiensis* and *B. contaminans* are present at 1.9% and 1.3%, respectively. The less prevalent species include *B. Multivorans, B. Metallica, B. Glandeao,* and *B. Ambifara*, each making up 0.6% of the population. This breakdown of bacterial species distribution is valuable for understanding the prevalence and composition of these microorganisms within the studied group.

In terms of the ward, the majority of patients, comprising 52.6%, were admitted to the CCM (Critical Care Medicine) ward. Smaller percentages were admitted to the GW (General Ward) at 4.5%, PCCM (Pulmonary and Critical Care Medicine) at 7.8%, TVU (Thoracic and Vascular Surgery Unit) at 26.6%, and QMH (Queen Mary Hospital) at 3.2%. Additionally, a further 5.2% were admitted

Table 4: Association of *Burkholderia* Species with Age.

Species		Age						
		10 - 19 yr	20 - 29 yr	30 - 39 yr	40 - 49 yr	50 - 59 yr	60 - 69 yr	>= 70 yr
B. Cenocepacia	No.	5	25	27	19	21	16	1
	%	71.4%	71.4%	73.0%	67.9%	87.5%	88.9%	20.0%
B.Cepacia	No.	0	5	8	7	2	2	2
	%	0.0%	14.3%	21.6%	25.0%	8.3%	11.1%	40.0%
B.Species	No.	0	2	2	1	0	0	0
	%	0.0%	5.7%	5.4%	3.6%	0.0%	0.0%	0.0%
B. vietnamiensis	No.	0	2	0	0	0	0	1
	%	0.0%	5.7%	0.0%	0.0%	0.0%	0.0%	20.0%
B. contaminans	No.	0	1	0	0	0	0	1
	%	0.0%	2.9%	0.0%	0.0%	0.0%	0.0%	20.0%
Other	No.	2	0	0	1	1	0	0
	%	28.6%	0.0%	0.0%	3.6%	4.2%	0.0%	0.0%
Significance		chi sq = 61.83, p < 0.001						

to other, unspecified wards. The sample types collected from these patients include blood, with the highest representation at 85.7%, followed by pus at 9.1%, fluid at 1.9%, urine at 1.3%, and ET aspirate at 1.9%. These different sample types are crucial for diagnostic and investigative purposes, aiding healthcare professionals in determining the underlying causes of various medical conditions. Furthermore, this dataset highlights the critical factor of ICU (Intensive Care Unit) admission, with 90.9% of patients requiring intensive care, while 9.1% did not require ICU admission.

The association between *Burkholderia* species and patient age was examined in this study. The results indicate a significant relationship between these variables, with a chi-square statistic of 61.83 and a p-value less than 0.001. *B. cenocepacia* was the predominant species across all age groups, with the highest prevalence observed in the 60 - 69 years age group at 88.9%, followed by the 50 - 59 years age group at 87.5%. In contrast, this species was less prevalent in the youngest age group (10 - 19 years) at 71.4%. *B. cepacia* showed an increasing trend in prevalence with age. While it was not detected in the 10 - 19 years age group, its prevalence steadily increased in older age groups, reaching 40.0% in individuals aged 70 years and above. *B. vietnamiensis*, and *B. contaminans* were less prevalent overall and did not show consistent patterns across age groups. Lastly, the "Other" category exhibited some variation in prevalence across age groups, with the highest prevalence in the 60 - 69 years age group at 4.2%. Overall, these findings suggest a statistically significant association between *Burkholderia* species and age, with varying prevalence patterns among different species.

Table 5: Association of *Burkholderia* Species with Sex.

Species		SEX	
		Male	Female
B. Cenocepacia	No.	83	31
	%	72.2%	79.5%
B. Cepacia	No.	20	6
	%	17.4%	15.4%
B. Species	No.	5	0
	%	4.3%	0.0%
B. vietnamiensis	No.	1	2
	%	.9%	5.1%
B. contaminans	No.	2	0
	%	1.7%	0.0%
Other	No.	4	0
	%	3.5%	0.0%
Significance		chi sq = 6.72, p = 0.242	

The association between *Burkholderia* species and patient sex was explored in this study. The results reveal no statistically significant relationship between these variables, as indicated by a chi-square statistic of 6.72 and a p-value of 0.242. Among the *Burkholderia* species examined:

- *B. cenocepacia* was more prevalent in both males (72.2%) and females (79.5%).
- *B. cepacia* was found in 17.4% of males and 15.4% of females.
- *B. species* was detected in 4.3% of males but was absent in females.
- *B. vietnamiensis* was present in 0.9% of males and 5.1% of females.
- *B. contaminans* was identified in 1.7% of males and was not found in females.
- The "Other" category had a prevalence of 3.5% in males and was absent in females.

In summary, the study did not find a significant association between *Burkholderia* species and patient sex, with similar prevalence patterns observed in both males and females across the different species.

The association between *Burkholderia* species and the ward in which patients were admitted was investigated. The results show that there is no statistically significant association between these variables, as indicated by a chi-square statistic of 24.79 and a p-value of 0.491.

The distribution of *Burkholderia* species across different wards is as follows:

- CCM (Critical Care Medicine) had the highest prevalence of *B. cenocepacia* at 75.3%, *B. cepacia* at 16.0%, *B. species* at 2.5%, *B. vietnamiensis* at 2.5%, and Other at 3.7%.
- GW (General Ward) had *B. cenocepacia* at 85.7%, *B. cepacia* at 0.0%, *B. species* at 0.0%, *B. vietnamiensis* at 0.0%, and Other at 14.3%. PCCM (Pulmonary and Critical Care Medicine) had *B. cenocepacia* at 75.0%, *B. cepacia* at 8.3%, *B. species* at 8.3%, *B. vietnamiensis* at 0.0%, and *B. contaminans* at 8.3%. TVU (Thoracic and Vascular Unit) had *B. cenocepacia* at 70.7%, *B. cepacia* at 22.0%, *B. species* at 4.9%, *B. vietnamiensis* at 0.0%, and *B. contaminans* at 2.4%. QMH (Queen Mary Hospital) had *B. cenocepacia* at 60.0%, *B. cepacia* at 40.0%, *B. species* at 0.0%, *B. vietnamiensis* at 0.0%, and Other at 0.0%. Other wards had *B. cenocepacia* at 75.0%, *B. cepacia* at 12.5%, *B. species* at 0.0%, *B. vietnamiensis* at 12.5%, and Other at 0.0%.

Table 6: Association of *Burkholderia* Species with Ward.

Species		Ward					
		CCM	GW	PCCM	TVU	QMH	Other
B. Cenocepacia	No.	61	6	9	29	3	6
	%	75.3%	85.7%	75.0%	70.7%	60.0%	75.0%
B. Cepacia	No.	13	0	1	9	2	1
	%	16.0%	0.0%	8.3%	22.0%	40.0%	12.5%
B. Species	No.	2	0	1	2	0	0
	%	2.5%	0.0%	8.3%	4.9%	0.0%	0.0%
B. vietnamiensis	No.	2	0	0	0	0	1
	%	2.5%	0.0%	0.0%	0.0%	0.0%	12.5%
B. contaminans	No.	0	0	1	1	0	0
	%	0.0%	0.0%	8.3%	2.4%	0.0%	0.0%
Other	No.	3	1	0	0	0	0
	%	3.7%	14.3%	0.0%	0.0%	0.0%	0.0%
Significance		chi sq = 24.79, p = 0.491					

Table 7: Association of *Burkholderia* Species with Sample Type.

Species		Sample				
		Blood	Pus	Fluid	Urine	ET ASPIRATE
B. cenocepacia	No.	100	10	2	0	2
	%	75.8%	71.4%	66.7%	0.0%	66.7%
B. cepacia	No.	21	3	0	2	0
	%	15.9%	21.4%	0.0%	100.0%	0.0%
B. species	No.	5	0	0	0	0
	%	3.8%	0.0%	0.0%	0.0%	0.0%
B. vietnamiensis	No.	2	0	1	0	0
	%	1.5%	0.0%	33.3%	0.0%	0.0%
B. contaminans	No.	2	0	0	0	0
	%	1.5%	0.0%	0.0%	0.0%	0.0%
Other	No.	2	1	0	0	1
	%	1.5%	7.1%	0.0%	0.0%	33.3%
Significance		chi sq = 40.62, p = 0.004				

The association between *Burkholderia* species and the type of sample collected from patients was examined. The analysis revealed a statistically significant association between these variables, with a chi-square statistic of 40.62 and a p-value of 0.004. Here's a breakdown of the distribution of *Burkholderia* species among different sample types: Blood: *B. cenocepacia* at 75.8%, *B. cepacia* at 15.9%, *B. species* at 3.8%, *B. vietnamiensis* at 1.5%, *B. contaminans* at 1.5%, and Other at 1.5%. Pus: *B. cenocepacia* at 71.4%, *B. cepacia* at 21.4%, *B. species* at 0.0%, *B. vietnamiensis* at 0.0%, *B. contaminans* at 0.0%, and Other at 7.1%. Fluid: *B. cenocepacia* at 66.7%, *B. cepacia* at 0.0%, *B. species* at 0.0%, *B. vietnamiensis* at 33.3%, *B. contaminans* at 0.0%, and Other at 0.0%. Urine: *B. cenocepacia* at 0.0%, *B. cepacia* at 100.0%, *B. species* at 0.0%, *B. vietnamiensis* at 0.0%, *B. contaminans* at 0.0%, and Other at 0.0%. ET Aspirate (Endotracheal Aspirate): *B. cenocepacia* at 66.7%, *B. cepacia* at 0.0%, *B. species* at 0.0%, *B. vietnamiensis* at 0.0%, *B. contaminans* at 0.0%, and Other at 33.3%.

In summary, the type of sample collected from patients is significantly associated with the prevalence of different *Burkholderia* species. This suggests that the species distribution varies depending on the type of sample analyzed.

Table 8: Association of *Burkholderia* Species with ICU Admission.

Species		ICU ADMISSION	
		No	Yes
B. cenocepacia	No.	7	107
	%	50.0%	76.4%
B. cepacia	No.	5	21
	%	35.7%	15.0%
B. species	No.	0	5
	%	0.0%	3.6%
B. vietnamiensis	No.	1	2
	%	7.1%	1.4%
B. contaminans	No.	0	2
	%	0.0%	1.4%
Other	No.	1	3
	%	7.1%	2.1%
Significance		chi sq = 8.49, p = 0.131	

The analysis explored the relationship between *Burkholderia* species and the admission of patients to the Intensive Care Unit (ICU). The results revealed that there is no statistically significant association between these variables, as determined by a chi-square test (chi sq = 8.49, p = 0.131). Breaking down the distribution of *Burkholderia* species among patients with and without ICU admission, we found the following patterns: For patients without ICU admission: *B. cenocepacia* was present in 50.0% of cases. *B. cepacia* was identified in 35.7% of cases. *B. species* was not detected in any cases. *B. vietnamiensis* accounted for 7.1% of cases. *B. contaminans* was absent in this group. Other *Burkholderia* species made up 7.1% of cases. For patients with ICU admission: *B. cenocepacia* was the most prevalent species, found in 76.4% of cases. *B. cepacia* was identified in 15.0% of cases. *B. species* was present in 3.6% of cases. *B. vietnamiensis* was detected in 1.4% of cases. *B. contaminans* was also identified in 1.4% of cases. Other *Burkholderia* species made up 2.1% of cases among ICU-admitted patients. In conclusion, the analysis suggests that the specific *Burkholderia* species present in patients does not significantly affect their likelihood of ICU admission. The admission to the ICU appears to be influenced by factors other than the type of *Burkholderia* species present in the patients' samples.

Discussion

B. cepacia is one of the major causes of HCAI outbreaks owing to its resistance to a number of antimicrobial agents and disinfectants. According to many studies, most of septicemia in ICUs are because of ESKAPE organisms, but occurrence of *B. cepacia* is rare [13]. This was similar to a study done by Hu Yan Jian who had an isolation rate of 39% of *Burkholderia* cepacia similar to our study [14]. High similarity of *B. cepacia* complex (BCC) (typically above 98%) are measured indicating that BCC species are phylogenetically very closely related [15,16]. A combination of phenotypic and molecular tests such as 16S rRNA are recommended for differentiation among the genomovars of the *Burkholderia* cepacia complex [17]. Sudden outbreaks are increasing both in immunocompromised and hospitalized patients, mostly because of various contaminations during hospitalization [18]. There are a few Bcc outbreaks published describing extrinsic substance contamination, such as Ringer lactate solution as multiple-dose vial for catheter flushing, diluted heparin solution, and antiseptics solutions prepared in the hospital [19-21]. Other, nonsterile aqueous medical products implicated in health care–associated outbreaks due to BCC contamination include nasal sprays, mouthwashes, preoperative skin solutions, and hand sanitizers, among others [22]. Most outbreaks occurred either exclusively in ICUs or with the involvement of the ICU, which is in line with our results. The most recent report from the United States revealed contaminated saline flush syringes being associated with 162 BCC bloodstream infections across 59 nursing facilities in five states and occurring during September 2016–January 2017. It led to a nationwide recall of the product [23]. Relationship between occupation and melioidosis showed that there was an increase in the incidence of melioidosis among the people with occupational and recreational exposure to surface water and soil particularly with flooding of rice fields [24]. Underlying risk factors associated with infection by *Burkholderia spp*. has been studied, as this organism is known to cause disease in the immunocompromised [25]. Maximum number of *Burkholderia* isolated was from blood specimen in our study, this finding is similar to many other studies done. Our study also shows that male population is more affected as compared to female population as males are more actively involved in outdoor activities. Age group wise division shows no significant association with the different species involvement in causing infection. *Burkholderia* cepacian (BCC) is an intrinsically resistant to antimicrobial agents such as aminoglycosides, first and second generation cephalosporins anti-pseudomonal penicillin's and

polymyxins. As per the CLSI guidelines, the drugs recommended against BCC are levofloxacin, meropenem, cotrimoxazole, ceftazidime, minocycline and chloramphenicol [26]. Drug combinations such as meropenem with ciprofloxacin and tobramycin as well as ceftazidime-tobramycin were reported in successful treatments [27]. Therefore, basic public health measures include the primary provision of safe drinking water and sanitation, prevention of contact with contaminated soil or water, early case detection and appropriate management, including eradication therapy [28].

Conclusion

The prevalence of *B. cepacia* in hospital is not so high but they were mostly responsible for septicaemia. Ongoing surveillance and prompt investigation of unusual diseases outbreak are vital for identifying sources of contamination of *B. cepacia* and avoiding undesirable consequences for immunocompetent and immunocompromised patients. Effective antibiogram is needed to control the *B. cepacian* like opportunistic infections.

Conflict of Interest

Authors have no conflict of interest.

Authors Contribution

All authors have contributed equally in drafting the manuscript.

Bibliography

1. De Smet B., *et al.* "*Burkholderia stagnalis* sp. nov. and *Burkholderia territorii* sp. nov. two novel *Burkholderia cepacia* complex species from environmental and human sources". *International Journal of Systematic and Evolutionary Microbiology* 65 (2015): 2265-2271.

2. Burkholder W. "Sour skin a bacterial rot of onion bulbs". *Phytopathology* 40 (1950): 115-118.

3. Parke JL and Sherman DG. "Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains". *Annual Review on Phytopathology* 39 (2001): 255-258.

4. Gautam V., *et al*. "Identification of lysine positive non-fermenting gram negative bacilli (*Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex)". *Indian Journal of Medical Microbiology* 27 (2009): 128-133.

5. Valvano MA., *et al*. "Survival and persistence of opportunistic *Burkholderia* species in host cells". *Current Opinion on Microbiology* 8.1 (2005): 99-105.

6. Kutty PK., *et al*. "Multistate outbreak of *burkholderia cenocepacia* colonization and infection associated with the use of intrinsically contaminated alcohol-free mouthwash". *Chest* 132.6 (2007): 1825-1831.

7. Mariana Tavares., *et al*. "*Burkholderia cepacia* Complex Bacteria: a Feared Contamination Risk in Water-Based Pharmaceutical Products". *ASM Journals Clinical Microbiology Reviews* 33.3 (2015).

8. Dancer SJ and King MF. "Systematic Review on Use, Cost and Clinical Efficacy of Automated Decontamination Devices". *Antimicrobial Resistance and Infection Control* 10 (2021): 34.

9. Mao N., *et al*. "Transmission Risk of Infectious Droplets in Physical Spreading Process at Different Times: A Review". *Building and Environment* 185 (2020): 107307.

10. Jimenez L., *et al*. "*Burkholderia cepacia*: a problem that does not go away!" *EC Microbiology* 2 (2015): 205-210.

11. McDonnell G and Russell AD. "Antiseptics and disinfectants: activity, action, and resistance". Clinical Microbiology Review 12 (1999): 147-179.

12. Croxatto A., *et al*. "Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology". *FEMS Microbiology Review* 36 (2012): 380-407.

13. Mangram A and Jarvis WR. "Nosocomial Burkholderiacepacia outbreaks and pseudo-outbreaks". *Infection Control and Hospital Epidemiology* 17.11 (1996): 718-720.

14. Yan JH. 2006-2007 Mohnarin report. Antibiotic Resistance of Non fermenting Gram Negative Bacilli (2008).

15. Attia MAM., *et al*. "A study on Burkholderia cepacia in susceptible pharmaceutical products: semi-nested PCR". *PDA Journal of Pharmaceutical Science and Technology* (2016).

16. Coenye T., *et al*. "Taxonomy and Identification of the Burkholderia cepacia Complex". *Journal of Clinical Microbiology* 39.10 (2001): 3427-3436.

17. Henry DA., *et al*. "Phenotypic Methods for Determining Genomovar Status of the Burkholderia cepacia Complex". *Journal of Clinical Microbiology* 39.3 (2001): 1073-1078.

18. LiPuma JJ., *et al*. "Burkholderia, Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia, and Acidovorax". *Manual Clinical Microbiology* 10 (2011): 692-713.

19. De Smet B., *et al*. "Outbreak of Burkholderia cepacia bloodstream infections traced to the use of Ringer lactate solution as multiple-dose vial for catheter flushing, Phnom Penh, Cambodia". *Clinical Microbiology Infectious* 19 (2013): 832-837.

20. Yang CJ., *et al*. "Nosocomial outbreak of two strains of Burkholderia cepacia caused by contaminated heparin". *Journal of Hospital Infection* 69 (2008): 398-400.

21. Lee S., *et al*. "An outbreak of Burkholderia cenocepacia associated with contaminated chlorhexidine solutions prepared in the hospital". *American Journal of Infection Control* 41 (2013): e93-96.

22. Matthew J., *et al*. "Outbreak of Burkholderia stabilis Infections Associated with Contaminated Nonsterile, Multiuse Ultrasound Gel — 10 States, May-September 2021". *MMWR* 71.48 (2022): 1517-1521.

23. Akinboyo IC J., *et al*. "Multistate outbreak of an emerging *Burkholderia cepacia* complex strain associated with contaminated oral liquid docusate sodium". *Infection Control and Hospital Epidemiology* 39 (2018): 237-239

24. Currie BJ J., *et al*. "The Epidemiology and Clinical Spectrum of Melioidosis: 540 Cases from the 20 Year Darwin Prospective Study". *PLOS Neglected Tropical Diseases* 4.11 (2010): e900.

25. Tavares M J., *et al*. "*Burkholderia cepacia* complex bacteria: A feared contamination risk in water based pharmaceutical products". *Clinical Microbiology Review* 33 (2020): 0139-119.

26. Levy., *et al*. "A prospective study of gram negative bacteremia in children". *The Pediatric Infectious Disease Journal* 15 (1996): 117-122.

27. Antony Beena., *et al*. "A sporadic outbreak of Burkholderia cepacia complex bacteremia in pediatric intensive care unit of a tertiary care hospital in coastal Karnataka, South India". *Indian Journal of Pathology and Microbiology* 59.2 (2016): 197-199.

28. Henry DA., *et al*. "Phenotypic Methods for Determining Genomovar Status of the Burkholderia cepacia Complex". *Journal of Clinical Microbiology* 39.3 (2001): 1073-1078.