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Characterisation of Burkholderia Cepacia Complex Isolates From Clinical Samples

KEYWORDS: Burkholderia cepacia complex – phenotypic characterization - genomic species - antibiogram

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ABSTRACT *Burkholderia cepacia complex (BCC), is an emerging pathogen which is capable of causing bacteremia and other invasive infections, predominantly in patients with cystic fibrosis and also in non cystic fibrosis patients. The objectives of the present study were to isolate, speciate and to find out the antibiogram of BCC from clinical samples. A total of 8929 samples were processed during seven months in a tertiary care centre in central Kerala. All the isolates morphologically resembling BCC were subjected to phenotypic characterization including antibiotic susceptibility testing by conventional and automated methods. We could obtain BCC isolates from 49 patients. Based on the biochemical reactions performed on 22 BCC isolates, 20 identified as Burkholderia cepacia, one as B. cenocepacia and the remaining one as B. stabilis genomic species [1,2] 97.96% of the isolates were sensitive to ceftazidime, 91.84% to co-trimoxazole, 71.43% to meropenem and all were resistant to colistin.*

INTRODUCTION

In spite of being a plant pathogen, Burkholderia cepacia has emerged as an opportunistic nosocomial pathogen since 1980's, particularly in patients with debilitating diseases. The old Pseudomonas cepacia, has been separated from Pseudomonas and renamed as B.cepacia, based on molecular analysis. B.cepacia is a cluster of at least ten closely related genomic species, named as Burkholderia cepacia complex (BCC) , which includes B.cepacia, B.multivorans, B.cenocepacia and others which can be differentiated by molecular and biochemical methods[3]

Non-fermenting Gram-negative bacilli (NFGNB) occur as saprophytes in the environment and some are also found as commensals in the human gut.[4]NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory.[5] In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important nosocomial pathogens and BCC is the fourth most common pathogenic NFGNB worldwide[1] BCC survives and multiplies in aqueous hospital environment, including detergent solutions and intravenous fluids. BCC has been reported as a cause of bacteremia, particularly in patients with indwelling catheters, UTI, septic arthritis, peritonitis and respiratory tract infections[.1, 6]

It has always been a tedious task for a routine microbiological laboratory to identify the NFGNBs, and poor laboratory proficiency in identification of BCC prevails worldwide, including our own country. For this reason, reports of disease due to this organism are less in India.[7] Early detection and treatment of infections with this organism is important because of its high transmissibility in the hospital setting, and association with a poor prognosis [8] .

MATERIALS AND METHOD

The present study was conducted in a tertiary care centre in Kerala, during the period from January 2012 - July 2012. A total number of 8929 samples including blood, sputum, urine, catheter tips and pus were processed as per the standard procedures [9]. Identification of the isolates by phenotypic methods and antibiotic sensitivity were carried out.

Non lactose fermenting colonies on Mac Conkey agar which were motile, Catalase and Oxidase positive, and slender Gram negative bacilli with bipolar staining were subjected to biochemical reactions like Indole production, Citrate Utilization, Nitrate Reduction, Urea Hydrolysis, Oxidation Fermentation Test(Hugh and Leifson medium), Decarboxylase Test and Esculin Hydrolysis. After confirming as BCC, tests for genomic speciation: Growth at 42°C, ONPG Test, Pigment Production, 10% Lactose utilization were also performed. Motility is tested with hanging-drop preparation since the semisolid agar medium for detecting motility of fermentative organisms may not be suitable for this NFGNB [2]. Phenazine pigments (red, maroon, yellow) are produced by BCC that impart distinctive colour to the colonies, which are helpful in making identification[.2]

The identification was confirmed with Vitek 2 automated bacterial identification and susceptibility test system (Biomerieux). Antibiotic susceptibility testing (AST) was done using conventional (Kirby Bauer disc diffusion method) and automated method (Vitek 2) as per CLSI guidelines.

RESULT

Table No: 1.Sample wise distribution of BCC isolates

Sample	No. of samples	No. of BCC isolates	Percentage (%)
Blood	2771	35	1.26
Sputum	1026	4	0.39
Urine	3688	6	0.16
Pus	1444	4	0.28
Total	8929	49	0.55

Among the 49 clinical isolates of BCC, 35 were from blood cultures , six from urine and four each from sputum and pus. We could obtain BCC isolates with same sensitivity pattern, repeatedly from six of the 49 patients. From three blood samples of a patient and from two samples each from another two patients as well as from the urine culture of two patients (two samples each) and from the pus sample of a patient twice.

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Table No: 2. Biochemical reactions of BCC isolates (n=22)

Biochemical test	No: of positive isolates	No: of negative isolates
Oxidase	22	0
Catalase	22	0
Indole	22	0
Citrate	22	0
Nitrate reduction	17	5
Urease test	14	8
OF glucose utilization	22	0
OF maltose	20	2
OF sucrose	21	1
Lysine decarboxylase	22	0
Ornithine	22	0
Arginine	0	22
Esculin hydrolysis	19	3
ONPG	20	2
Growth at 42^0c	22	0
Pigment production	19	3
10% Lactose utilization	22	0

Based on the biochemical reactions performed, of the 22 BCC isolates, 20 have been identified as Burkholderia cepacia, one as B. cenocepacia and the remaining one as B. stabilis genomic species.[1,2]

Table No: 3. Antimicrobial susceptibility pattern of BCC isolates

Antimicrobial agent	No. of sensitive strains	Percentage (%)
Ceftazidime	48	97.96
Chloramphenicol	10	20.41
Colistin	0	0
Co-trimoxazole	45	91.84
Levofloxacin	20	40.82
Meropenem	35	71.43
Tetracycline	4	8.16

DISCUSSION

Non-fermenting Gram negative bacilli including BCC, are emerging as important nosocomial pathogens causing blood stream infections worldwide particularly in immunocompromised patients, patients with hematological malignancies and patients admitted in ICUs.[10] Being one of the most antibacterial resistant organisms encountered in the clinical laboratory, BCC infections can prove very difficult to treat and, it needs to be correctly identified and differentiated from Pseudomonas aeruginosa.[11]

BCC infections are seen mostly in CF patients. Infections in immunocompetent patients occur only sporadically, but several cases of pseudo epidemics and nosocomial infections, often caused by contaminated disinfectants and anesthetic solutions, have been reported.[12]

The present study was undertaken to identify and speciate BCC isolates and to find out their antibiogram from patients admitted in a tertiary care centre in Kerala. Out of a total number of 8929 samples processed we obtained 56 isolates of BCC from 49 patients (seven repeat isolates). In a similar study conducted at PGIMER, approximately 150 isolates of BCC were obtained within four years (2006-2009).[13] Among the total number of BCC isolates 71.44% were from blood. The study of ours correlates well with the study conducted by Gautam V, et al who reported that all BCC isolates from Escorts Heart Institute and Research Centre, Delhi were from blood cultures.[11]

Bacteremia, including catheter related BSI, were the most frequent (87.76%) among BCC infections in the present study. BCC bacteremia, most often in association with polymicrobial catheter-related infection, has been reported in patients with cancer and in patients undergoing hemodialysis.[12]

Repeated isolations from the same patients reinforce the genuineness of the isolates. Similarly all the sputum, urine and pus samples which grew BCC showed numerous pus cells along with short Gram negative bacilli in the smears. Of the 49 patients, 6 patients expired during the period of hospitalization, 43 patients recovered and got discharged. Most of our patients were immunocompromised and debilitated and had undergone invasive procedures that predisposed them to infection. Majority of the patients were SIRS criteria positive and the others may represent cases of pseudobacteremia.

All the isolates which were preliminarily identified as BCC were further identified using automated ID system . Such isolates were again subjected to various biochemical tests for confirmation of the identification and categorization into different genomic species. Twenty isolates (91%) were identified as B. cepacia genomic species,. In cystic fibrosis (CF) cases, B. cenocepacia and B. multivorans predominate [14,15] and the isolates recovered from non-CF infections, B. cenocepacia III A is again the most dominant.[14].According to Mahenthiralingam E. et al, it has been observed that B. cenocepacia can replace other Burkholderia spp. and were associated with a poor clinical outcome and high mortality[.3]

Performance and interpretation of biochemical reactions in the case of NFGNB is slightly different from that of the fermentative bacteria. Many non-fermenters including BCC, display only weak decarboxylase activity and may produce insufficient amines to convert the pH indicator system which necessitates the usage of small quantities of substrates (1 -2 ml) and a heavy inoculum .The initial conversion of the medium to a yellow colour from the small amount is not seen with non-fermenters; rather the end point reactions are read: the strong alkaline purple colour reaction.

As per the CLSI guidelines, the antibiotics recommended for BCC includes ceftazidime, co-trimoxazole, chloramphenicol, minocycline, meropenem and levofloxacin.[12] 97.96% were sensitive to ceftazidime ,91.84% to co-trimoxazole,71.43% meropenem, 40.82% levofloxacin, 20.41% chloramphenicol and 8.16% to tetracycline. Our study correlates well with the study conducted by Palash Samanta, et al, for whome ceftazidime showed the highest sensitivity (85%), followed by co-trimoxazole (80%), meropenem (60%), levofloxacin (58%) and tetracycline (45%).[16]

As the number of BCC isolated cases increased alarmingly in this institution during the last several months, the infection control committee formulated strategies to investigate the source of BCC isolates. As a part of this, a surveillance study was conducted and samples were collected from the hospital environments.We could isolate BCC twice from a hand rub used in the ICUs and instructions were given to change the hand rub. There after the isolation of BCC had decreased to a significant level. Outbreaks have been reported originating from diverse sources such as contaminated nebulisers, chlorhexidine solution, alcohol-free

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mouth wash, tap water, bottled water, cosmetics, napkins, nasal sprays and ultrasound gel.[11]

Continuous environmental surveillance and strict infection control policies have to be taken in all the health care settings in order to prevent infections with this saprophyte which is seen ubiquitously including the hospital premises.

CONCLUSION

The speciation of BCC with the conventional phenotypic methods is cumbersome and inconclusive too. Identification through commercial kits and automated systems is not fool-proof as many non- Burkholderia betaproteobacteria are misidentified as BCC and some BCC strains as Pseudomonas aeruginosa. The laboratories which are tentatively identifying Burkholderia species using an automated system should confirm isolate identity by conventional biochemical testing and, if necessary molecular techniques[.1,2] Hence molecular methods are preferred, among which restriction fragment length polymorphism analysis of the rec A gene can serve as a primary means of identifying taxonomic diversity of BCC isolates.

REFERENCES

1. LiPuma JJ,Currie BJ, Lum G,Vandamme P. Burkholderia, Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia and Acidovorax. In:Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, and Yolken RH, editors. Manual of clinical microbiology, ASM Press, Washington, DC 2007:749-69
2. Winn W Jr, Allen S, Janda W, Koneman E,Procop G, Schrreckenberger P, et al., editors.NFGNB. In: Koneman's Color Atlas and textbook of Diagnostic Microbiology, 6th edition, USA: Lippincott Williams and Wilkins Company; 2006.p.305-91
3. Mahenthiralingam E, Vandamme P, Campbell ME, Henry DA, Gravella AM, Wong LT, et al. Infection with BCC genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace Bukholderia multivorans. Clin Infect Dis 2001; 33:1469-75.
4. Steinberg JP, Rio DC. Other Gram negative and Gram variable bacilli. In: Mandell GL, Dolin R, editors. Principles and Practice of Infectious diseases, 6th edition.vol.2. Philadelphia, USA: Elsevier Publication; 2005.p.2751-68.
5. Rubin SJ, Granato PA, Wasilauskas BL. Glucose NFGNB. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, editors. Manual of clinical Microbology, 4th edition.Wasington, DC: American Society for Microbiology: 1985.p.330-49.
6. Doit C, Loukil C, Simon AM, Ferroni A, Fontan JE, Bonacorsi S, et al. Outbreak of Burkholderia cepacia bacteremia in a pediatric hospital due to contamination of lipid emulsion stoppers. J Clin Microbiol 2004; 42:2227-30.
7. Gautam V, Ray P, Vandamme P, Chatterjee SS, Das A, Sharma K, et al. Identification of lysine positive NFGNB (Stenotrophomonas maltophilia and BCC).Indian J Med Microbiol 2009;27:128-33.
8.
9. Murat Dizbay, Ozlem Guzel Tunccan, Busra Ergut Sezer, Firdevs Aktas, Dilek Arman. Nosocomial Burkholderia cepacia infections in a Turkish university hospital: a five-year surveillance. J Infect Dev Ctries 2009; 3 (4):273-277.
10. Collee .Mackie & Mccartney Practical Medical Microbiology, 14th edition, Edinburgh: Churchil Livingstone
11. Ramphal R. Infections due to Pseudomonas species and related organisms, Chapter 145. Harrison's Principles of Internal Medicine, 17th Ed. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. USA: McGraw-Hill Medical 2008.p.949-56.
12. Gautam V, Singal L, Ray P.BCC : Beyond pseudomonas and acinetobacter. Indian J Med Microbiol 2011; 29:4-12.
13. Maschmeyer G Mandell: Mandell, Douglas and Bennett's Principles and Practice Of Infectious Diseases. Philadelphia, Pennsylvania: Elsevier Churchill Livingstone.2009.Chapter 220.
14. Gautam V, Ray P, etal. Investigation of BCC in septicaemic patients in a tertiary care hospital, India. Nepal Med Coll J 2009; 11:222-224.
15. Mahenthirlingam E, Baldwin A, Dowson CG. BCC bacteria opportunistic pathogens with important natural biology. J Appl Microbiol 2008; 104: 1539-51
16. Reik R, Spilker T, Li Puma JJ. Distribution of BCC species among isolates recovered from persons with or without cystic fibrosis. J Clin Microbiol 2005; 43:2926-8.
17. Palash Samanta, Vikas Gautam, et al. Emerging resistance of NFGNB in a tertiary care centre. Indian Journal of Pathology and Microbiology 2011; 54:666-667.