Investigation of *Burkholderia cepacia* complex in septicaemic patients in a tertiary care hospital,India

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ABSTRACT

Burkholderia cepacia complex (BCC) is being increasingly recognized as an important pathogen of humans. During the year 2007-8, 39 putative BCC isolates were obtained from 21 cases and subjected to recA PCR RFLP. Twenty-four isolates were confirmed as *Burkholderia cenocepacia* IIIA (nineteen isolates, recA PCR RFLP type G and five isolates, recA PCR RFLP type I), six were confirmed as *B. cepacia* (recA PCR RFLP type E). BCC were isolated from inpatients of different wards of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh with increased isolation from children admitted to different wards of Advanced Pediatric Centre (11/21 cases). BCC isolates are often resistant to most commonly used antibiotics and an early use of effective antimicrobial therapy can decrease morbidity and mortality.

Keywords: *Burkholderia cepacia*, septicaemia, investigation.

INTRODUCTION

Burkholderia cepacia complex (BCC), a phytopathogen first described in 1950 as the causative agent of onion rot,[1] is increasingly recognized as an important cause of morbidity and mortality associated with infections in immunocompromised patients and hospitalized patients, probably because of intrinsic resistance to antimicrobial agents.[2,3] BCC causes a wide range of infections from superficial to deep-seated and disseminated infections[4] and the complex consists of ten recognized species and five newer species recently suggested.[5] Reliable identification of different species (formerly genomovars) becomes important in this complex since some genomovars (particularly genomovar IIIA) have been associated with high transmissibility between patients and a poor prognosis. BCC bacteremia is infrequently reported and is found mainly in immunocompromised and hospitalized patients.[6,7] However, BCC bacteremia is not uncommon in Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. An upsurge of the bacteremia caused by this complex has been observed for consecutive four years (2004-07) in the 1460 bedded tertiary care hospital that caters to 1 million outpatients and 50,000 inpatients a year.[8]

MATERIAL AND METHODS

This study was carried out in the clinical bacteriology laboratory at PGIMER, Chandigarh (India), on isolates from blood and other specimens during the period April, 2007 to March, 2008. Blood for bacterial culture was carried out in the clinical bacteriology laboratory of the department of Medical Microbiology as part of routine diagnostic services to the patients admitted to the Nehru Hospital and advanced Pediatric centre, Postgraduate Institute of Medical Education and Research, Chandigarh (India). Approximately 5 ml of blood was aseptically collected and added to each of the two bottles containing 50 ml of Tryptone Soy broth and Bile broth (Hi-Media Labs, India). Both the bottles were incubated aerobically at 37ºC for seven days and subcultured on sheep blood agar and MacConkey agar after overnight, 48hours and 7days or in between when visible turbidity appeared. Body fluid was subcultured only once after overnight incubation. For cerebrospinal fluid (CSF), sample was inoculated on sheep blood agar and MacConkey agar for 48h and incubated aerobically and in 5.0-10.0% CO_2. In positive cases, isolates were identified to species level by conventional biochemical tests. Gram-negative, oxidase-positive, motile, non-fermenting gram-negative bacilli (NFGNB) were selected and a few biochemical reactions were put up for the identification that included triple sugar iron agar, lead acetate paper strip for hydrogen sulphide production, decarboxylases, aerobic low-peptone basal medium containing glucose. BCC isolates were lyophilized and stored at 4ºC for further reference. Antibiotic susceptibility testing was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standards Institute, 2007.[9] Bacterial isolates were identified by the conventional phenotypic methods and recA polymerase chain reaction (PCR) based restriction fragment length

polymorphism (RFLP) was performed to identify BCC isolates to species level.[10]

RESULTS

Out of 25,585 blood cultures performed at the hospital during the year 2007-8, 21.0% (5373) tested positive for bacterial culture and 30 (0.6%) of these positive cultures grew BCC. In addition to these, BCC was also isolated from four respiratory samples, twice from CSF in a patient of meningitis, and one each from two body fluid samples and a pus sample. Out of 39 putative BCC isolates during the year 2007-8, recA PCR RFLP was performed in 34 isolates, and of these 30 (88.2%) were finally confirmed to be BCC. Twenty-four isolates were identified as *Burkholderia cenocepacia* IIIA (nineteen isolates, recA PCR RFLP type G and five isolates, recA PCR RFLP type I), six were identified as *B. cepacia* (recA PCR RFLP type E). Besides 23 blood isolates, two isolates each were obtained from cerebrospinal fluid and sputum samples; one isolate each was obtained form pleural fluid, continuous ambulatory peritoneal dialysis fluid and endotracheal aspirate. recA PCR could not be performed on five strains that lost viability on storage of which four were blood culture isolates and one was a pus culture isolate. Amongst the four other isolates obtained from blood, two isolates tested negative by recA PCR, one showed a faint band and a different band was obtained in another isolate. Isolates that yielded a double product, a faint product, or a product with the wrong size on recA PCR were not considered BCC.

By the disk diffusion method, isolates of BCC were susceptible to piperacillin-tazobactam (26/30, 86.7%), levofloxacin (25/30, 83.3%), ceftazidime (24/30, 80.0%), and tetracycline (23/30, 76.7%). Amongst these 30 isolates, maximum resistance was observed against meropenem (11/30, 36.7%) and co-trimoxazole (7/30, 23.3%).

DISCUSSION

BCC is being increasingly recognized as an important pathogen of humans in both immunocompromised and hospitalized patients who are infected by contact with contaminated equipment during hospitalization.[2] BCC bacteremia should be considered in febrile patients with nosocomial infections, especially those who have an indwelling catheter, are on ventilators, have cystic fibrosis or have immune dysfunction.

In this study, 30/34 (88.2%) isolates putatively identified by conventional methods were confirmed to be BCC by recA PCR. In a recent Brazilian study also, 41 cystic fibrosis (CF) isolates of BCC were identified by culture, and confirmation of identity and genomovar determination was obtained in 32 isolates (74.0%) by recA PCR.[11] Thirty isolates were obtained from 21 cases (some had repeated isolation – data not shown), 16 (70.0%) were males and 3 (10.0%) females (age and sex was not available for two patients); and included neonates as well as elderly patients. These were nosocomially acquired infections in these 21 patients of whom 7 (33.3%) died during hospital stay. We had isolation of BCC from the patients admitted in different wards of PGIMER with increased isolation from children admitted in different wards of the Advanced Pediatric Centre (nine typed, and isolates from two cases lost viability on storage; 11/21 cases).

Twelve patients had a clinical diagnosis of sepsis (details not available for another 9 cases) as per the Systemic Inflammatory Response Syndrome (SIRS) criteria to evaluate the systemic response to infection as developed by a consensus committee of American experts in 1992. The spectrum of infections caused by BCC included bacteremia, meningitis, endocarditis, pneumonia, surgical wound infections and urinary tract infections.[2,12] Five bacteremic patients and one case of meningitis in this study had repeated isolation of BCC. One fifth of the patients were admitted in intensive care units (ICU). Eleven out of 21 (52.4%) of these were children, and four were admitted to the emergency. Three of these children admitted to Pediatric emergency had expired by the next day when the blood culture was taken. Several predisposing factors have been suggested as the major determinants for developing BCC bacteremia. These include staying in an ICU, having undergone major surgery, and having an intravascular catheter.[2,12] It's difficult to point out at a predisposing factor in this study with small no. of BCC isolates. BCC has been isolated throughout the year from blood cultures, and from different wards during the same month. The predominance of RFLP type G in our center could suggest case clustering. To show the strain relationships among the isolates within each species, pulse field gel electrophoresis and/or multilocus sequence typing is desired which has not been conducted in this study.

Out of the five isolates from a 50 year old female admitted to ICU, the three isolates (four isolates processed) from this patient were later confirmed to be *B. cepacia* (RFLP type E) and the last isolate was *B. cenocepacia* (RFLP type G). It has been observed that *B. cenocepacia* can replace other *Burkholderia* spp. as stated in a previous study where *B. cenocepacia* strains replaced *B. multivorans* in 6 patients. *B. cenocepacia* strains were associated with a poor clinical outcome and high mortality.[13] BCC was not isolated in this patient from respiratory specimens and urine. Patient died of sepsis after a long ICU stay of 19 days.

BCC exhibits a broad range of resistance to many antimicrobial agents in vitro. The high level of antibiotic resistance limits the therapeutic options. BCC is intrinsically resistant to antimicrobial agents such as polymyxin, aminoglycosides, first and second generation cephalosporins and anti-pseudomonal penicillins.[2] Some antibiotics such as ceftazidime, carbapenem and ciprofloxacin display some in vitro activities against this bacterium.[12] With the exception of the epidemic *B. cenocepacia* ET12 lineage, previous studies of the BCC have shown greatest susceptibility to meropenem irrespective of species status.[14] However, in our study, amongst these 30 isolates, maximum resistance was observed against meropenem (11/30, 36.7%) followed by co-trimoxazole (7/30, 23.3%). This percentage is comparable to the resistance observed in an Italian study in 31 CF patients where less than half of the strains were sensitive to meropenem, ceftazidime, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole determined by minimum inhibitory concentration (MIC).[15] In this study, piperacillin with tazobactam had been shown to have best activity.

B. multivorans and *B. cenocepacia* account for the majority of isolates from CF patients, with patient-to-patient spread and higher mortality mainly being associated with genomovar III and *B. dolosa*.[2,8] To conclude, all of the isolates obtained during this year from different types of samples were of predominantly single species only – *B. cenocepacia* (RFLP type G and I) except six isolates (from four patients of different species – *B. cepacia* (RFLP type E). Treatment of BCC is a challenge as isolates are often resistant to most commonly used antibiotics. Early use of effective antimicrobial therapy can nevertheless decrease morbidity and mortality.

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