Correspondence

Burkholderia cepacia complex in septicaemic non-cystic fibrosis cases from two tertiary care hospitals in north India

Sir,

Burkholderia cepacia complex (BCC) can be particularly devastating, highly virulent, cystic fibrosis (CF) pathogens that are also able to cause nosocomial infections among other groups of debilitated patients\(^1\). They cause a wide variety of infections ranging from superficial to deep-seated and disseminated infections, such as pneumonia (especially in patients with cystic fibrosis), meningitis, peritonitis (in patients undergoing peritoneal dialysis), septicaemia and bronchiectasis\(^2\). BCC survives and multiplies in aqueous hospital environments where it may persist for long periods\(^1,3,4\). Due to high intrinsic resistance of the BCC to antibiotics and antimicrobial compounds, all of these infections can prove very difficult to treat and may be fatal\(^3,4\). BCC, though reported worldwide, has not been reported from India, and isolation of only two strains has been published from another tertiary care centre in north India\(^5\). The identification of BCC based on phenotypic analysis alone should be confirmed using molecular methods\(^6\). Their classification has undergone considerable taxonomic changes over the last two decades. The group is now known to encompass at least ten distinct well known species (formerly genomovars), and five other novel species, whose laboratory identification can often prove difficult\(^2,7,8\). In order to develop rapid tests to determine the genomovar status of BCC isolates, nucleotide sequence polymorphism in two genes, the 16S \(rRNA\) gene (16S \(rDNA\)) and \(recA\) gene has been examined, and only \(recA\) based typing has been found useful to identify BCC strains from both environmental samples and sputum as well as to identify other *Burkholderia* species. Restriction fragment length polymorphism (RFLP) analysis of the \(recA\) gene can serve as a primary means of identifying taxonomic diversity among isolates and \(>50\) BCC RFLP types have now been found when the gene is cut with the restriction enzyme *HaeIII*\(^1\).

A sudden upsurge of this rare non fermenting Gram-negative bacillus (NFGNB) has been observed in septicaemic patients of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, during the four consecutive years (2004-2007), and during 2006 in Escorts Heart Institute and Research Centre (EHIRC), Delhi. We analysed the consecutively obtained BCC isolates of septicaemic non-cystic fibrosis cases from these two centres of north India using standard phenotypic and a molecular \(recA\) polymerase chain reaction (PCR) based RFLP method to identify BCC isolates to the species level.

Blood culture for bacterial infections was carried out in two bottles containing 50 ml of Tryptone Soy broth and Bile broth (Hi-Media, Mumbai) as a part of routine diagnostic services to the inpatients admitted at PGIMER, Chandigarh. In EHIRC, blood culture was performed in BacT/Alert FA blood culture bottles (bioMerieux, France). For cerebrospinal fluid (CSF), body fluids and respiratory specimens (sputum and bronchoalveolar lavage, BAL), sample was inoculated on sheep blood agar and MacConkey agar for 48 h and incubated aerobically and in 5-10 per cent \(CO_2\). For pus specimens, sample was inoculated on sheep blood agar and MacConkey agar for 18 h and incubated aerobically at 37\(^0\)C. In positive cases, isolates were tentatively identified to the species level by conventional biochemical tests\(^9\). In EHIRC, the isolates were identified by mini API identification system (bioMerieux). Systemic inflammatory response syndrome (SIRS) criteria were included to evaluate the systemic response to infection\(^10\). The Ethical clearance was obtained for the study from Institute Ethics Committee, PGIMER, Chandigarh.

Genomic DNA was isolated using the Avgene system as per the protocol recommended by the supplier (Avgene, Taiwan). Genomovar status was determined by amplification of the entire \(recA\) gene followed by RFLP\(^11\). Fifty two BCC clinical isolates from two centers in northern region of India (PGIMER, Chandigarh = 40, Escorts Heart Institute and Research
Centre, Delhi = 12) were subjected to recA-based PCR tests to assign them to a genospecies. The strains of PGIMER consisted of isolates obtained from blood culture (n = 32), pus specimen (n = 1), respiratory specimens (n = 3), body fluids (n = 2) and CSF (n = 2 isolates of the same patient). All the 12 isolates obtained from EHIRC, Delhi, were blood culture isolates. In five septicaemic patients, there was repeated isolation of BCC. A positive control strain, with a known genomovar status (ATCC 25609), was used in each PCR reaction. The isolates were subjected to recA PCR amplification using the oligonucleotide primers BCR1 and BCR2. Thirty eight of the 52 isolates were recA positive, and isolates that did not yield a PCR product of 1,043 bp were not considered BCC. Digestion of the recA amplicon with the endonuclease HaeIII generated three distinct recA RFLP types, two of which represented B. cenocepacia (recARFLP type G & AN) (Fig.) and one represented B. cepacia (recARFLP type E).

In 2002 at PGIMER, Chandigarh, of the 414 representative blood and body fluid samples, only 9 isolates (2.2%) of BCC were isolated and were identified by the conventional identification tests and rapid automated system – API20NE (bioMerieux). Of the 5,437 blood specimens received during three months (November 2004 to January 2005) from various inpatient departments, 170 (13.7%) NFGNB were isolated from 1235 positive blood culture specimens. Seventy (41.2%) isolates were identified as lysine-positive NFGNBs (BCC and Stenotrophomonas maltophilia), and others identified as Alcaligenes spp. (40, 23.5%), Pseudomonas aeruginosa (31, 18.2%), Chryseobacterium indologenes (1, 0.6%) and Brevundimonas diminuta (1, 0.6%) respectively. Twenty seven (16%) isolates could not be identified by the limited available conventional biochemical tests. Initially, there was overlapping of BCC and S. maltophilia by conventional methods and therefore, the term lysine-positive NFGNB has been used. During the later years, the conventional methods was refined for the clear distinction of S. maltophilia from BCC and supported by recAPCR based identification of BCC (Table). Within two years (2006 and 2007), 69 isolates of BCC were obtained in PGIMER. This is in comparison to a study by Reik et al who reported 90 isolates over the span of eight years from various clinical specimens of non-cystic fibrosis patients.

Most of our patients were immunocompromised and debilitated patients and had undergone invasive procedures (indwelling catheters, endotracheal intubation and mechanical ventilation) that predisposed them to sepsis and contributed to their high risk status. All the patients were SIRS criteria positive. BCC infections in immunocompetent patients occur only sporadically, but several cases of pseudo-epidemics and nosocomial infections have been reported. Of the 31 recA PCR positive (PGIMER inpatients) isolates, 12 isolates of BCC were from the children. Three children had died by the next day of isolation of BCC. All 12 isolates were blood isolates except two CSF isolates obtained from the same child.

Environmental surveillance every three months over the last four years in the main operation theatre area, cardiothoracic wards and in bone marrow transplant (BMT) was unsuccessful in the isolation of B. cenocepacia, but oxidase positive NFGNB have been isolated from BMT and cardiothoracic ward. In two BMT patients, B. cenocepacia was isolated twice and thrice respectively from blood cultures and both the patients expired. In the second patient admitted under BMT, three BCC isolates were obtained from red and white lumens of the cannula and the sample withdrawn from the peripheral line. All the three isolates were positive by recA PCR. Isolates obtained from white lumen and peripheral line gave RFLP type G pattern. Isolate from red lumen gave faint band on recA PCR and RFLP pattern could not be obtained though repeated twice. Surveillance was conducted immediately the next day to exclude product contamination and find the environmental niche. Cultures from three taps and corresponding soaps grew oxidase positive NFGNBs but BCC could not be isolated.

Nine isolates from PGIMER and five isolates form EHIRC, Delhi remained unidentified by recA-based PCR. Unidentified isolates have also been reported.

Fig. RFLP analysis (HaeIII) of the B. cepacia complex recA amplified by PCR. The most common pattern G is shown in all the lanes. Molecular size standard (50-bp ladder) is in the first lane.
in previous epidemiological studies.\textsuperscript{14,15} In a recent Brazilian study also, 41 CF isolates of BCC were identified by culture, and confirmation of identity and genomovar determination was obtained in 32 isolates by recA-based PCR.\textsuperscript{16} It seems likely that the number of genospecies constituting the BCC will continue to rise. Recently, a tenth species, \textit{B. ubonensis} has also been identified.\textsuperscript{7} Because of the devastating potential impact of a false-positive or false-negative diagnosis, especially since some BCC species are of a greater clinical significance than others, BCC infections must be identified both rapidly and accurately in a clinical setting.\textsuperscript{17}

\textbf{BCC} is usually resistant to aminoglycosides, antipseudomonal penicillins, and antipseudomonal third-generation cephalosporins.\textsuperscript{2} But we found that the non-CF isolates were behaving similar to the non-CF nosocomial Italian isolates showing susceptibility to ceftazidime.\textsuperscript{18} The percentage of resistance observed against co-trimoxazole, ceftazidime, tetracycline, piperacillin-tazobactam, meropenem and levofloxacin was 21.7, 10.8, 77.8, 13.0, 56.52 and 13.0 per cent respectively by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI).\textsuperscript{9}

To conclude, our study showed that \textit{B. cepacia} (genomovar IIIA) was the most prevalent species of BCC among a population of Indian non-CF patients screened over a period of four years. This is a cause of concern as patients with \textit{B. cepacia} (genomovar IIIA) infections face the highest mortality and have higher transmission rate.

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\begin{table}
\caption{Relative isolation of \textit{B. cepacia} complex (BCC) over the years and the genomospecies isolated}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Year of isolation (PGIMER) & No. of blood cultures & Positives No. (%) & No. of isolates & Genomospecies & RFLP & source \\
\hline
2002 (identified by API 20 NE) & 414 (blood and body fluid cultures) & 9 BCC & & & & \\
\hline
2004 (Nov, 2004 - Jan 2005) & 5,437 & 1235 (22.71) & 70 & Lysine positive NFGNB & & \\
\hline
2005 (Sept-Dec) & 7,779 & 1797 (23.1) & 74 & Lysine positive NFGNB & 1 - \textit{B. cepacia} & 1 - G \\
\hline
2006 & 25,887 & 6109 (23.6) & 30 BCC & 8 - \textit{B. cepacia} & & \\
\hline
2006 (EHIRC, Delhi) & 11,883 & 1390 (11.7) & 59 BCC & 5 - \textit{B. cepacia} (2 recAPCR positive but no RFLP obtained) & 6 - G (1-body fluid, 1–pus) & \\
\hline
2007 & 28,491 & 7065 (24.8) & 30 BCC + 9 from other specimens & 15 - \textit{B. cepacia} & 15 - G (1-BAL, 1-sputum, 2-CSF) & \\
\hline
& & & & 7 - \textit{B. cepacia} & 7 - E (1-body fluid, 1-sputum) & \\
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B, bronchoalveolar lavage, CSF, cerebrospinal fluid, EHIRC, Escorts Heart Institute and Research Centre, NFGNB, non fermenting Gram-negative bacillus; RFLP types obtained were G, AN and E.
References


