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Correspondence

Burkholderia cepacia complex in Indian cystic fibrosis patients

Sir,

In India, there are no precise reports of the prevalence of *Burkholderia cepacia* complex (BCC) infections due to the lack of awareness and difficulty in identification by routine clinical laboratories. In most cases, BCC has been ambiguously reported as non-fermentative Gram-negative bacilli (NFGNB) or simply *Pseudomonas* spp.[1,2]. For this reason, reports of disease due to BCC are rare and BCC has been reported from only a few tertiary care centres in north India[2-6]. BCC is an established pathogen in two patient populations with genetic diseases *viz.* cystic fibrosis (CF) and chronic granulomatous disease, where it causes increased morbidity and mortality[7]. Moreover, BCC has become an increasingly common nosocomial pathogen due to its high intrinsic and acquired antimicrobial resistance, lack of effective antibiotics, and survival ability in the environment for prolonged periods of time.

BCC has been observed in non CF-septicaemic patients of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh during 2005-2010. However, though known to be associated frequently, there are no reports of isolation of BCC from Indian cystic fibrosis patients. CF itself was thought to be rare in India but published reports indicate that CF is probably far more common in people of Indian origin than previously thought but is underdiagnosed or missed in the majority of cases[8]. In 1968, six CF cases were reported from PGIMER[9]. At present, there are approximately 60 CF patients on regular follow up in Advanced Paediatric Centre (APC). We hereby report isolation of BCC from six CF cases admitted in PGIMER between April 2009 and March, 2010. The ethics committee of PGIMER, Chandigarh, approved the study protocol.

Blood culture was performed using BACTEC 9240 (Becton Dickinson, USA). Specimens were inoculated onto sheep blood agar and MacConkey agar, incubated aerobically at 37^{o}C for up to 48 h. BCSA (*B. cepacia* selective agar) was used in addition for the recovery of *B. cepacia* from respiratory specimens [expectorated sputum, induced sputum, bronchoalveolar lavage (BAL), endotracheal aspirates] of CF patients (diagnosed as per the guidelines of CF Foundation)[10]. In positive cases, isolates were identified using standard biochemical tests and confirmed using *recA* PCR based RFLP (restriction fragment length polymorphism) to identify BCC isolates to the species level[2,11]. Genomic DNA was isolated using the Avgene system as per the protocol recommended by the manufacturer (Avgene, Taiwan). Antibiotic susceptibility testing was done on Mueller-Hinton agar by Kirby Bauer's disk diffusion method according to CLSI guidelines[12]. BCC susceptibility was performed for ceftazidime, tetracycline, co-trimoxazole, meropenem and levofloxacin (Oxoid)[12].

Amongst the six CF infants (five male and one female), a total of 12 BCC isolates were obtained. A four month old male infant with history of bronchopneumonia, presented with fever, cough and respiratory distress of 4 wk duration. *B. cenocepacia* was isolated twice from BAL and the RFLP types (G and I) were obtained in this child. He was treated with intravenous ceftazidime followed by meropenem. However, the patient deteriorated, his blood culture also grew BCC twice and he died of septic shock. In another two month old infant, BCC was isolated twice each from BAL specimens and blood cultures, with three isolates having the same RFLP types (type G - *B. cenocepacia* IIIA). First isolate from blood specimen gave faint band on recA PCR and RFLP pattern could not be obtained though repeated thrice. The other four infants diagnosed with CF presented with fever, cough and respiratory distress. *B. cenocepacia* (RFLP types G and I) was isolated from BAL in two of these cases. *B.*

cenocepacia is genetically highly heterogeneous, being composed of at least four phylogenetic lineages (IIIA, IIIB, IIIC, and IIID) based on the polymorphism of the *recA* gene. In our cases, *B. cenocepacia* IIIA (*recA* RFLP types G) and IIIB (*recA* RFLP types I) were isolated. Though *recA* lineage IIIA has very limited environmental reservoir, *recA* lineage IIIB has been found in both clinical specimens and natural habitats[13]. All BCC isolates were found to be susceptible to ceftazidime, meropenem and co-trimoxazole. Appropriate antimicrobial therapy was initiated based on the susceptibility pattern. All infants except one responded well with improvement in condition.

Since BCC is difficult to eradicate after colonization, initial screening plays a pivotal role and patients may be segregated accordingly. *B. cenocepacia* and *B. multivorans* are more predominant amongst CF patients than non-CF patients as reported from United States, Canada, Italy and Australia[14-16]. Other than *B. cenocepacia* and *B. multivorans*, the remaining formally named species account for less than 10 per cent of all CF infections caused by the complex[13]. Isolation of *B. cenocepacia* from all our CF patients is a cause of concern, as these patients suffer from high mortality and have higher rate of transmission[2-5].

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