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Fatal infection in adults by pneumolysin & autolysin producing, non-vaccine serotype *Streptococcus pneumonia*

Sir,

Despite variable levels of antibiotic resistance in clinical isolates of *Streptococcus pneumoniae* reported from India, invasive pneumococcal infection continues to pose a challenge in children[1]. It is a paradox that the susceptibility of pneumococci to antibiotics is not as major a problem as it is observed with staphylococci, enterococci and the Gram-negative bacteria, though outcome of some of the pneumococcal infections are fatal[2]. Rapidly progressive disease in the vulnerable population namely, the very young and the elderly is a challenge to the treating physician, a factor which is compounded by lack of confirmatory diagnosis due to poor laboratory support. Sporadic reports of adult pneumococcal infections have been documented[3]. Factors predisposing this group to fatal pneumococcal infection are unknown[4]. Vaccination of the adult population is limited to the very elderly and those with risk of underlying immunosuppression and splenectomized persons[5]. We encountered two cases of invasive pneumococcal infections in adult patients with progressive illness with a fatal outcome in a tertiary care centre in south India during 2012. An attempt was made to detect virulence genes coding for pneumolysin (*ply*) and autolysin (*lytA*), as these two antigens are known to be associated with virulence of *S. pneumoniae* in experimental animals[6].

The first case was a 72 years old male with loss of consciousness for 12 h, brought to the emergency unit of Pondicherry Institute of Medical Sciences, Puducherry, India. He had no history of fever, breathlessness or seizures. He did not have any known risk factors. At admission he had respiratory distress and was transferred to the intensive care unit (ICU) for intubation. Chest X- ray showed bilateral infiltrates with pneumothorax on the left side. Computed tomography (CT) of the brain revealed dilated ventricles. Initial laboratory investigations revealed the following values: total WBC count of 9800/mm^3, differential count of 89 per cent neutrophils, 8 per cent lymphocytes and 3 per cent monocytes. A preliminary diagnosis of sepsis with pneumonia and pyogenic meningitis was made. He was started empirically on cefoperazone sulbactam, metronidazole and doxycycline (due to the area being endemic for scrub typhus). Subsequently, blood and CSF cultures grew *S. pneumoniae* (IBT 1960) (serotyped as 33C- Pneumotest- Statens Serum Institute, Solna, Sweden), which was susceptible to ampicillin, penicillin (by oxacillin screen), ceftriaxone, cefotaxime and vancomycin. Following the culture report antibiotics were changed to vancomycin and ampicillin. He developed hypotension and shock, and despite ventilatory support, administration of intravenous fluids, antibiotics and vassopressor agents his condition deteriorated and he died within 48 h of admission.

The second case was a 47 year old male who was conscious but restless and disoriented, and was brought to the casualty of this hospital. There was history of fever and shortness of breath for two weeks, cough for one week and chest pain for two days. There was no other co-morbid condition. Chest X-ray revealed right lower lobe consolidation. Complete blood count revealed total WBC count to be 24000/mm^3 and a differential count suggestive of pyogenic infection (95% neutrophils, 4% lymphocytes, 1% monocytes). A diagnosis of right lower lobe pneumonia associated with acute respiratory distress syndrome, sepsis and multi-organ dysfunctional syndrome was made. He was empirically started on linezolid, imipenem and piperacillin-tazobactam. Sepsis was confirmed by a positive blood culture for *S. pneumoniae* (IBT -1975; serotype 7C), which was sensitive to penicillin, ciprofloxacin, ceftriaxone, erythromycin, gentamicin and vancomycin. The

patient was continued with linezolid, imipenem while azithromycin was added. His condition continued to deteriorate and he succumbed to the infection within 72 h of admission.

The two isolates were further tested for the presence of different virulence determinants such as genes encoding for pneumolysin (*ply*), autolysin (*lytA*) and to document presence of virulence factors and penicillin binding protein (*pbpA)*[7]. The primer details are given below: *lytA* (size 319 bp)[4] forward primer F-5'AACCGTACAGAATGAAGCGG-3' and reverse primer R-5' TATTCGTGCAATACTCGTGCG-3'; *ply* (size 348 bp) forward primer F-5'ATTTCTGTAACAGCTACCAACGA3' and reverse primer R- 5'GAATTCCCTGTCTTTTCAAAGTC3'; *pbp*A (size-789 bp) forward primer F-5'CCGTATCCTGGGAGCTTTCTT-3' and reverse primer R-5'-TCGCGGTTTGTTTCTACTGC-3'. All primers used in the study were designed using GeneTool software and custom synthesize by Eurofins (Bengaluru, India), used in previously described study[7]. All three genes were detected in both the isolates (Figure).

Rapidly fatal pneumococcal disease is known to occur in immunocompromised individuals, splenectomized individuals and HIV infected patients are at increased risk of developing severe pneumococcal disease[8,9]. Fatal outcome in the immunocompetent patients have been infrequently reported in literature[10]. Rapidly progressing pneumococcal sepsis with metabolic acidosis and disseminated intravascular coagulation has been reported in two adult individuals by Iinuma *et al*[3].

Analysis of the isolates revealed two interesting observations. Both the isolates that were responsible for fatal outcome belonged to non-vaccine serotypes *i.e.* 33C and 7C. The capsular polysaccharide-23 valent vaccine used for adult vaccination does not contain these two serotypes[11,12]. Studies in the West have shown that infections due to vaccine serotypes have declined in adults following vaccination of children with PCV-7 while non-vaccine serotypes causing invasive infections in high risk adults has risen[13-15]. In India, where pneumococcal vaccines have not been included in the routine immunization schedule in children, and adult vaccination is sporadic, and serotyping of invasive isolates remains a challenge. Routine typing to know the incidence of infections due to vaccine or non-vaccine serotypes both in children and adults, is not undertakn by most clinical laboratories across the country. Fatal infections caused by non-23 valent vaccine serotypes such as 33C and 7C of *S. pneumoniae* observed in this study raise the question on current strategies of adult pneumococcal

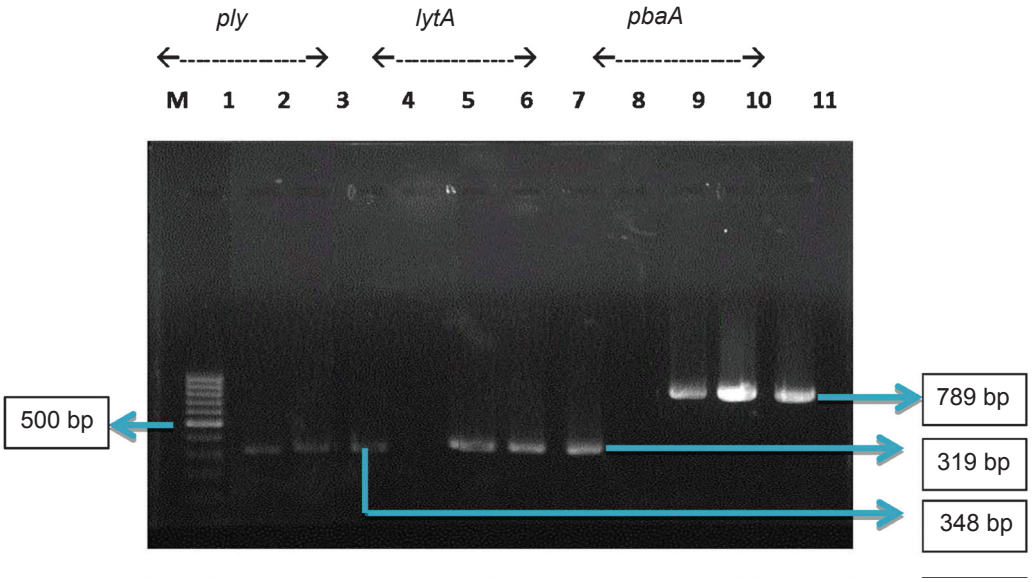

Figure. Pneumolysin (*ply*), autolysin (*lytA*) and modified penicillin binding protein (*pbpA*) genes amplified in the two isolates from cases of fatal pneumococcal infection. Amplicon sizes : *ply* - 348 bp; *lytA* - 319 bp and *pbpA* - 789 bp. M - 100 bp ladder (Gene ruler); Lanes 1, 5, 9; IBT-1960 (serotype 33C); Lanes 2, 6 ,10 - IBT -1975 (serotype 7C) and Lanes 3,7,11 - *S. pneumoniae* ATCC 49619; Lanes 4, 8, negative control.

vaccination. As the patients were healthy adults, preventive vaccination would not have played a role in protecting these individuals.

Pathogenesis of invasive pneumococcal disease depends on both host inflammatory response as well as the organism's virulence. Several virulence factors have been incriminated in the pathogenesis of pneumococcal disease, including autolysin, pneumolysin (a key inducer of apoptosis) and pneumococcal surface adhesin A (PsaA)[16]. Capsular polysaccharide is the most commonly attributed factor. Pneumolysin is a cholesterol dependent cytolysin, that binds to cells and induces apoptosis[17]. Pneumolysin is known to reduce ciliary action, phagocytic function of polymorphonuclear cells and induce acute inflammatory reaction[18]. Both the isolates in this study were positive for genes coding for autolysin and pneumolysin. Though *pbp* gene was detected, penicillin resistance was not detected phenotypically, suggesting non-phenptypic expression of the gene.

Our observation highlights two points. First, the involvement of non-vaccine serotypes in fatal invasive pneumococcal infection in adults. Some studies have shown a decline in adult pneumococcal disease following introduction of multi-valent pneumococcal conjugate vaccine in children[13,18]. But adult invasive infection and protecting the high risk groups still remains a major problem in several countries[19]. The US advisory committee on invasive pneumococcal disease (ACIP) has recommended the use of 23 valent polysaccharide vaccine (PPSV23) and the PCV13 (conjugate vaccine) for use in the elderly population aged >65 yr[7,20]. Secondly, could presence of pneumolysin and autolysin genes in the isolates obtained from these patients have any diagnostic or prognostic indications? Severity of pneumococcal pneumonia associated with bacterial genomic load determined by the copies of *lytA* genes has been reported[21].

In patients with no underlying risk factors, a strong suspicion of invasive pneumococcal disease with rapid assessment needs to be done to prevent grave outcome. A delay in treatment can occur in young and middle-aged patients, who are otherwise healthy leading to serious consequence. Accurate management in a timely manner can improve outcome. Rapid laboratory tests are essential for confirmatory diagnosis. A rapid diagnostic tool has been developed to detect pneumococcal antigen in urine and other body fluids which shows high sensitivity and specificity in adults[19]. The two isolates from our patients had the *lyt A* and *ply* genes, although we did not attempt to detect this from the patients' blood or body fluids. High level of clinical suscpicion with laboratory support by conventional blood culture, effective case management with appropriate antibiotics will decrease morbidity and mortality due to invasive pneumococcal disease in adults.

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***Conflicts of Interest*:** None.

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