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Aetiological spectrum of severe community-acquired pneumonia in HIV-positive patients from Pune, India

Sir,

Pulmonary infections are a major cause of morbidity and mortality in HIV-positive individuals, of which bacterial community-acquired pneumonia (BCAP) is reported to be 25 times more common than in the general community[1-3]. Studies also indicate increased BCAP-related mortality rates in HIV-positive patients[2,4]. Selection of initial empirical treatment depends on the common pathogens identified in previous aetiological studies and relevant treatment trials; however, the aetiological profile of CAP is different in different countries, and hence it is imperative that these recommendations are based on epidemiological data obtained from particular geographic location. Severe CAP (SCAP) occurs in approximately 18-36 per cent of all CAP cases with mortality rate of <5 per cent in outpatient cases, 10 per cent in hospitalized patients and can exceed 30 per cent in patients admitted to Intensive Care Units[5]. There is a paucity of data on the aetiological profile of SCAP from the South Asian countries, especially amongst HIV-positive patients. The present study was thus conducted to determine the aetiological spectrum of severe CAP and to assess the mortality predictors associated with BCAP in HIV-positive patients from Pune, India.

This prospective observational study was conducted in collaboration with the department of Chest and Tuberculosis, Sassoon General Hospitals, Pune, India, attached to B. J. Government College, during January 2012-2014. A total of 121 consecutive HIV-positive patients presented with SCAP during the study period, of whom 111 were enrolled. Seven patients were critically ill, whereas three refused to consent and hence were not included. The inclusion criteria for SCAP were patients presenting with symptoms of CAP[6] and associated with either two or more of the criteria listed in parenthesis (systolic BP ≤90 mmHg, bilateral pneumonia or multilobar pneumonia and PaO_2/FIO_2 ≤250 mmHg) or with the presence of septic shock (hypotension not responding to adequate fluid resuscitation and required vasopressor/ionotropic support). Patients <18 yr of age, reporting hospitalization within seven days, critically ill and those refusing to consent were excluded from the study. The study was approved by the Institutional Ethics Committees of the B. J. Government College and ICMR-National AIDS Research Institute (NARI), Pune. Written informed consent was obtained from all participants.

All patients underwent complete history taking in structured questionnaires and physical examination by the attending physician. Chest radiography was done followed by the collection of induced sputum or bronchoalveolar lavage (BAL) and blood samples for microbiological workup, before instituting empirical therapy. The samples were sent to the Microbiology Laboratory, NARI, maintaining the cold chain for laboratory testing. The quality of the samples of induced sputum and BAL samples was assessed[7,8]. Bacterial identification, reporting of significant cultures and antibiotic sensitivity testing were performed using standard microbiological techniques[9-11]. The criteria for labelling an organism as multidrug-resistant (MDR) were as per Magiorakos *et al*[12]. Atypical bacteria were detected by polymerase chain reaction (PCR) using primers that amplified 630bp fragment of *mip* gene for *Legionella pneumophila*, 277 bp fragment of 16S rRNA gene for *Mycoplasma pneumoniae* and 438 bp fragment of *Pst*I fragment for *Chlamydophila pneumoniae*[13].

Mycobacterial detection was done by Ziehl-Neelsen acid-fast staining and Lowenstein Jensen culture. *Pneumocystis jiroveci, Cytomegalovirus* and *Herpes simplex virus* were detected by PCR using primers targeting the mitochondrial large-subunit rRNA

gene, IRL 11 region and simplex herpes virus (HSV) glycoprotein B gene, respectively[14,15]. The aetiological cause to each case was assigned as per the criteria of Park *et al*[16]. Mortality was defined as death due to BACP during hospital stay.

Statistical analysis: Statistical analysis was done using SPSS statistical software version 15.0 (SPSS, Chicago, USA). Frequencies of aetiological agents were reported according to their monomicrobial or polymicrobial status. Mortality-associated factors with BCAP were analyzed by logistic regression. All variables found to be significant in univariate analysis were included in multivariate analysis.

Of the 111 cases, 92 (82.9%) had identifiable aetiologies, of which 73 (79.3%) were monomicrobial and 19 (20.7%) were polymicrobial infections. The aetiological distribution is presented in Table I. To restrict the analysis to BCAP, patients with diagnosis of mycobacterial, fungal and viral aetiologies were excluded from further analysis.

Amongst the cases with identifiable aetiologies, 47 (51.1%) were definitive BCAP cases. BCAP patients were predominantly males (63.8%, n=30), with median age 39 [interquartile range (IQR) 35, 44] yr, 55.3 per cent (n=26) were on antiretroviral therapy (ART) and 21.3 per cent (n=10) were receiving co-trimoxazole prophylaxis. The median CD4+ count was 338 (IQR 238, 441) cells/µl in patients on ART and 92 (IQR 65, 122) cells/µl in ART-naive ones. Co-morbid conditions [chronic obstructive pulmonary disease (n=2), chronic liver disease (n=4), asthma (n=1) and diabetes mellitus (n=2)] were present in 19.1 per cent (n=9) patients. There were no significant differences in the overall characteristics of patients with and without BCAP. The presenting symptoms were cough (95.7%, n=45), fever (80.8%, n=38), breathlessness (42.5%, n=20) and chest pain (17.02%, n=8). Radiological involvement revealed monolobar involvement in 35 (74.5%) cases, whereas 12 (25.5%) had multilobar involvement of whom four had pleural effusion.

Streptococcus pneumoniae was the predominant pathogen isolated (14 of 47, 29.8%). A total of 35.8 (n=5), 28.6 (n=4) and 7.2 (n=1) per cent isolates were resistant to penicillin, erythromycin and co-trimoxazole, respectively, while all were sensitive to tetracycline, cefotaxime and ciprofloxacin. MDR was seen in 7.1 per cent (n=1) isolates. Gram-negative bacilli (GNB) including *Klebsiella pneumoniae* (n=9), *Escherichia coli* (n=5) and *Pseudomonas aeruginosa* (n=4) were detected in 40.4 per cent (n=19) cases. A total of 68.4 (n=13), 52.7 (n=10), 47.4 (n=9), 26.4 (n=5), 21.1 (n=4) and 15.8 (n=3) per cent isolates were resistant to co-trimoxazole, amoxicillin-clavulanic acid, tetracycline, cefotaxime, ciprofloxacin and gentamicin, respectively, whereas all were sensitive to imipenem. Extended spectrum beta-lactamase production was seen in 5 of 15 (33.3%) isolates. MDR was seen in 31.6 per cent (n=6) isolates. *S. aureus* was isolated in 8 of 47

Table I. Aetiological spectrum in severe community-acquired pneumonia

Aetiological agent	Number of cases (n=111), n (%)
Monomicrobial infections	73 (65.8)
S. pneumoniae	13 (17.8)
S. aureus	7 (9.6)
S. pyogenes	5 (6.8)
K. pneumoniae	9 (12.3)
E. coli	5 (6.8)
P. aeruginosa	4 (5.5)
H. influenzae	1 (1.4)
M. tuberculosis	17 (23.3)
P. jiroveci	7 (9.6)
Cytomegalovirus	5 (6.8)
Polymicrobial infections	19 (17.1)
M. tuberculosis + *K. pneumoniae*	3 (15.8)
M. tuberculosis + *E. coli*	1 (5.3)
M. tuberculosis + *Cytomegalovirus*	1 (5.3)
M. tuberculosis + *C. albicans*	3 (15.8)
M. tuberculosis + *P. jiroveci*	4 (21.1)
P. jiroveci + *S. aureus*	1 (5.3)
P. jiroveci + *S. pneumoniae*	1 (5.3)
P. jiroveci + *Cytomegalovirus*	1 (5.3)
S. pneumoniae + *M. pneumoniae*	1 (5.3)
S. aureus + *M. pneumoniae*	1 (5.3)
K. pneumoniae + *M. pneumoniae*	1 (5.3)
E. coli + *Cytomegalovirus*	1 (5.3)
Unidentified aetiologies	19 (17.1)

S. pneumoniae, *Streptococcus pneumoniae*; *S. aureus*, *Staphylococcus aureus*; *S. pyogenes*, *Streptococcus pyogenes*; *K. pneumoniae*, *Klebsiella pneumoniae*; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *H. influenzae*, *Haemophilus influenzae*; *M. tuberculosis, Mycobacterium tuberculosis*; *P. jiroveci*, *Pneumocystis jiroveci*; *C. albicans*, *Candida albicans*; *M. pneumoniae*, *Mycoplasma pneumoniae*

(17.02%) cases. Methicillin resistance was observed in 37.5 per cent (n=3) isolates, whereas 50 per cent isolates (n=4) were resistant to erythromycin and co-trimoxazole, 37.5 per cent (n=3) to amoxicillin-clavulanic acid and tetracycline, 25 per cent (n=2) to ciprofloxacin and 12.5 per cent (n=1) to gentamicin, whereas all were sensitive to vancomycin. MDR was seen in 12.5 per cent (n=1) isolates. Beta-haemolytic streptococci and *Haemophilus influenzae* were seen in 10.6 (n=5) and 2.1 per cent (n=1) cases, respectively. A total of 40 per cent (n=2) streptococcal isolates were resistant to co-trimoxazole and amoxicillin-clavulanic acid and 20 per cent (n=1) to erythromycin and tetracycline, while all were sensitive to cefotaxime, ciprofloxacin and vancomycin. The single *H. influenzae* isolate was sensitive to amoxicillin-clavulanic acid, erythromycin, tetracycline, cefotaxime and ciprofloxacin.

Overall MDR was observed in 23.4 per cent (n=26) isolates, whereas bacteraemia was seen in 7.2 per cent (n=8) cases. Atypical bacteria, all *M. pneumoniae,* were detected in 6.4 per cent (n=3) cases. Mortality during hospital stay in BCAP cases was 29.8 per cent (14/47). The bacterial isolates in the 14 fatal cases were *S. aureus* (n=5), *K. pneumoniae* (n=4), *P. aeruginosa* (n=2) and *S. pneumoniae* (n=3). Fatality was seen in 62.5 per cent *S. aureus,* 50 per cent *P. aeruginosa,* 40 per cent *K. pneumoniae* and 21.4 per cent *S. pneumoniae* infections. Table II shows the risk factors associated with BCAP mortality. On multivariate analysis, infection with MDR bacteria [*P*=0.012, adjusted odds

Table II. Mortality predictors associated with bacterial community-acquired pneumonia

Variable	Survived (n=33), n (%)	Died (n=14), n (%)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	*P*	AOR (95% CI)	*P*
Gender						
Male	24 (72.7)	6 (42.9)	1		1	
Female	9 (27.3)	8 (57.1)	3.56 (0.96-13.13)	0.057	4.83 (0.80-29.18)	0.086
Age (yr)						
>40	12 (36.4)	5 (35.7)	1			
≤40	21 (63.6)	9 (64.3)	1.03 (0.28-3.79)	0.966		
CD4 count (cells/µl)						
>100	18 (54.5)	6 (42.9)	1			
≤100	15 (45.5)	8 (57.1)	1.6 (0.45-5.65)	0.465		
Antiretroviral treatment						
Yes	21 (63.6)	5 (35.7)	1			
No	12 (36.4)	9 (64.3)	3.15 (0.86-11.59)	0.084		
Infection with MDR bacteria						
Present	3 (9.1)	8 (57.1)	1		1	
Absent	30 (90.9)	6 (42.9)	0.08 (0.02-0.37)	0.001	0.07 (0.01-0.55)	0.012
Involvement on chest X-ray						
Multiple	5 (15.2)	7 (50.0)	1		1	
Single	28 (84.8)	7 (50.0)	0.18 (0.04-0.73)	0.017	0.12 (0.02-0.95)	0.044
Septic shock						
Present	1 (3.0)	4 (28.6)	1		1	
Absent	32 (97.0)	10 (71.4)	0.078 (0.01-0.78)	0.030	0.04 (0.001-0.94)	0.046
Co-morbidities						
Present	5 (15.2)	4 (28.6)	1			
Absent	28 (84.8)	10 (71.4)	0.45 (0.10-2.00)	0.292		

OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval; MDR, multi drug resistant

ratio (AOR)=0.07, 95% confidence interval (CI)=0.01-0.55], multilobar involvement (*P*=0.044, AOR=0.12, 95% CI=0.02-0.95) and septic shock (*P*=0.046, AOR=0.04, 95% CI=0.001-0.947) were independently associated with mortality.

In the present study, aetiological diagnosis was possible in 82.9 per cent (n=92) cases. Worldwide, variable rates of aetiological diagnosis in CAP have been reported and these differences can be attributed to factors such as geographical locales, patient groups studied, samples used, aetiologies assessed and diagnostic assays employed. The ability to investigate an array of pathogens and collection of induced sputum or BAL allowed us to better define the aetiologies in our patients.

Overall 51.1 per cent CAP cases (n=47) were attributable to bacterial aetiologies. Worldwide, prevalence rates of BCAP ranging from 10.7 to 52.7 per cent have been reported from HIV-positive individuals[17-21]. In confirmation with previous reports, *S. pneumoniae* was the most common bacterial isolate and exhibited considerable resistance to routinely prescribed antibiotics[17,20-26]. Bacteraemia and high recurrence rates complicating pneumococcal pneumonia amongst HIV-positive individuals have been reported[27], warranting an urgent need for evaluation of pneumococcal vaccine for its potential benefits in Indian HIV-positive patients. Although reported to account for approximately five per cent pneumonias in HIV-positive persons, GNB were detected in a considerable number of cases. GNB constitute the common pathogens reported in HIV-negative CAP cases in Indian settings[22-24]. The high mortality observed with *S. aureus* infection was in corroboration with earlier studies[17,28]. Our results were in contrast with the only study from India that reported a high prevalence of *M. pneumonia* infection amongst HIV-positive individuals[22].

Mortality during hospital stay was 29.8 per cent. Studies worldwide have reported mortality rates ranging from 5 to 30 per cent in HIV-positive patients with BCAP[1-5]. The association between septic shock and radiological progression has been reported previously[3,21,29]; however, there are limited data on the impact of drug-resistant bacteria on clinical outcome of BCAP. We found a significant association between initial infection with MDR organism and mortality in HIV-positive patients. High *in vitro* resistance was observed towards commonly prescribed antibiotics with about one-fourth MDR isolates, highlighting the need to institute appropriate initial antimicrobials directed towards the pathogen, which may lead to favourable clinical outcome and prevent the chronology to death.

This study was a single centre study; hence the results may not be generalizable to the entire country. However, all patients were enrolled prospectively and consecutively, lending consistency to data in contrast to the retrospective reports published earlier[3,29]. To conclude, this study identified the predominance of bacterial aetiologies and high *in vitro* resistance to commonly prescribed antibiotics in SCAP amongst HIV-positive patients, highlighting the need for choosing appropriate initial pathogen-directed antimicrobial therapy for proper management of BCAP.

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