RESEARCH ARTICLE

Antimicrobial sensitivity pattern, demographic findings and risk factors amongst meningitis and non-meningitis invasive *Streptococcus pneumoniae* at Aga Khan University Hospital Clinical Laboratory, Karachi, Pakistan

Seema Irfan,[1] Joveria Farooqi,[2] Haresh Kumar,[3] Afia Zafar[4]

Abstract

Objective: To determine the resistance rate of penicillin and ceftriaxone amongst invasive meningitis and non-meningitis isolates of *streptococcus pneumoniae*.

Methods: The prospective cross-sectional study was conducted from January 2011 to March 2014 at the Clinical Microbiology Laboratory of Aga Khan University, Karachi, and comprised all invasive strains of streptococcus pneumoniae. Penicillin and ceftriaxone susceptibilities were performed and interpreted based on minimum inhibitory concentration breakpoints recommended by Clinical and Laboratory Standards Institute guidelines. Data was analysed using Stata 12.

Result: There were 163 strains isolated from sterile body fluids of 109 patients. Of the total, 46(28%) samples were meningitic while 117(72%) were non-meningitic. Of the meningeal isolates, 12(26%) were resistant to penicillin, while none was resistant to ceftriaxone and vancomycin. None of non-meningeal isolates showed resistance to penicillin, ceftriaxone or vancomycin.

Conclusions: There was considerable penicillin resistance among meningeal strains of *streptococcus pneumoniae,* but here appeared to be no need to add vancomycin for empirical treatment of invasive *streptococcus pneumoniae* infection.

Keywords: Meningitis, Invasive *streptococcus pneumoniae,* Penicillin resistance, Ceftriaxone, Vancomycin. (JPMA 69: 1124; 2019)

Introduction

Streptococcus (S.) pneumoniae is a part of normal flora of nasopharynx, but can cause a variety of infections in the general population.[1] Majority of these infections are non-invasive, such as otitis media, but some invasive infections, like meningitis, bacteraemia and pneumonia, can be life-threatening.[2] Invasive pneumococcal disease (IPD) is defined as an infection confirmed by the isolation of S. pneumoniae from a normally sterile site (e.g. blood or cerebrospinal fluid [CSF] but not sputum).[3]

IPD, particularly meningitis, is associated with substantial rates of morbidity (20-30%) and mortality (10%).[4-6] Pneumococcal Global Burden of Disease Study Team concluded that Pakistan, among six other countries of Asia, has the highest number and proportion of S. pneumoniae cases.[7-9] Changing trends in the antimicrobial susceptibility pattern of S. pneumoniae in invasive infections have been reported globally.[10,11] There is an increase in the rate of resistance to various classes of antibiotics, especially β-lactams, which have traditionally been an effective therapy.[10] This makes the treatment more difficult and costly. Resistance rates vary widely among different geographic regions. Published data from different countries reports variable prevalence of penicillin and ceftriaxone resistance amongst S. pneumoniae strains. For example, a study from South Africa revealed penicillin resistance in 33% of their invasive strains,[12] while a centre in Baltimore, United States[13] reported 48.6% penicillin-resistant strains. India, one of Pakistan's neighbouring countries, has reported just 2.7% penicillin resistance.[14] Resistance to penicillin, ceftriaxone or any other third generation cephalosporin among invasive isolates of S. pneumoniae has not been documented yet in Pakistan.[15] However, a recent study on pneumococcal nasal carriage showed penicillin minimum inhibitory concentration (MIC)90 of 0.25 mcg/ml, suggesting there are some strains circulating in the community which could be considered resistant using meningitic breakpoints.[16] There is also paucity of IPD demographic and risk factor data reported from Pakistan. Therefore, the current study was planned to identify differences in demographic, co-morbid and other clinical parameters as well as antimicrobial susceptibility between meningitic and non-meningitic IPD strains.

[1,2,4]Department of Pathology and Laboratory Medicine The Aga Khan University Hospital, [3]Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Correspondence: Seema Irfan. Email: seema.irfan@aku.edu

Materials and Methods

The prospective cross-sectional study was conducted from January 2011 to March 2014 at the Clinical Microbiology Laboratory of Aga Khan University (AKU), Karachi. The laboratory receives specimens from patients admitted to AKU Hospital (AKUH), a tertiary care 700-bed private facility. In addition, the laboratory receives samples from more than 200 collection points spread all over the country. All invasive strains of S. *pneumoniae*, defined as those isolated from sterile body fluids, such as blood, CSF, pleural fluid, synovial fluid, ascitic fluid and splenic drain specimens, were included. S. pneumoniae strains from both CSF and blood cultures in the same patient were considered duplicates, and in that case, the CSF isolate was included for analysis. Multiple isolates from the same patient were also considered duplicates. All non-invasive S. pneumoniae strains and duplicate isolates were excluded.

The sample size was calculated on the basis of a previous study[17] which showed penicillin resistance of 44% and ceftriaxone 15% among invasive pneumococcal isolates. It was calculated using World Health Organisation (WHO) software,[18] using lowest proportion, i.e. ceftriaxone 15%, and an absolute precision of 6%, at 95% confidence level.

Clinical and demographic information on the patients was obtained via telephonic communication with respective clinicians. It was collected as part of routine clinical reporting of cultures at the AKU laboratory, and was retrieved from laboratory records. No additional information was obtained. S. pneumoniae isolates were considered meningitic if they were isolated from CSF. In case of blood culture isolates, only those from patients who had a clinical diagnosis of meningitis were considered meningitic. In case of growth of S. pneumoniae from both CSF and blood samples of the same patient, preference of susceptibility was given to the CSF isolate. Exemption from ethical approval was obtained from the institutional ethics review committee.

Clinical isolates of S. pneumoniae were identified using American Society of Microbiology (ASM) guidelines.[19] Initial suspicion of pneumococcus was based on centrally depressed colony morphology, i.e. Draughtsman colony, α-haemolysis, gram staining and catalase test. Final identification was confirmed by conventional tests, such as optochin and bile salt susceptibility. Fresh isolates of S. pneumoniae growing on enriched media were initially saved at -80°C in 1ml aliquots containing glycerol-phosphate buffer.

Susceptibility testing of antibiotics recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines,[20] including vancomycin, chloramphenicol, were performed using Kirby-Bauer disk diffusion method.

For testing penicillin and ceftriaxone MICs, organisms were revived on the sheep blood (SB) agar. To perform susceptibilities, 0.5 McFarland suspension was made, sterile swab dipped in the suspension was squeezed to remove the excess fluid, and lawn was made on Mueller-Hinton agar (MHA) with 5% sheep blood (SB-MHA). E-test strip of penicillin (Oxoid) and ceftriaxone (Biomerieux) were placed over the lawn on two separate 90mm SB-MHA plates incubated in 5% carbon di-oxide (CO2) enriched atmosphere for 24h. MICs were read and interpreted according to CLISI breakpoints[20] and the isolate was categorized as sensitive (S), intermediate (I) or resistant (R). In the 21st Informational Supplement of CLSI, the recommended MIC breakpoints of penicillin and ceftriaxone are different for S. pneumoniae isolates growing from meningeal CSF and other invasive non-meningeal isolates.[20] For meningeal strains, penicillin MIC $\leq$0.06µg/ml isolate is considered susceptible while MIC of $\geq$0.12 µg/ml is categorised as resistant. For non-meningeal isolates, penicillin MIC of 2µg/ml, 4µg/ml and 8µg/ml is regarded as S, I and R, respectively. Similarly, for meningeal isolates, ceftriaxone MIC of $\leq$0.5 µg/ml, 1µg/ml and $\geq$2µg/ml are defined as S, I and R. Finally, MIC of 1 µg/ml, 2µg/ml and 4µg/ml are categorized as S, I and R, respectively for non-meningeal ones. Multidrug-resistance (MDR) was defined as penicillin (beta-lactam) resistance along with resistance to 2 or more antibiotics from among co-trimoxazole, ofloxacin, erythromycin and chloramphenicol.[12] CSF isolates were tested against ofloxacin, erythromycin and tetracycline only for categorisation as MDR or non-MDR, and these antibiotics were not reported for clinical use.

Data was coded and entered into Microsoft Excel 2010, transferred to Stata 12 software for statistical analysis. Mean and standard deviation of the continuous variables, i.e. age, and penicillin and ceftriaxone MICs, were calculated. Frequency and percentage of the categorical variables, like age group, gender, source of specimen, antibiotic susceptibility, clinical information on prior antibiotic history and vital status, was also calculated. Fischer's exact test was used to determine whether there was any statistically significant difference between the meningitic and non-meningitic isolates in terms of susceptibility profile and clinical characteristics. Binary logistic regression was used to calculate odds of mortality in the presence of certain risk factors by performing univariate and then multivariable analysis.

Results

There were 163 strains isolated from sterile body fluids of

Table-1: Minimum inhibitory concentration (MIC) and susceptibility data of meningitic and non-meningitic isolates of Streptococcus pneumoniae (N=163).

Beta-lactam drugs MIC	Meningitic (n=46)	Non-meningitic (n=117)	
Penicillin			
MIC50	0.06 µg/ml	0.03 µg/ml	
MIC90	0.125 µg/ml	0.25 µg/ml	
Ceftriaxone			
MIC50	0.032 µg/ml	0.023 µg/ml	
MIC90	0.094 µg/ml	0.094 µg/ml	
Antibiotics (no. tested)	No. of resistant (R) meningitic strains, %R	No. of resistant (R) non-meningitic strains, %R	Fisher's exact (p-value)
Penicillin (n=163)	12/46, 26.09	0/117, 0	<0.001*
Ceftriaxone (n=163)	0/46, 0	0/117, 0	-
Ofloxacin (n=159)+	8/42, 19.05	8/117, 6.84	0.022*
Chloramphenicol (n=159)	6/42, 14.29	5/117, 4.27	0.069
Erythromycin (n=159)+	12/42, 28.57	29/117, 24.79	0.361
Tetracycline (n=159)+	20/42, 47.62	59/117, 50.43	0.033*
Co-trimoxazole (n=159)+	31/42, 73.81	97/117, 82.91	0.336
Vancomycin (n=159)	0/42, 0	0/117, 0	-
MDR (n=159)	8/42, 19.05	0/117, 0	<0.001*

MIC: Minimum inhibitory concentration, R: Resistant. MDR: Multi-drug resistent.
+These antibiotics are not used for treatment of meningitis and the resistance rates given here are only of epidemiological significance. *Statistically significant at p-value <0.05.

Table-2: Descriptive statistics: clinical differences between meningitic and non-meningitic streptococcus pneumoniae strains in 109 patients admitted in Aga Khan University Hospital.

Risk Factor	Meningitic (N=31) (N, %)	Non-meningitic(N=78) (N, %)	Fisher's exact p-value
Age Group			0.003*
≤5	6, 19.4	24, 30.8	
≥5 to ≤15 years	7, 22.6*	2, 2.6*	
>15 to ≤64 years	8, 25.8	35, 44.9	
≥65 years	10, 32.3	17, 21.8	
Male gender	19, 61.3	41, 52.6	0.523
Diabetes	4, 12.9	17, 21.8	0.42
Hypertension	4, 12.9	13, 16.7	0.774
COPD	2, 6.5	4, 5.1	1
Renal disease	0, 0	1, 1.3	1
Cancer	1, 3.2	4, 5.1	1
Liver disease	0, 0	12, 15.3	0.018*
Smoker	1, 3.2	3, 3.8	1
Hospital Unit			0.474
Ward	17, 56.7	51, 68.0	
ICU	5, 16.7	8, 10.7	
ER	8, 26.7	16, 21.3	
Pneumonia	7, 22.6	57, 73.1	<0.001*
Death	7, 22.6	11, 14.1	0.391
Antibiotic Therapy			
Vancomycin	12, 41.4	13, 18.3	0.022*
3rd Gen Cephalosporin	14, 48.3	41, 56.9	0.51
Ampicillin	0, 0	6, 8.3	0.178
Carbapenem	6, 20.7	5, 6.9	0.073*
Betalactam-inhibitor combination	6, 20.7	11, 15.3	0.561
Macrolides	4, 13.8	12, 16.7	1

*Statistically significant difference between the meningitic and non-meningitic strains. *Statistically significant at p-value <0.05. NS: Not significant.
In multivariable analysis, male gender, admission in ICU and emergency room had higher odds of mortality while erythromycin resistance was found to be protective against it, after adjusting for age groups.
ICU: Intensive care unit. ER: Emergency room.

Table-3: Predictors of mortality: binary logistic regression -univariate and multivariable analysis.

Risk factor	Expired (N, %)	Univariate OR, CI (p-value)	Multivariable analysis OR, CI (p-value)
Male	20, 33.3	3.6, 1.3-9.8 (0.01)*	5.3, 1.5-18.7 (0.009)*
Female	6, 12.2		
Age group			
>15y to <64y (reference)	10, 38.5		
<5y	9, 34.6	1.4, 0.5-4.1 (0.52)	2.7, 0.65-11.3 (0.17)
>5y to <15y	0, 0	-	-
>65y	7, 26.9	1.2, 0.4-3.5 (0.80)	1.3, 0.3-4.9 (0.69)
Cancer	3, 60.0	5.3, 0.8-33.5 (0.08)*	NS
Non-cancer	23, 22.1		
Hospital Unit			
Ward (reference)	9, 13.2		
ICU	6, 46.2	5.6, 1.5-20.5 (0.009)*	6.1, 1.2-28.4 (0.022)*
ER	10, 41.7	4.6, 1.9-13.7 (0.005)*	5.0, 1.4-18.3 (0.014)*
Antibiotic Resistance			
Penicillin	0,0 vs. 4, 4.8	-	
Ofloxacin	1, 3.9 vs. 11, 13.3	0.3, 0.0-2.0 (0.19)	
Chloramphenicol	1, 3.9 vs. 7, 8.4	0.4, 0.1-3.7 (0.45)	
Erythromycin	2, 7.7 vs. 28, 33.7	0.2, 0.0-0.7 (0.02)*	0.1, 0.02-0.7 (0.017)*
Co-trimoxazole	21, 80.8 vs. 69, 83.1	0.7, 0.2-2.3 (0.59)	
Tetracycline	11, 42.3 vs. 52, 62.7	0.4, 0.2-1.0 (0.06)*	NS
MDR	0,0 vs. 4, 4.8	-	
Antibiotic Therapy (n, %)			
Vancomycin	8, 32.0 vs. 17, 22.7	1.6, 0.6-4.3 (0.35)	
3rd Gen Cephalosporin	13, 50.0 vs. 42, 56.0	0.7, 0.3-1.9 (0.60)	
Ampicillin	2, 7.7 vs. 4, 5.3	1.5, 0.3-8.6 (0.66)	
Carbapenem	5, 19.2 vs. 6, 8.0	2.7, 0.8-9.9 (0.12)*	NS
Betalactam-inhibitor combination	3, 11.5 vs. 14, 18.7	0.6, 0.1-2.2 (0.41)	
Macrolides	3, 11.5 vs. 13, 17.3	0.6, 0.2-2.4 (0.49)	
De-escalation in<3 days	23, 88.5 vs. 65, 78.3	2.1, 0.6-7.9 (0.26)	

OR: Odds ratio
CI: Confidence interval
ICU: Intensive care unit
ER: Emergency room.

109 patients. Of the total, 46(28%) samples were meningitic while 117(72%) were non-meningitic. Among the patients, 57(52.3%) had presented with signs and symptoms of pneumonia alone, 24(22%) with meningitis alone, 7(6.4%) with both pneumonia and meningitis, and 21(19.2%) had neither pneumonia nor meningitis symptoms. In terms of age, 30(27.5) patients were 5 years or younger, 9(8.25%) were aged 5-15 years, 43(39.4%) were 15-65 years and 27(24.7%) >65 years. Overall, 60(55%) patients were male.

Of the 46 meningitic isolates, 23(50%) grew from CSF samples, and, in 19(82.6%) of them, S. pneumoniae were yielded only from CSF samples, while in 4(17.4%) cases, both CSF and blood yielded the same isolate. In the remaining 23(50%) cases, isolates grew from blood cultures, and, based on clinical suspicion, these isolates were considered meningitic. Among the 46 meningitic isolates, 42(91.3%) belonged to patients from Karachi, and, of them, 24(57%) patients were hospitalised at AKUH. Only 4(8.6%) samples were received from other cities of Pakistan.

At AKUH, 7(29%) patients were admitted in intensive care unit (ICU), while 17(71%) were treated in general wards. In the 24 in-patients, empirical ceftriaxone was started in 14(58%) patients, and 5(36%) of them were given the drug alone and 9(64%) in combination with other drugs. Besides, 10(42%) patients received other broad-spectrum beta-lactam antibiotics; 7(70%) meropenem and 3(30%) piperacillin-tazobactam. In 15(62.5% patients, vancomycin was added along with ceftriaxone, meropenem or piperacillin-tazobactam. Of the 24 patients at AKUH, 6(25%) died in hospital, 8(33%) were discharged and 10(42%) were transferred to other facilities. No clinical history was available in

meningitis patients whose samples were submitted as outpatients.

Out of the 46 meningeal strains, 12(26 %) isolates were found resistant to penicillin with MIC50= 0.046 µg/ml and MIC90= 0.125 µg/ml. None of them was resistant to ceftriaxone with MIC50= 0.023 µg/ml and MIC90= 0.094 µg/ml. Chloramphenicol resistance was found in 6(14.3%) isolates.

Out of 117 non-meningitic IPD strains, 100(85.5%) were isolated from blood cultures, while the remaining 17(14.5%) were from other sterile sites. None of non-meningeal IPD isolates showed resistance to penicillin, ceftriaxone or vancomycin. Penicillin and ceftriaxone MICs of meningitic and non-meningitic isolates were compared and it was noted that penicillin resistance, and, hence, multi-drug resistance (MDR), was seen only in meningitic isolates ($p<0.001$). Ofloxacin resistance was significantly low, while tetracycline resistance was high in meningitic isolates, suggesting that the strains causing meningitis were different from non-meningitic ones, and, hence, serotypes could also be different (Table-1).

Underlying liver disease, absence of pneumonia, vancomycin and carbapenem usage and age 5-15 years were significantly associated with meningitic strains ($p<0.05$ each) (Table-2).

After adjusting for age, patients presenting to emergency department (ED) requiring ICU admission and having male gender were found to have higher odds of mortality than female patients who were neither admitted through ED nor ended up in ICU; and erythromycin resistance appeared to be protective against mortality (Table-3), suggesting that strains isolated from patients who expired were less likely to be exposed to macrolides, which are the first-line antibiotics used in respiratory tract infections.

Discussion

The current laboratory-based study clearly indicates that penicillin resistance was high (26%) amongst meningitic S. pneumoniae isolates from Karachi. However, ceftriaxone resistance was not detected in any of these tested isolates. On the other hand, regional data from India and Bangladesh has reported very low percentage of penicillin non-susceptible isolates in studies ranging from 1.3 to 2.9.[12,20] Regarding ceftriaxone non-susceptibility, international data again shows wide variation.[21-24] In Pakistan surveillance data for susceptibility of invasive S. pneumoniae isolates is limited. The current study comprised highest number of meningitis-causing S. pneumoniae isolates analysed for susceptibility pattern. A previous community-based study evaluated 15 strains of S. pneumoniae from Karachi and Hyderabad, and the susceptibility of S. pneumoniae to penicillin was 26% which is consistent with our findings. It also corroborates the available antibiogram data published by different tertiary care centres of Karachi, reporting penicillin resistance ranging 8-30% during 2006-12.[25]

Current recommendations by international guidelines prescribe a combination of ceftriaxone and vancomycin as empiric antibiotic therapy for suspected bacterial meningitis in all age groups except neonates.[26] These guidelines are closely followed at the AKUH and the data as such indicates that 63% (15/26) of patients admitted in our setting were empirically started on IV vancomycin along with either ceftriaxone or other broad-spectrum antibiotics i.e. meropenem or piperacillin-tazobactam. Out of 46 isolates, 12(26%) revealed resistance to penicillin and justified the need of empiric ceftriaxone in cases of suspected bacterial meningitis. However, all of the meningitis-causing S. pneumoniae strains revealed ceftriaxone MIC within susceptible range with MIC50 and MIC90 of 0.023, 0.094 µg/ml respectively. This collection had isolates with penicillin MIC90 of 0.125µg/ml which further supports sole use of ceftriaxone in our setting. Therefore, our finding points out the overuse of empiric vancomycin for S. pneumoniae meningitis.

In addition to AKUH hospitalised patients, 43% (20/46) of the tested S. pneumoniae isolates were from samples that were submitted from different areas of Karachi. This finding further highlights the susceptibility pattern of common strains circulating in the community.

Looking at age and gender distribution, S. pneumoniae meningitis was found to be common in all age groups with almost 30% of cases occurring in <5 years of age. This study supports the need of vaccination in this age group.

Male gender and severe disease necessitating ED presentation and ICU stay were significantly associated with patient demise even after adjusting for age as confounder. This emphasises the role of early disease recognition and timely institution of appropriate antibiotic therapy. The study has its limitations. For instance, the data is four years old and the majority of S. pneumoniae isolates belonged to Karachi city. Only four clinical samples belonged to other cities of Pakistan; 2 from Hyderabad and one each from Quetta and Abbottabad. Although all four S. pneumoniae isolates

belonging to three different cities of Pakistan were susceptible to ceftriaxone, due to a small number of isolates this statement cannot be generalised for the whole of Pakistan. Due to cost constraints, the study could not to identify pneumococcal serotypes which could have helped in the selection of future vaccine type in Pakistan.

For a wider and clearer picture, countrywide evaluation of S. pneumoniae susceptibility data using standard sensitivity testing methods is required and needs to be reported from other centres of Pakistan. Key role of antibiotic stewardship must be emphasised for the control of antimicrobial resistance. In this regard active participation of clinical microbiology laboratory is crucial. Prompt reporting of antimicrobial susceptibility is essential to curtail overuse of broad-spectrum antibiotics, including vancomycin. In 15 AKUH-hospitalised patients, initial empiric therapy included vancomycin, but de-escalation of vancomycin within 3 days was seen in all cases. In the 3 remaining patients, discontinuation of vancomycin was performed at 5th, 6th and 10th days. Similarly, correct methodology to detect penicillin and ceftriaxone resistance is an essential requirement for correct reporting. CLSI recommends broth or agar dilution-based MIC reporting for penicillin, and ceftriaxone for meningeal S. pneumoniae strains as disk diffusion-based Kirby Bauer oxacillin screening method lacks sensitivity for detecting penicillin resistance in meningeal strains. Therefore, to minimise reporting errors, clinical laboratories should strictly follow this recommendation. We performed E-strip testing for penicillin and ceftriaxone MIC determination. E-strip method is an easy, reliable and widely used method in clinical studies.[26,27] Finally, in order to strictly monitor emerging resistance, sharing of resistance data amongst different hospitals is also required.

Conclusion

Penicillin resistance was found to be high in S. pneumoniae meningitis isolates from Karachi, justifying the empiric use of ceftriaxone. However, resistance to ceftriaxone was not found which suggests that the addition of vancomycin in empiric therapy of pneumococcal meningitis is currently not required. Continuous monitoring of antimicrobial susceptibilities is necessary by clinical laboratories to capture any emerging ceftriaxone resistance. Countrywide surveillance programme, including pneumococcal serotyping, is urgently needed to support curtailing of invasive pneumococcal disease.

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