Anti-biogram of pneumococcal isolates in a tertiary care hospital

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Abstract

Streptococcus pneumoniae are gram positive normal flora of the upper respiratory tract in humans but are also the primary causative bacterial agents of pneumonia and otitis media mostly in children. They are also known to cause sinusitis, bronchitis, meningitis as well as bacteremia. They are differentiated from other Streptococci by their special characteristics such as their morphology, bile solubility as well as their sensitivity to optochin in addition to their display of a capsule. They are sensitive to most antibiotics and the beta lactams are considered the best drugs of choice in their treatment. Antimicrobial resistance currently considered as a serious public health challenge, not only in hospital settings but also in the community at large.

Aims and Objectives

1. To find out the occurrence of pneumococcal etiology in Community Acquired Pneumonia (CAP) and Hospital Acquired Pneumonia (HAP).
2. To find out Penicillin resistance among these isolates by: Penicillin E-strips.
3. To identify Macrolide resistance among these isolates by: Erythromycin E- strips.

Materials and Methods: Pneumococcal isolates were collected from the respiratory samples such as sputum, endotracheal secretions, throat swab, broncho alveolar lavange, and also from other samples such as ear swab and the bacteremia suspected blood samples.

Results: A total of 30 pneumococcal isolates were collected from respiratory samples namely: sputum, endotracheal secretions, throat swab, broncho alveolar lavange, and other samples like ear swab and blood. The collected isolates were confirmed to be *Streptococcus pneumonia.* Out of 30 samples, 18 (60%) were collected from male patients and 12 (40%) samples from female patients.

Conclusion: The need of MIC determination for antimicrobial susceptibility pattern testing of Pneumococci is a better method because it was found that the isolates which were sensitive for disc diffusion method even had the resistant MIC as 40% and 56.6% for Penicillin and Erythromycin.

Keywords: Streptococcus *pneumonia*, Optochin sensitive, Antimicrobial resistance.

Introduction

Streptococcus pneumoniae (Pneumococcus) is a gram positive, lanceolate diplococci, capsulated, facultative anaerobe with a spectrum of illness such mild otitis media, fatal meningitis and it is the most common cause of community acquired pneumonia.[1,2] *Streptococcus pneumoniae* (pneumococcus) is among the bacteria of international concern, ranked 9th in the according to the report by world health organization (WHO).[3] In 2014, the mortality by influenza and pneumonia were ranked eighth by the National Center for Health Statistics3. Pneumonia is the single largest cause of death worldwide in children. In 2015, pneumonia killed about 920,136 children under the age of 5, accounting for 15% of all deaths of children under five years old.[4] The development of penicillin resistance in the pneumococcus round about the 1980s–1990s resulted in the shift from antibiotics treatment to the treatment with macrolides. In the past, macrolide resistance was rare, and that made macrolides the traditional alternatives to penicillin.

However, due to asymptomatic nasopharyngeal carriage, the increase in the number of macrolide-resistant strains has also become a public health concern. There are two major mechanisms mediating resistance to the macrolides, and are based on ribosomal modification and active drug efflux. The former, when caused by methylation of rRNA, is associated with much high-level resistance to macrolides, lincosamides, and also streptogramin type B antibiotics, the latter is associated with lower levels of resistance to 14- and 15-membered macrolides (M phenotype).[6] The first is known as M-phenotype which is an efflux pump associated with mefE gene that results in the efflux of the macrolides from the cells.[6]

Pneumococcal resistance to macrolides is a problem because macrolides are among the most common oral drugs used in treating community-acquired pneumonia.[5] Many studies have examined the etiology of CAP. All studies in a compilation of 15 trials showed that *S. pneumoniae* was the most common pathogen accounting for 20%–60% of cases, followed by *Haemophilus influenzae* accounting for 3%–10%. The atypical pathogens (*Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Legionella pneumophila*) were sometimes implicated depending on the study, and viruses have also become common causes of pneumonia primarily in adult patients. Other such as tuberculosis, *Pneumocystis carinii*, Q fever, fungi, and also severe acute respiratory syndrome–associated coronavirus)[8] were less considered. The antibiogram is an essential component of studies in detecting the

susceptibility pattern of the organism to antibiotics which in turn helps in the effective treatment of the infection it causes. This study was carried out to document the recent antibiotic resistance in macrolides and penicillin by e-strip method among *Streptococcus pneumonia* isolated in a tertiary care hospital.

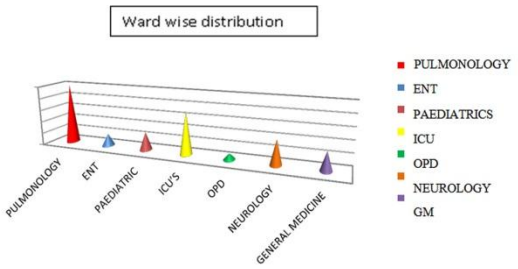
Materials and Methods

All the samples were collected from the suspected cases of pneumococcal infection. Respiratory samples such as sputum, endotracheal secretions, throat swab, broncho alveolar lavange, and also from other samples such as ear swab and the bacteremia suspected blood samples.

Bacterial identification and Isolation: Respiratory samples were collected from the patients by using sterile wide mouth container. The presence of bacteria was identified by direct microscopy, 0.5-1ml of the samples was inoculated in to Blood agar, chocolate agar and MacConkey agar were incubated overnight at 37ºC in 5-10 % CO_2, thus providing the required capnophilic environment for its growth. Incubated at $37^{.c}$ for 24-48 hours the culture media for bacterial growth and isolation, thus providing the required capnophilic environment for its growth. The isolated organisms were identified by using standard biochemical reactions and antimicrobial susceptiblity testing (ABDD and E-test) was performed as per CLSI guidelines.

1. A total of 30 pneumococcal isolates were collected from respiratory samples such as; sputum, endotracheal secretions, throat swab, broncho alveolar lavange, and other samples like ear swab and blood. The collected isolates were confirmed to be *Streptococcus pneumoniae* by biochemical identifications and were then subjected to culture and antibiotic susceptibility testing by Kirby bauer disc diffusion and E-strips test method (CLSI guidelines 2017).
2. Out of 30 samples, 18 (60%) were collected from male patients and 12 (40%) samples from female patient. The highest number of samples were from Pulmonology Ward; 10 (33.33%), followed by different ICU'S;07(23.3%) and the least sample were from Out Patient Department;01(3.33%), followed by ENT;02(6.66%) respectively.

Graph 1:



Results

Table 1: Showing sample wise distribution

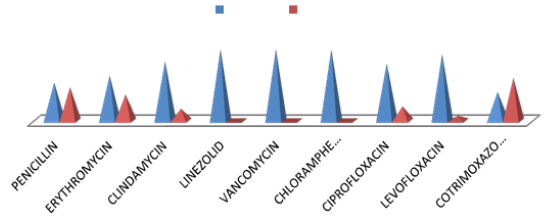
S.No	Sample	Number	Percentage
1	SPUTUM	11	36.66%
2	Endotracheal Secretions	08	26.8%
3	Throat Swab	03	33.3%
4	BAL	02	6.60%
5	Ear Swab	02	6.60%
6	Blood	03	33.3%
Total	-	30	100%

Among 30 isolates collected, 11 (36.66%) isolates were from Sputum sample, followed by 08 (26.8%) from Endotracheal secretions and the least were from 02 (6.60%) from BAL and Ear swab each

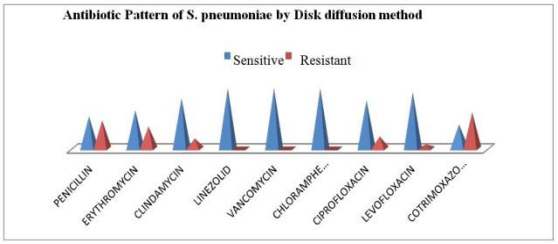
Table 2: Showing antibiogram of all the isolates by disc diffusion method

DRUG	Sensitive	Percentage	Resistant	Percentage
Pencillin	16	53.4 %	14	46.6 %
Erythromycin	19	63.4 %	11	36.6 %
Clindamycin	25	83.4 %	05	16.6 %
Linezolid	30	100 %	00	00 %
Vancomycin	30	100 %	00	00 %
Chloromphenicol	30	100 %	00	00 %
Ciprofloxacin	24	80 %	06	20 %
Levofloxacin	28	93.3 %	02	6.7 %
Cotrimoxazole	12	40 %	18	60 %

Graph 2:



Graph 3:



Among 30 isolates, majority of the isolates were resistant to cotrimoxazole 60%, followed by penicillin 46.6% and no resistance shown in linezolid, vancomycin and chloramphenicol with 100% sensitive strains, followed by levofloxacin 93.3% sensitivity.

Table 3: Resistance pattern of PRP and PSP against other antibiotics

DRUG (n=30)	*PRP=12 (40%)	*PSP=10 (33.3%)
Erythromycin	5 (16.6%)	3 (10%)
Clindamycin	2 (6.66%)	2 (6.66%)
Linezolid	00	00
Vancomycin	00	00
Chloramphenicol	00	00
Ciprofloxacin	03 (10%)	01 (3.33%)
Levofloxacin	07 (23.3%)	05 (16.6%)
Cotrimoxazole	01 (3.33%)	00
*PRP=Penicillin resistant Pneumococci, *PSP=Penicillin sensitive Pneumococci		

Table 4: MIC determination of Penicillin and Erythromycin using e-strips

Pencillin			Erythromycin		
S<=2	I =4	R>=8	S<=0.25	I= 0.50	R >=1
10 (33.3%)	**08 (27%)**	**12 (40%)**	**09 (30%)**	**04 (13.3%)**	**17 (56.6%)**
S=Sensitive, I= Intermediate, R =Resistant					

1. The isolates were tested for susceptibility of penicillin and erythromycin among which the highest resistance was observed in erythromycin with 56.66% with sensitivity of 30%, 04 isolates were found to be intermediate of 13.33% with MIC of 0.50µg/ml.
2. Penicillin resistance was observed in 40% strains, 33.3% were sensitive and 27% were intermediate with MIC 3-7µg/ml.

Graph 4: MIC determination of Penicillin and Erythromycin using e-strips

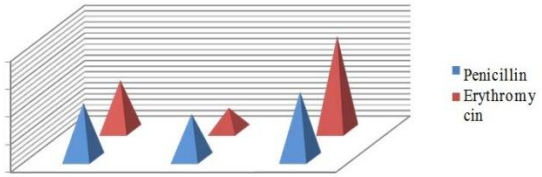


Table 5: Distribution of 30 strains by MICs of Penicillin and growth inhibition zone diameter around 1-µg oxacillin disk

No. of isolates inhibited by Penicillin MIC (µg/ml) of:			
Inhibition zone diameter (mm) around 1-µg oxacillin disk	Sensitive (<=2)	Intermediate (4)	**Resistant(>=8)**
6	00	03 (10%)	05 (16.6%)
7-19	02 (6.66%)	05 (16.6%)	06 (20%)
>=20	09 (30%)	00	00

Oxacillin, sensitive>=20mm.

1. Oxacillin disc diffusion test shows 9 isolates to have zone size >=20mm and were also found susceptible to Penicillin by MIC findings. 06 intermediate and 05 resistant Isolates were present with no zone around Oxacillin disc (1µg). 13 isolates were found to have zone less than 20mm
2. This study shows that in 30 isolates, 9 were found to be multidrug resistant (resistance to more than two classes of antibiotics). The groups of antibiotics in which this MDR pneumococci were seen are pencillin, erythromycin, clindamycin, and cotrimoxazolr.

Discussion

Streptococcus pneumoniae (pneumococcus) is known to be one of the most important and common human pathogens. It is a major cause of invasive pneumococcal disease (IPD), refers to as pneumonia, meningitis, bacteremia, sinusitis, and otitis media, and it occasionally infects tissues at other sites in the human body too. Pneumococcus is presumed to be one of the primary bacterial causes of community-acquired lower respiratory infections (LRI) among children in developed countries. Pneumococci spread from one individual to another by direct or droplet transmission as a result of close contact; transmission can also be enhanced by crowding or poor ventilation. The susceptibility profile of *S. pneumoniae* to antibiotics can be determined through a number of various methods, but determination of a minimum inhibitory concentration (MIC) constitutes the gold-standard.

Conclusion

Antimicrobial resistance is now acknowledged as a serious public health issue not only in hospital settings but in the community as well. The prevelance of *Streptococcus pneumoniae* (Pneumococci) that are resistant to antimicrobial agents is on the rise globally. Therefore, the aim of this study was to analyze the patterns of resistance to the antimicrobial drugs particularly for β-lactams (Penicillin) and Macrolides (Erythromycin) in Pneumococci, which may be possibly produce antibiotic treatment success in relation to β-lactam macrolide usage. The need of MIC determination for susceptibility pattern testing of Pneumococci is essential because was found that the isolates which were sensitive for disc diffusion method even had the resistant MIC as 40% and 56.6% for Penicillin and Erythromycin respectively. It was then concluded that the surveillance to pneumococcal susceptibility pattern is crucial in monitoring the development of antimicrobial resistance.

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