International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Antibiotic Susceptibility Pattern of *Streptococcus Pneumoniae* in South India by Using E-Test Techniques

R. Kadhar Nivas[1], Dr. M. Boominathan[2]

Research Scholar, Department of Biotechnology, Marudupandiyar College, Bharathidasan University, Thanjavur, Tamilnadu, India

[2]Head of the Department, Department of Biotechnology, Marudupandiyar College, Bharathidasan University, Thanjavur, Tamilnadu, India.

Abstract: ***Objective**: To analyse, estimate the consequence and antibiotic susceptibility pattern of Streptococcus Pneumoniae isolates various clinical specimens from different places. **Materials and Methods**: 105 clinical strains were recovered from different hospital in thanjavur, tamilnadu state at India from January 2014 to Jun 2014. All the isolates of streptococcus pneumonia were obtained from fresh material using routine methods and were identified using conventional methods by optochin susceptibility. These Strains were reidentified by conventional test after arriving biotechnology laboratory at thanjavur. Antibiotic Susceptibility test for isolated organism was done using by E-Test method. **Results**: Out of 105 isolates of Streptococcus pneumonia 69 (65.71%) were sputum/respiratory in origin.18 (17.14%) Ear swabs, 3 (2.85%) Throat swabs, 6 (5.71%) Eye swabs, 3 (3.15%) pus swabs, 2 (1.90%) CSF, 4(3.80%). **Conclusion**: This aim is not only to reduce the morbidity and mortality in the patients but also to control the emergence and spread of resistance among Streptococcus pneumoniae. Continuous surveillance of the use of antibiotics helps in preserving the effectiveness of antibiotics. The results from our study strongly emphasize the need for continuous epidemiological monitoring of antibiotic resistant.*

Keywords: *Streptococcus Pneumoniae*, Antibiotic Susceptibility Pattern, Multi Drug Resistance

1. Introduction

Pneumococcal disease is an infection caused by streptococcus pneumonia bacteria (pneumococcus) [(1)]. The genus streptococcus belongs to the non-spore forming aerobic to facultative anaerobic bacteria [(2)]. Streptococcus pheumoniae or pneumococcus is a gram positive, alpha-haemolytic, facultative anaerobic member of the genus streptococcus[(3)]. They are gram positive occurring in pairs or in chains [(4)] These bacteria can cause many types of illness including: pneumoniae (infection of the lungs), ear infections, sinus infections, meningitis (infection of the covering around the brain and spinal cord) and bacteraemia (blood stream infection). Pneumococcus bacteria are spread through coughing, sneezing, and close contact with an infected person. Bacteria are developing different means to overcome the activity of antibacterial. The production of modifying enzymes and the alteration of binding proteins in the bacterial membrane are the important mechanism by which resistance is expressed in bacteria. Reports of Pneumococci with increased resistance to penicillin have appeared during the last 10 years (Morbidity and \mortality Weekly Report, 1977, vol 26, no.35;4-6, 8-10,12) Most of the non-Blactamase resistance among gram positive bacteria is mediated by change in binding proteins [(5)]. There are differences in the antibiogram of the same organism reported throughout the world, the need for current in vitro susceptibility data is therefore apparent. These differences may be caused by inappropriate self medication, over or under prescription all using oral agents. It is important to monitor these changes to provide local information for therapy. The E. Test[2] method has been described as a highly accurate method for detection of resistance to B-Lactam drugs [(6).]

2. Materials and Methods

105 clinical strains were recovered from different hospital in thanjavur, tamilnadu state at India from **January 2014 to Jun 2014.** All the isolates of streptococcus pneumonia were obtained from fresh material using routine methods and were identified using conventional methods by optochin susceptibility. These Strains were reidentified by conventional test after arriving biotechnology laboratory at thanjavur. For testing, isolates were removed from storage, streaked onto Mueller Hinton Agar supplemented 5% sheep blood. The direct colony suspension method was recommended and applied. The colonies harvested from 16-18hr. blood agar plate emulsified in normal saline and the turbidity adjusted to match the 0.5% McFarland standard as described by Jorgensen et al3[(7)]. Within 15 minutes the surface of agar (about 4 cm in thickness) was inoculated with the aid of a swab and allowed to dry for 15 minutes. All the five antibiotics **E-test** [(8)] strips (5cm plastic strip to which an antibiotic gradient has been incorporated) were applied using the template provided. An Oxacillin disc was also applied to the agar surface at the periphery between two of the strips. The plates were incubated at **35^{0}C within 15minutes of application.** After 18 hours of incubation the **MIC reading value** written on the **E-test strip** was noted by an elliptical zone of inhibition produced and **MIC value** read from the strip where the inhibition zone intersects the strip.

3. Results

Out of 105 isolates of Streptococcus pneumonia 69 (65.71%) were sputum/respiratory in origin.18 (17.14%) Ear swabs, 3 (2.85%) Throat swabs, 6 (5.71%) Eye swabs, 3 (3.15%) pus swabs, 2 (1.90%) CSF, 4(3.80%) All were associated with clinical infections, specimen and sex wise

given below (Table 1). The MIC of different antibiotic was taken according to the NCCLS criteria (Table 2)

Table 1: Specimen type & Sex wise distribution of *Streptococcus Pneumoniae* isolates

Sex	*Specimen type*						
	Sputum	*Respiratory*	*Ear swabs*	*Throat swabs*	*Eye swabs*	*Pus swabs*	*CSF*
Male	40	8	0	5	3	2	3
Female	29	10	3	1	0	0	1
Total	69	18	3	6	3	2	4

Table 2: MIC reading for *Streptococcus Pneumoniae*

Antibiotics	*Susceptible*	*Intermediate*	*Resistant*
Amoxycillin–Clavulanate	<0.5/0.25	2/1	>4/2
Ampicillin	<0.25	0.5	>1
Cefurozime	<0.5	1	>2
Ceftriaxome	<0.5	1	>2
Clarithromycin	<0.25	0.5	>1
Ciprofloxacin	<0.5	1	>2
Erythromycin	<0.5	1	>2
Penicillin	<0.06	0.1-1	>2

Strains that have MIC <0.06 µg/ml are designated as susceptible to penicillin. Strains that have MIC between 0.12-1.0mg/ml are considered relatively resistant to penicillin or intermediate. Those with MIC >1.0 µg/ml are designated as resistant.

Table 3: The percentage of susceptibility for different antibiotics was as follows (

Antibiotics	*Susceptible*		*Intermediate*		*Resistant*	
	n	*%*	*n*	*%*	*n*	*%*
Amoxycillin–Clavulanate	105	100	0	0	0	0
Ampicillin	23	21.9	0	0	82	78
Cefuroxime	96	91.4	0	0	9	8.5
Ceftriaxome	105	100	0	0	0	0
Clarithromycin	93	88.5	5	4.7	7	6.6
Ciprofloxacin	89	84.7	9	8.5	7	6.6
Erythromycin	58	55.2	12	11.4	35	33.3
Penicillin	85	80.9	20	19	0	0

All the strains showed (n=105) 100% susceptibility (MIC < 0.5-0.25) to Amoxycillin-Clavunate and Ceftriaxone (MIC <0.5). For Cefuroxime strains showed (n=96) 91.4% susceptibility i e., (MIC <0.5) and (n=9) 8.5% were in resistant (MIC-2). Strains of *Streptococcus Pneumoniae* showing susceptibility (MIC <0.25) to Clarithromycin (n=93) was 88.5% and (n =5) 4.7% were in intermediate category while (n=7) 6.6% showed definite resistance (MIC>1) to Clarithromycin. Ampicillin strains showed (n=23) 21.9% susceptibility (MIC <0.25) and (n=82) 78% showed definite resistance and no intermediate. Ciprofloxacin (n=89) was 84.7% and (n =9) 8.5% were in intermediate category while (n=7) 6.6% showed definite resistance (MIC>2) to Ciprofloxacin. Erythromycin (n=58)55.2% and (n=12) 11.4% were intermediate category while (n=35) 33.3% definite resistance (MIC>2) to Erythromycin. Penicillin susceptibility (n=85) was 80.9%) and (n=12)11.4%) of the strains were intermediate category. No strain was found to be resistant to penicillin in this study.

4. Discussion

It is generally accepted that penicillin-resistance is associated with a limited number of Streptococcus pneumoniae serotypes, against which can be protected by use of conjugated vaccines [8, 9]. Penicillin has been the drug of choice for pneumococcal infection for many years and susceptibility testing was not required. The emergence of strains those are resistant to multiple antibiotics including Penicillin has been his conception [10]**.** The disc diffusion test does not differentiate strains that are relatively resistant (MIC of 0.12-1.0µg/ml) from strains that are resistant (MIC >1.0 µg/ml). The E-test strips have antibiotic gradient incorporated is a sensitive method for the detection of resistant strains [11]. The NCCLS recommends a 1 mg disc of Oxacillin for detection of penicillin resistance and strains having a zone as Oxacillin as Oxacillin is a better predictor of Penicillin resistance. All penicillin resistant isolates in this study were also resistant to one or more of the commonly used antimicrobials which is consistent to the findings of researches conducted in Malaysia, Ethiopia, and USA [12, 13]**.** A study conducted in Israel showed that there was an increasing rate of ceftriaxone resistance which rose from 0% to 10% of intermediate resistance in 1998-1999 [14]. The prevalence of pencillin resistant pneumococci is being reported recently [15], there have been reports of an increase in the percentage of DRSP (Drug Resistant Strept. Pneumoniae) strains in the Far East. Saudi Arabia. Thailand and Turkey. In a study in Denver [16], 1.1% showed complete resistance (MIC >2.0 jig/mi) to penicillin [17]**.** In these studies 105 strains of Streptococcus pneumoniae isolated from different sites showed no drug resistance to Amoxycillin Clavunate and Penicillin. Intermediate sensitivity or relative resistance to Clarithromycin 4.7%, Ciprofloxacin 8.5%, Erythromycin 11.4% and Penicillin 19% respectively. Definite resistance to Ampicillin 78%, Cefuroxime 8.5%, Clarithromycin 6.6%, Ciprofloxacin 6.6% and Erythromycin 33.3%. The percentage of strains depicting intermediate sensitivity to penicillin was 19% whether some of these strains will develop definite resistance or not is a matter of time. Definite resistance to penicillin was not found in our study.

5. Conclusion

Drug- resistant Streptococcus Pneumoniae continues to increase, causing significant morbidity and mortality. The three major factors that have led to development of drug-resistance include: inappropriate use of antibiotics, prolonged dosage regiments, under dosing**.** Incidence of *Streptococcus pneumoniae* infection is increasing worldwide affecting the children and aged adult population which may lead to severe invasive infection by dissemination to other organs of the body if not treated adequately. The indiscriminate and inadvertent use of antibiotics has led to the emergence of multidrug resistance among commonly used antibiotics. Our investigation aims to guide medical officer on appropriate use of antibiotics. This aim is not only to reduce the morbidity and mortality in the patients but also to control the emergence and spread of resistance among *Streptococcus pneumoniae*. Continuous surveillance of the use of antibiotics helps in preserving the effectiveness of antibiotics. The results from our study strongly emphasize

the need for continuous epidemiological monitoring of antibiotic resistant.

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