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## Antibiogram Sensitivity Pattern of *Streptococcus pyogenes* and *Streptococcus pneumoniae* Isolated from Patients with Sore Throat and Pneumonia Infections

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**Abstract:** Strains of *Streptococcus pyogenes* and *Streptococcus pneumoniae*, isolated from clinical samples (125 throat swabs and 100 sputum) from patients suffering from sore throat and pneumonia infections, were subjected to a series of culture sensitivity tests against known antibiotics to determine their antibiogram patterns. Of the *S. pyogenes* isolates, all were found to be sensitive to penicillin and ceftriaxone, whereas some of the strains showed complete resistance against cotrimoxazole (88.9%), oxacillin (22.2%), cephadrine (22.2%) and erythromycin (11.1%). As for the *S. pneumoniae* strains, all the isolates in this study were sensitive to vancomycin and cephadrine, whereas 80% showed complete resistance to kanamycin, 60% to gentamycin, 20% to cotrimoxazole, 20% to tetracycline and 10% to penicillin. Strains of *S. pneumoniae* (20%) displayed intermediate resistance to erythromycin. Inadequate diagnostic procedures, un-supervised, improper use of antibiotics and easy access to prescription drugs may contribute to the rise of resistant strains. To combat such trends approved strategies must encompass legislative enforcement through strict enforcement of the laws related to sales of prescription drugs, involvement of clinicians, pharmacies and civil society pressure groups to ensure the rational and correct use of prescription drugs.

**Key words:** Antibiotic resistant patterns, *Streptococcus pyogenes*, *Streptococcus pneumoniae*

### INTRODUCTION

The development of antimicrobial resistance has almost invariably accompanied the therapeutic use of antimicrobial agents. Newer antimicrobials have succeeded partly but not entirely in overcoming the problem of resistance. Antimicrobial resistance in gram-positive cocci has achieved its greatest prominence in past 15 years among which macrolide resistance in gram-positive cocci and penicillin resistance in pneumococci were of great importance<sup>[1]</sup>.

Respiratory tract is usually involved in general and localized infections involving the mouth, oropharynx, nose, nasopharynx, larynx, trachea and lungs<sup>[2]</sup>. Upper respiratory infections are one of the most common health hazards that primary health care physicians have to deal every day<sup>[3]</sup>. Like wise lower respiratory infections are the major cause of morbidity and mortality reported from all parts of the world. These infections are caused by wide varieties of bacteria and viruses specially *Streptococcus* sp. among which *Streptococcus pyogenes* and *Streptococcus pneumoniae* are major pathogens. In United States, each year about 10 million cases of septic

sore throat, due to Group A *Streptococci* and 500,000 cases of pneumonia, due to *S. pneumoniae*, have been reported<sup>[4]</sup>. In Pakistan, about 250,000 child deaths are attributed to acute respiratory infections of which pneumonia accounts for 28.5%<sup>[5]</sup>. There has been a significant increase in streptococcal diseases in developing countries over last 30 to 40 years. This change may be due to rapid industrialization, which brought about a shift in population from rural areas to crowded urban slums, an environment favoring the spread of streptococcal infections<sup>[6]</sup>.

Diagnosis of streptococcal infection is based on both clinical and laboratory findings, however, several rapid tests are available<sup>[7]</sup>. Treatment is based on antibiotic therapy with the choice therapeutic agent being penicillin and erythromycin<sup>[8]</sup>.

Although penicillin has extensively been used over the past 50 years, fortunately, Group A *Streptococci* remain susceptible to this antibiotic<sup>[9,10]</sup>. However, recently erythromycin resistant Group A *Streptococci*<sup>[10-17]</sup> and the escalating rate of the emergence of penicillin resistant strains of *Streptococcus pneumoniae* have been reported<sup>[18-26]</sup>. The extensive use of large number of

antimicrobial agents has fueled the crises of antibiotic resistance in the era of modern chemotherapy<sup>[1]</sup>.

In Pakistan, researchers have conducted studies to check the efficacy of different antimicrobial agents against Group A *Streptococcus* sp. and have reported only moderate resistance to penicillin and no resistance to erythromycin<sup>[9,27-31]</sup>, whereas in other Asian countries and Gulf States, high resistance to penicillin and other antibiotics have been documented and reported<sup>[32,33]</sup>.

In Rawalpindi/Islamabad, despite the fact that numerous studies have been conducted, few have been formally documented, most are published in the form of reports only, with limited to no access to the public at large. Keeping in view the importance of these two microbes causing health hazards in human population, this study was designed to determine the antibiotic efficacy against *S. pyogenes* and *S. pneumoniae* isolated from patients with Acute Respiratory Tract Infections (ARIs)

#### MATERIALS AND METHODS

For this study a total of 225 clinical samples of throat, nasopharyngeal swabs and sputum were collected, over a one-year period, from suspected patients of sore throat and pneumonia visiting at National Institute of Health and Pakistan Institute of Medical Sciences, Islamabad, from August 2001 till July 2002.

After collection, these samples were processed for bacteriological isolation and characterization at the bacteriology laboratory of the National Institute of Health.

Clinical samples were inoculated on blood, chocolate and MacConkey agar. Inoculated plates were incubated at 37°C for 24 h. Colonies of bacterial pathogens were identified on the basis of their morphological and color pattern characteristics.

After identification the samples were subjected to different biochemical tests for confirmation viz., Catalase test, Bacitracin sensitivity test, Optochin sensitivity test, Bile solubility test, Coagulase test, Satellitism test, Oxidase test, Indole test, Citrate test, Urease test, Motility test, Triple sugar iron test and Germ tube test.

**Antibiotic sensitivity test:** The identified isolates of *S. pyogenes* and *S. pneumoniae* were then subjected to the antibiotic sensitivity test, using the Disc Diffusion Technique<sup>[34]</sup>. The microorganisms were mixed in peptone water and their turbidity matched with standard solution (Barium chloride solution). The organisms were then streaked, using sterile swabs, to form a lawn culture on blood agar. Afterwards, commercially available discs were

placed on plates firmly by means of sterile forceps, under aseptic conditions, plates incubated for 24 h at 37°C. After incubation diameter of zone of inhibition were measured and the degree of sensitivity/resistance were determined with the help of list of break points of antibiotics provided by the manufacturer. The antibiotics used were penicillin, ceftriaxone, erythromycin, cephradine, oxacillin, cotrimoxazole, vancomycin, tetracycline, gentamycin and kanamycin.

#### RESULTS AND DISCUSSION

Although streptococcal disease is a pediatric problem yet it can be a significant, even disabling disease, in adults when favored by adverse environmental and hygienic conditions<sup>[34-36]</sup>.

The results of this study revealed that all the isolates of *S. pyogenes* were susceptible to penicillin (Table 1), reinforcing the fact that strains in Pakistan still remain susceptible to penicillin, despite the presence of resistance in other species of *Streptococci*. Similar results were obtained in other studies, both in Pakistan<sup>[9,27-31]</sup> and in worldwide surveys<sup>[15,16,37]</sup>. Although this antibiotic has been used extensively over past 50 years, still Group A *Streptococci* are susceptible to this antibiotic. Among the most likely reasons for this remarkable state of continued susceptibility may be that beta lactamase may not be expressed or may be toxic to organisms and/or that low affinity penicillin binding protein either are not expressed or render organisms nonviable<sup>[38]</sup>.

On the other hand, erythromycin resistance amongst the *S. pyogenes* strains was observed in 11.1% of the cases reported in this study (Table 1), which shows a remarkable increase from a previous study carried out in Pakistan, where 4% isolates were reported to be resistant to erythromycin<sup>[29]</sup>. These findings are, however, in accordance with other studies carried out in Europe and the United States<sup>[14,15,17,37]</sup>. Erythromycin has traditionally been regarded as an effective and very safe treatment for sore throat infections and has been the drug of choice for patients who are allergic to penicillin, but this increased clinical use has been associated with increased resistance of *S. pyogenes* to this antibiotic<sup>[37]</sup>.

The results of this study under discussion also revealed that ceftriaxone was also an effective therapeutic, agent against *S. pyogenes*, as the isolates were found sensitive to this antibiotic. Researchers have also documented mixed findings for ceftriaxone, with some reporting on its effectiveness<sup>[10]</sup>, while others have found *S. pyogenes* resistant to this particular antimicrobial agent<sup>[15,29]</sup>.

A majority of the isolates of *S. pyogenes* were also found to be susceptible to cephradine and oxacillin

Table 1: Antibiogram pattern of *Streptococcus pyogenes* isolated from patients of sore throat

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin	100.0	-	-
Ceftriaxone	100.0	-	-
Erythromycin	88.9	-	11.1
Cephadrine	77.8	-	22.2
Oxacillin	66.7	11.1	22.2
Cotrimoxazole	11.1	-	88.9

(77.8 and 66.7%), however, some resistant strains were also identified, while a larger majority of the *Streptococcus pyogenes* isolates were found to be resistant to cotrimoxazole (88.9%) (Table 1). Researchers have reported increased resistance of *S. pyogenes* to cotrimoxazole<sup>[29]</sup>, yet this antibiotic remains the first choice for treatment of children with respiratory infections in developing countries because of its low cost, which may have contributed to the increased resistance to this particular antibiotic<sup>[29]</sup>.

The antibiogram patterns of *S. pneumoniae* in this study revealed interesting results particularly in terms of the resistance to penicillin. The study showed that 10% of the *S. pneumoniae* strains isolated from patients with pneumonia were resistant to penicillin (Table 2). This figure is higher than those previously reported for the local isolates<sup>[27,31]</sup>, who reported only intermediate resistant to penicillin, but follows the logical assumption that complete resistance develops gradually and over a period of time, subsequent to the development of intermediate resistance. However results of this study are in agreement with the surveys conducted in surrounding Asian countries, where researchers have reported penicillin resistance ranging from 3.8 to 9.8%<sup>[32]</sup>. Similar findings have also been reported in European studies, where penicillin resistance was seen in 6.6%<sup>[39]</sup> and 9.7%<sup>[24]</sup> of the *S. pneumoniae* isolated strains. Penicillin has been used as first choice antibiotic in the treatment of these infections, which may have lead to development of resistant strains and may be attributed to the molecular alteration of penicillin binding proteins ( $\beta$ -lactamases), present on peptidoglycan layers of the gram-positive bacterial cell wall<sup>[40]</sup>.

All isolates of *S. pneumoniae* were found to be sensitive to vancomycin and cephradine in this study, which is in conformity with previous studies<sup>[23,27,32]</sup>, however, researchers have reported the development of resistance strains against cephradine<sup>[29]</sup>.

In case of erythromycin no *S. pneumoniae* isolate showed complete resistance, however, 20% did display intermediate resistance, which is first step to the development of complete resistance over a period of time. Once again the use of this therapeutic agent must be monitored and controlled, much like that of penicillin, to

Table 2: Antibiogram pattern of *Streptococcus pneumoniae* isolated from patients of pneumonia

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Vancomycin	100	-	-
Cephadrine	100	-	-
Penicillin	90	-	10
Erythromycin	80	20	-
Tetracycline	80	-	20
Cotrimoxazole	40	-	60
Gentamycin	20	-	80
Kanamycin	10	10	80

ensure that the development of resistance is slow and the resistant strains identified through culture sensitive tests to enable clinicians to prescribe the correct therapeutic drug. This may help to avoid situations like that of tetracycline, where this study revealed that 20% of the *Streptococcus pneumoniae* isolates showed resistance to this drug. These findings are similar to those reported by other researchers, where resistance to tetracycline has ranged from 10.2%<sup>[23]</sup> to 32.1%<sup>[39,41]</sup>.

Cotrimoxazole, gentamycin and kanamycin were shown to be the least effective antimicrobials against *S. pneumoniae*, as the findings of this study, under discussion, revealed that 60 and 80% of the isolated strains were resistant to cotrimoxazole, gentamycin and kanamycin, respectively (Table 2). These findings are in accordance with other studies carried out in Pakistan<sup>[27,29]</sup> as well as in Saudi Arabia<sup>[42]</sup>.

Despite the fact that there may be some variations in drug resistance between the two streptococcal pathogens under discussion, there are some important similarities as well, that have to be addressed by clinicians as well as pharmaceutical companies and the civil society organizations who have an interest in public health, particularly child health. The fact that 10% of the *S. pneumoniae* isolated in this study were resistant to penicillin is an eye-opener. Furthermore, both *S. pneumoniae* and *Streptococcus pyogenes* seem to be on the verge of developing resistance to the cheaper antimicrobials. The cumulative effect of these aspects would lead to the loss of efficacy of the cheap-easily available drugs, which would be catastrophic in terms of provision of primary health care and medicines, particularly in countries like Pakistan where poverty is rampant and public awareness is minimal.

Antibiotics are cheaply available at medical stores and may be bought without prescription, or supervision, which leads to their inappropriate use. This over and misuse has lead to death of sensitive strains leaving resistant strains to survive to infect new hosts. Laboratory facilities are also unavailable in many areas to isolate pathogen and perform sensitivity tests thus leading to misuse of antibiotics. Another reason of

resistance might be inadequate control procedures in hospitals resulting in spread of infectious diseases and resistant strains.

In the light of present study it may be concluded that there are various reasons for the rise in resistant strains in Pakistan, from inadequate diagnosis procedures, un-supervised and improper use of antibiotics. However, what is very clear that the plan of action to combat such trends must be holistic, encompassing legislative enforcement through strict implementation of the laws pertaining to prescription drugs, coupled with the active involvement of clinicians, pharmaceuticals and civil society pressure groups to ensure the rational use of drugs. Furthermore, it is always more economical to promote prevention that seek the cure, therefore, factors that may spread the infection, or cause it in the first place have to be addressed simultaneously. Such factors include, densely populated urban centers, lack of adequate primary health care, poverty, tobacco smoking-especially among the youth-the use of narcotics, inter alia contribute, either directly, or indirectly to the spread of infection and development of resistant strains.

#### SUGGESTIONS AND RECOMMENDATIONS

With the easy access to antibiotics in the market in Pakistan, purchased without proof of prescription and the steady rise in population accompanied by a demand for urbanization, which more often than not results in the gradual deterioration of the environment, it has become imperative to know detailed knowledge about the current status of streptococcal resistance among inhabitants of Rawalpindi/Islamabad. The results of this study shall be of immense importance to the medical profession in determining the level of antimicrobial resistance amongst the various pathogens associated with ARIs and the best possible treatment to ensure minimal chances of development of resistance strains. The study will also generate general awareness among common people about the ill effects of respiratory infections and about the excessive and indiscriminate use of antibiotics, which actually leads to selection of resistant bacterial strains.

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