Antibiotic Sensitivity of *Streptococcus Pneumoniae* that Isolated from Different Pneumococcal Infections

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Absrtact

During the period from June of 2018 to February 2019 (150) isolates of *Streptococcus pneumoniae* were isolated from 600 patients with clinical symptoms of Lower respiratory tract infections (LRTI) (pneumonia), otitis media and meningitis obtained from Baqubah Hospitals. The results showed that not only *S. pneumoniae* causes pneumonia and it can causes diseases other than pneumonia such as otitis media and meningitis but with less frequency. *S. pneumoniae* showed different susceptibilities towards antibiotics used in this study. The total susceptibility was (65.8%) and the total resistance was (34.2%). The highest pneumococcal susceptibility was showed to the cell wall inhibitors (44.4%) followed by protein synthesis inhibitors (28%) and quinolones (17.3%), and the lowest susceptibility was to folate antagonists with 0%. The highest rates of susceptibility was to penicillin (100%), chloramphenicol (86%), vancomycin (80%) and moderate rates of susceptibility to levofloxacin (90) 60%, linezolid (42.7) %, cefotaxime (40%), ofloxacin (40%) whereas there was a relatively lower susceptibility rate towards other antibiotics such as ampicillin, imipenem, amoxicillin, trimethoprim/sulfamethoxazole. Therefore, it should be avoided in the treatment in addition to tazobactam, amikacin, gentamicin, which had lowest rates susceptibility against *S. pneumoniae.* As a result, it required more research to identify new antibiotic or vaccine to reduce the risk of pneumococcal infection.

Keywords: *Streptococcus pneumoniae**,** pneumonia, otitis media, antibiotic sensitivity.*

Introduction

Streptococcus pneumoniae is an important respiratory pathogen responsible for high rate of morbidity and mortality around the world. This bacterium causes many infections, which lead to die especially in children according to the World Health Organization [(1)].

The pneumococcus causes many different types of infections such as otitis media, sinusitis and conjunctivitis, and pneumonia. *S. pneumoniae* can invade the bloodstream to cause sepsis or septicemia and spread to other human sites to cause meningitis, collectively called invasive pneumococcal disease (IPD) [(2)], which most infect children less than 2 years old, adults over 65 years old and immunocompromised individuals [(3)]. The human nasopharynx is the only reservoir of pneumococci which spread by droplet between individuals [(3)]. This bacterium produces different virulence factors that assist its spread from the nasopharynx to other human sites such as the lungs, blood and brain [(4).] Antibiotics are the drug of choice for pneumococcal infection. In the last twenty years, the antibiotic resistance among pneumococci has become a big problem and is affected by the types of antibiotic use and population density [(3)]. However, vaccination can give protection against most of pneumococcal serotypes. On the other hand, there are different problems with current vaccination strategies, and horizontal gene transfer, which can reduce the efficacy of vaccines and antibiotics [(5)].

Antibiotics are one class of antimicrobials, which produced through chemically derived substances from different organisms, such as bacteria and fungi [(6)]. The antibiotic treatment relays on the infection human site and the age of patient [(7)]. Effectiveness and allergic of potential antibiotic will detect a sole or combined administration of such prophylactic drug agents. However, pneumococcal diseases still connected with significant morbidity and mortality around the world. A growing proportion of *S. pneumoniae* in the aetiology of

morbidity and mortality is highly correlated with a high incidence of individual (especially to penicillin) and multiple antibiotic resistance. Antimicrobial resistance of *S. pneumoniae* is not only local but also a global problem. Resistance of pneumococci leads to changes in the clinical presentation of diseases which in turn leads to more difficult diagnosis and treatment. The resistance may result from several different mechanisms, such as the inactivation of the antibiotic by bacterial enzymes, low permeability to antibiotics bacteria, changes in the target protein, leading to reduced binding of the antibiotic, or overproduction of the target protein structure and by passing the metabolic pathways of the target [8]. Therefore, the aim of this study was to detect the prevalence of sensitivity and resistance of *S. pneumoniae* to antibiotics that act on the cell wall synthesis, protein synthesis, folate antagonists and quinolones, in order to achieve proper treatment of pneumococcal disease and reduce morbidity and mortality.

Materials and Method

Study design

This study is of prospective-retrospective and analytical nature was conducted in the Bacteriology Unit of Baqubah teaching hospital. invasive and non-invasive *pneumococcal* samples were collected from adult patient(>16 years) suspected to have pneumonia and otitis media and meningitis, a total of 600 of samples were collected between June of 2018 to February 2019 From the hospital , non-invasive isolates such as sputum and ear swab.

Bacterial Strains and Growth Conditions

All the infectious samples were cultured on blood agar base (BAB) (Oxoid, UK) supplemented with 5% (v/v) defibrinated horse blood, Chocolate agar, and MacConkey agar (Oxoid, UK) and incubated at 37℃ for 24 hr. Blood agar plates and Chocolate agar Plates were incubated in the presence of 5-10% CO2 at 37°C for 34 hr. Sputum samples were homogenized and mixed with an equal volume of normal saline by a vortex. Then, it was take 0.1 ml of homogenized sputum samples and cultured on the plates. The cultures were made for all the infectious samples [9].

Identification of Bacteria

The diagnosis of *S. pneumoniae* was done according to colony morphology, sensitivity to optochin test and biochemical reactions as described in [10]. A blood agar plate was cultured with a pure isolate colony of the bacteria that need to be tested. Then, optochin disc was placed in the center of the plate and incubated at 37°C with CO_2 for 24 hr. The showing of a zone of inhibition growth around the disc more than 14 mm was considered the bacteria as sensitive [11].

Antibiotic Susceptibility Testing

Disk diffusion method was used according to the instructions of the Clinical Laboratory Standards Institute [12]. A pure isolated colonies of identified bacteria was used for culture on 5 ml of tryptic soy broth and then incubated for 2 hr to produce a turbidity that was the same with the turbidity of 0.5 McFarland standard tube. Inoculum from the standardized bacterial suspention was cultured by a sterile swab on a Muller–Hinton agar plate supplemented with 5% horse blood. The antibiotic discs were put on the surface of this plate and incubated at 37°C for 24 hrs. The inhibition zones around the antibiotic discs were measured and compared with standard zones to detect the sensitivity of the bacteria to each antibiotic [12].

Statistical analysis

Graphpad Prism software (Graphpad, California, USA) was used for statistical analysis**.** The results were expressed as means ± standard error of the mean (SEM), significance was defined as (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$).

Results

The samples for pneumococcal isolated were obtained from sputum (300) and (100) from ear swab, and the remaining was from CSF (100) and blood (100). As can be seen in Figure 1, most of *S. pneumoniae* was found in sputum (100) and ear swab (50) while a limited number of the microorganism isolated from CSF and blood (<25).

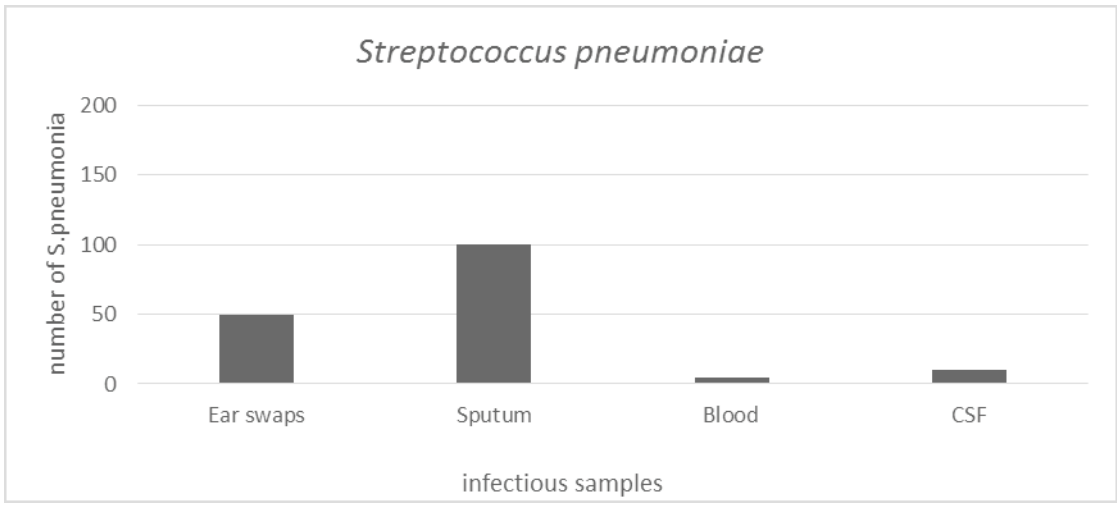

Figure 1. Number of *S. pneumoniae* that isolated from each sample.

In Figure 2 shows that of 300 sputum samples, 100 samples of sputum culture appeared *S. pneumoniae* and 150 of them produced no growth, the remaining results of sputum culture was another microorganisms such as *K. pneumonia*, *P. aerugenosa*, and *Acinetobacter*.

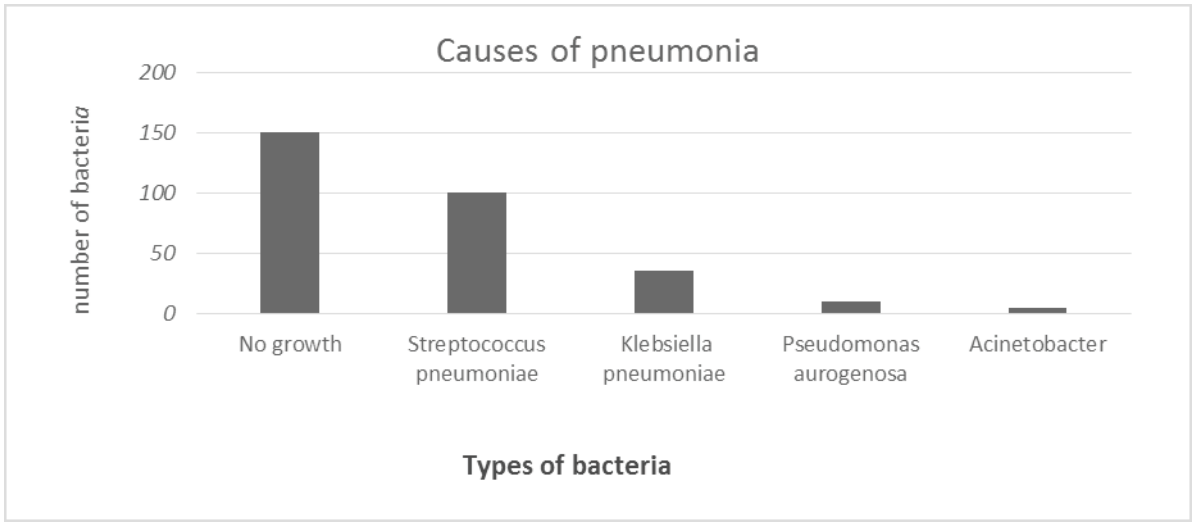

Figure 2. Types of bacteria that resulted from sputum culture.

In 65.8% of *Streptococcus pneumoniae* that isolated appeared sensitivity against antibiotics, while they were shown resistance in 34.2%.

The representation of the susceptibility of cell wall inhibitors as penicillin 14.7% ,vancomycin 11.8% ,cefotaxime 5.9% ,meropenem 4% , piperacillin 3% ,amoxi-clav 2% , amoxicillin 1.4% ,cefepime 1.5% , ceftriaxone 1.2% ,ampicillin 0.5% ,imipenem 0.2% ,tazobactam 0.2%, While susceptibility of protein synthesis inhibitors as chloramphenicol 12.8% , linezolid 6.4% ,tetracycline 3% ,erythromycin 2% ,azithromycin 1.6% , doxycycline 1.5% ,amikacin 0.8% ,gentamycin 0.6. Susceptibility of quinolones as levofloxacin 8.9% ,ofloxacin 5.9% , ciprofloxacin 1.6% , gatifloxacin 0.8% , trimethoprim/ sulfamethoxazole 0.2%.

The results of this study showed the susceptibility of each group antibiotics according to the number of susceptible *S. pneumonia*e (150). We will find that the susceptibility of cell wall inhibitors : penicillin (150) 100% ,vancomycin (120) 80% ,cefotaxime (60) 40% , meropenem (42) 28% , piperacillin (26) 17.3% , amoxi-clav (20) 13.3% , cefepime (14) 9.3% , ceftriaxone (12) 8% , amoxicillin (10) 6.7% ,ampicillin (4) 2.7% ,imipenem (2) 1.3% , tazobactam (2) 1.3% Figure 3.

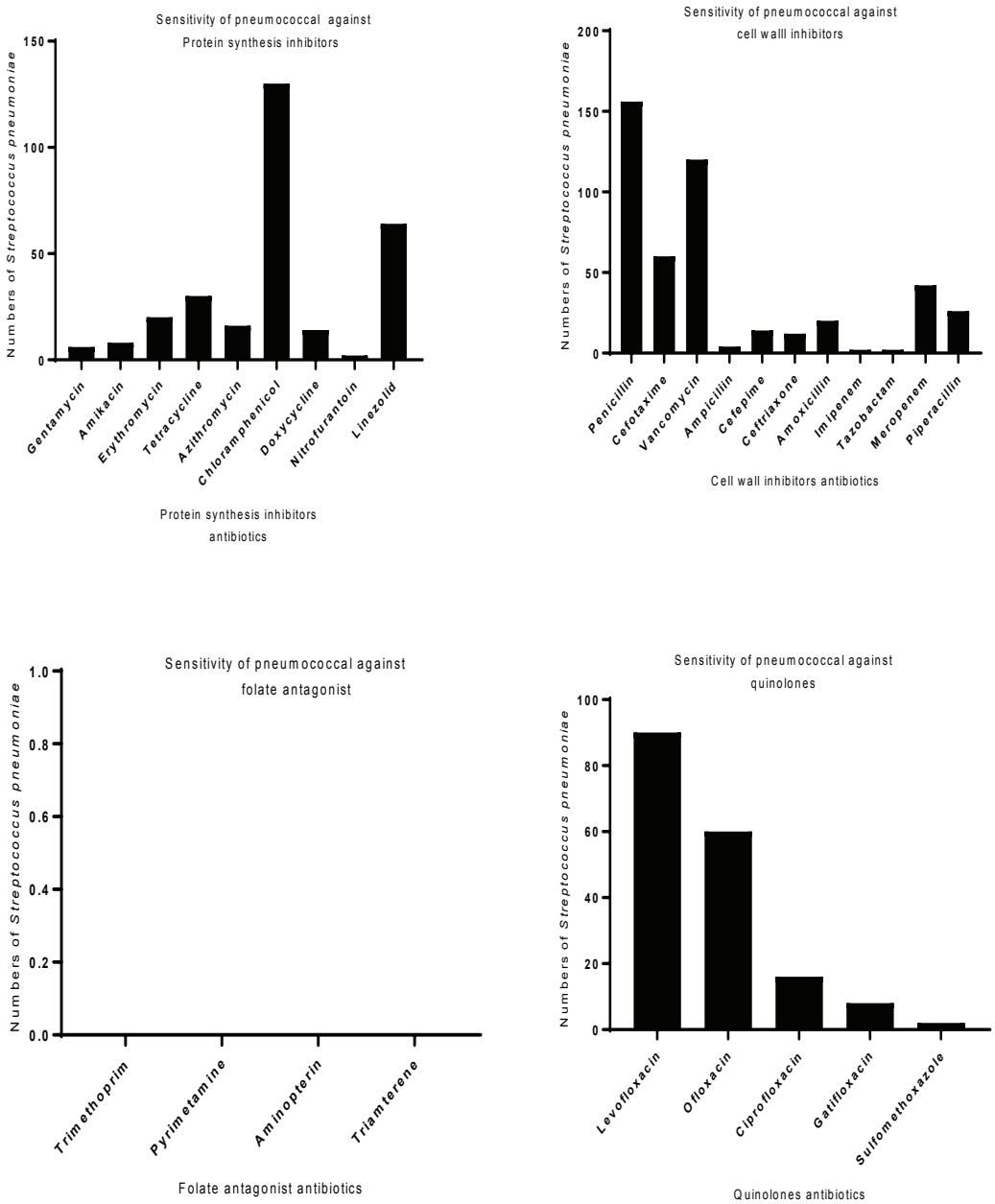

Figure 3. Antibiotic sensitivity of *S. pneumoniae* according to the number of bacterial sensitivity for each drug.

Protein synthesis inhibitors were shown sensitivity as chloramphenicol (130) 86%, tetracycline (30) 20%, erythromycin (20) 13.3%, azithromycin (16) 10.7%, doxycycline (14) 9.3%, amikacin (8) 5.3%, gentamycin (6) 4%, nitrofurantoin 1.3% Figure 3.

Quinolones were appeared sensitivity as levofloxacin (90) 60%, ofloxacin (60) 40%, ciprofloxacin (16) 10.7%, gatifloxacin (8) 5.3%, trimethoprim/sulfamethoxazole (2) 1.3% Figure 4, However, folate antagonists were shown full resistance against pneumococcus and the sensitivity was 0% Figure 3.

Discussion

Annually, invasive pneumococcal diseases (IPD)

cause an estimated 1.6 million deaths, including 1 million children less than 5 years old [3]. The available population-based surveillance shows significant differences in the incidence and mortality rates of IPD worldwide between industrialized and developing countries. Pneumonia cause death more in children under five years of old than other infection around the world. It was reported that the estimated 9 million child deaths were through pneumonia. The results of this study shows that most of *S. pneumoniae* infection presented as pneumonia and otitis media less commonly with meningitis (Figure 1). In addition, it can be seen that not only *S. pneumoniae* causes pneumonia, it can caused by other microorganisms such as *K. pneumoniae*, *S. aerugenosa*, and *Acinetobacter* (Figure 2). Not very low representation of resistance (34.2%) that was observed in this study may be due to inadequate prescription and consumption of medicines, predisposed specific serotypes of *S. pneumoniae* in the development of resistance to antibiotics and their different geographical distribution.

For many years penicillin has been the mainstay of therapy for pneumococcal diseases. Clinical resistance to penicillin was first reported in 1960's. Since this early reported, penicillin resistance has been encountered with increasing frequency in strains of Streptococcus pneumoniae from around the world. Our results showed that penicillin sensitivity is 100%, comparing our results with those of 2013-2014 at Iraq al-najaf [13] which was 18.9%, this shows a huge increase in penicillin sensitivity from 2013 to 2019. In addition, it was observed variations in the prevalence of susceptibility of *S. pneumoniae* to certain drugs. There has been a significant decline in the susceptibility of Amoxicillin, Amoxicillin-Clavulanic acid, Ceftriaxone, Meropenem, Imipenem, Vancomycin, Azithromycin, Tetracycline, Ciprofloxacin, Ofloxacin, trimethoprim/sulfamethoxazole, Linezolid, Gentamicin, This may be a result of the existence of barriers to the use of penicillin in the treatment of pneumococcal disease, due to fear of the possible dramatic allergic reactions including anaphylactic shock and greater recourse to other drugs in the treatment of pneumococcal infection.

MDR were identified as non-susceptible (NS) to at least 2 types of antibiotics. It was showed that *S. pneumoniae* have appeared decreasing in sensitivity to penicillin and macrolides but none against fluoroquinolones [14]. It was reported that 97.8% of *S. pneumoniae* were sensitive to erythromycin and chloramphenicol and this percentage is higher than our results in which 86% of chloramphenicol and very low sensitivity of erythromycin 13.3% [15]. The new fluoroquinolones are widely performed to treat respiratory tract infections. It was showed that a high rate 96.5% of ciprofloxacin susceptibility in *S. pneumoniae*, it was higher than rates found in a recent study. The high consumption of quinolones could explain the lower susceptibility rate [16].

The differences showed in the rates of sensitivity to ciprofloxacin and levofloxacin are within 16 samples with first-step mutations in the quinolone resistance-determining regions. These isolates (ciprofloxacin susceptible and levofloxacin resistant) may become highly resistant under selective fluoroquinolone pressure and are associated with treatment failure when quinolones are used [17].

The emergence of high-level resistance to antimicrobials is an increasing threat to global health [18], and even a small increase in antibiotic-refractory bacterial subpopulations or MIC could herald the emergence of higher-level resistance [19]. Therefore, any factor leads to rise the antibiotic resistance is essential.

The most important result of this study is the sensitivity level of Ceftriaxone (from 32.4 % to 8%), Meropenem (from 83.8% to 28%), Imipenem (from 100% to 1.3%) , cefepime (from 86.9% to 9.3%),While there is an increase in the results of erythromycin from 0% to 13.3% and chloramphenicol from 32.4% to 86% and penicillin from 18.9% to 100%. Therefore, this study showed that these antibiotics are the most common used in the Iraqi hospitals, and this is still a good antibiotic choice for the treatment of pneumococcal pneumonia, otitis media, and meningitis.

Conclusion

The lowest frequency in isolated pneumococci is recorded with imipenem 1.3%, trimethoprim/ sulfamethoxazole 1.3% and gentamicin 4%, while the higher incidence of isolated sensitive pneumococci is recorded at penicillin 100%, chloramphenicol 86% and vancomycin 80%. Defining representation of antibiotics groups in the overall susceptibility make it easy to suspect their representation in the total resistance which show therapeutic adequacy of the same. This point was of great importance, because it approximately can serve as a guide in the selection of a therapeutic agent in the case of impossibility of making susceptibility testing or therapeutic coverage waiting period. For example,

quinolones and folate antagonists have significantly lower representation in the overall susceptibility make them to have the greater representation in the overall resistance and are therapeutically inadequate. It is evident that the presence of inhibitors of the cell wall in the overall susceptibility is very high, but that also has a much significant share in the overall resistance.

The results obtained in this study indicate the existence of a great need for rational use of antibiotics and establishing adequate monitoring patterns of pneumococcal resistance. We should determine the serotypes and the spread of resistant strains. Physicians should keep local and regional patterns of resistance at the consideration during the selection of empirical therapy for diseases caused by this agent. For further control of the resistance development necessary is a multidisciplinary approach that includes the clinicians, epidemiologists, microbiologists and pharmacists.

Ethical Clearance: The project of this study was taken from the ethical committee of College of Medicine/ University of Diyala.

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Conflict of Interest: Nil

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