**Antimicrobial susceptibility of *Streptococcus pneumoniae* isolates causing LRTI in Najaf/Iraq**

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**Abstract**

During the period from February, 2013 to April 2014, 74 (12.3%) isolates of *Streptococcus pneumoniae* were isolated from 600 patients (359 males and 241 females) with clinical symptoms of Lower respiratory tract infections (LRTI) (pneumonia and COPD) obtained from Najaf/Iraq Hospitals. Patients in age group (51-60) years appeared a high percent of *S. pneumoniae* isolates (19.7%) comparing with other age group with a significant variation (P<0.05) between them. Males (54%) showed high percent of *S. pneumoniae* isolate than females (45.9%) with no significant variation (P>0.05). Smokers have been shown increased risk to LRTI than nonsmokers (P>0.05), and there was no significant variation between Urban and Rural (56.8%), (43.2%) patient. *S.**pneumoniae* showed different susceptibility towards antibiotics used in this study. The highest rate of resistance was against erythromycin (100%), azithromycin (83.8%), clindamycin (83.8%) and trimethoprim/sulfamethaxzol (81.1%) and moderately resistance to ceftriaxone (67.6%), cefotaxime (64.9%), chloramphenicol (64.9%), tetracycline (59.5%) and benzylpenicillin (45.9%) whereas relatively lower resistance appeared toward others. The results of this study showed that *S.**pneumoniae*isolates were found to be a remarkable sensitive to Vancomycin (100%) and Imipenem (100%). In the present study, sixsteen antibiotics were tested for (MIC) against 37 *S. pneumoniae* isolates by using Vitek-2 antibiotics susceptibility testing (AST) cards (41497) AST-GP74. 100% and 83.8% of *S. pneumoniae* isolates were resistant to erythroycin and SXT with MIC ≥1 mg/ml and 4/76 mg/ml of these antibiotic respectively, and moderately resistance to cefotaxime 64.9%, ceftriaxone 64.9% and chloramphenicol 64.9% with MIC 4 mg/ml for CTX and CRO each one, and MIC 8 mg/ml for C only. All isolates were showed sensitivity in 100% for each Vancomycin and Erythromycin with MIC mg/ml and ≤1 mg/ml and ≤2 mg/ml, respectively. *S. pneumoniae* isolates showed high rate of sensitivity to Ertapenem 97.3% with MIC ≤1 mg/ml, Telithromycin 89.2% with MIC ≤1, Meropenem 86.5% with MIC ≤0.25 mg/ml.

**الخلاصة:**

خلال الفترة ما بين بداية شهر شباط 2013 إلى نهاية شهر نيسان 2014عزلت 74 (12,3٪) عزلة من بكتريا *S. pneumoniae* من 600 مريض (359 ذكور و 241 إناث) ظهرت عليهم أعراض سريرية لاصابة الجهاز التنفسي السفلي (LRTI) (ذات الرئة ، مرض الانسداد الرئوي المزمن) ارتادوا الشعبة الصدرية في ثلاث مستشفيات رئيسية في محافظة النجف الاشرف. اظهرت الدراسة ان المرضى في الفئة العمرية (51-60) سنة اعطت نسبة عالية من *S. pneumoniae* (19.7٪) مقارنة مع غيرها من الفئات العمرية مع فروقات معنوية (P <0.05) بينهما. أظهر الذكور (54٪) نسبة عالية من عزلات *S. pneumoniae* من الإناث (45.9٪) مع عدم وجود فروقات معنوية (P> 0.05). وقد أظهرت المدخنين اكثر خطر للاصابة ب LRTI من غير المدخنين (P> 0.05)، وكان هناك فروقات معنوية بين مرضى المناطق الحضرية أو الريفية (56.8٪)، (43.2٪) على التوالي.     اختبار فحص الحساسية لمعظم المضادات الحيوية شائعة الاستخدام لعلاج *S. pneumoniae* باستخدام طريقة نشر القرص MIC وتم تفسير النتائج وفقا لمنطقة التثبيط الذي أدلى به CLSI, (2014). أظهرت نتائج الدراسة اختلافا في حساسية عزلات *S. pneumoniae* للمضادات الحيوية المستخدمة في هذه الدراسة. ويعتبر أعلى معدل للمقاومة مع الاريثروميسين (100٪)، أزيثروميسين (83.8٪)، الكليندامايسين (83.8٪) وميثوبريم / سلفلميثازول (81.1٪) ومتوسطة المقاومة للسيفترياكسون (67.6٪)، سيفوتاكسيم (64.9٪)، الكلورامفينيكول (64.9٪)، التتراسيكلين (59.5٪)، وبنزيل بنسلين (45.9٪) في حين هو مقاومة أقل نسبيا تجاه بقية المضادات. أظهرت نتائج هذه الدراسة حساسية عزلات  *S. pneumoniae* اللافتة للنظر لفنكمسن والامبنيم (100٪).    في هذه الدراسة، تم اختبار16 من المضادات الحيوية ل(MIC) ضد عزلات *S. pneumoniae* باستخدام تقنية Vitek-2 AST-GP74. وجد ان 100٪ و83.8٪ من عزلات *S. pneumoniae* كانت مقاومة للerythroycin وSXT مع MIC ≥1 ملغ / مل و 4/76 ملغ / مل من هذه المضادات الحيوية على التوالي، ومقاومة معتدلة إلى سيفوتاكسيم 64.9٪، 64.9٪ سيفترياكسون والكلورامفينيكول 64.9٪ مع MIC 4 ملغ / مل لCTX وCRO لكل واحد، وMIC 8 ملغ / مل للكلورامفنيكول فقط. وقد أظهرت جميع العزلات حساسية في 100٪ لكل فنكمسن والاريثروميسين حيث كان MIC ≤1 ملغ / مل و≤2 ملغ / مل على التوالي. اظهرت *S. pneumoniae* نسبة عالية من الحساسية للارتابنيم 97,3٪ مع MIC 1≤ملغ / مل، التليثرومايسين 89,2% عند MIC ≤1، لكل من مضاد الميروبنيم 86.5٪ عند MIC ≤0.25 ملغ / مل.

**Introduction**

*S. pneumoniae* is the major cause of community- acquired pneumonia in adults and serious respiratory infections in children in the United States. An estimated three to five million deaths occur annually in children under 5 years of age due to acute respiratory infections, for which *S. pneumoniae* is the most important pathogen (Obaro, 2000)*.* The incidence of pneumococcal disease and the occurrence of antibiotic resistant isolates have been positively correlated to the levels of carriage (Paul, 1997). Carriage can be influenced by age (highest in infants and decreasing with age), immune status, seasonal variation, socioeconomic factors, and other demographic features (Gray *et al.,* 1982).

 Penicillin is the antimicrobial agent of choice, and macrolides are the second most common alternative. Within the last two decades, the emergence of strains of *S. pneumoniae* that are resistant to penicillin, macrolides, and other antimicrobial agents has become a serious health care problem (Jorgensen *et al.,* 2004). The present study was designed to isolation and identification of *S. pneumoniae* from LRTI patients, by using conventional and vitek-2 techniques and studying the antibiotic susceptibility of *S. pneumoniae* to different antibiotics by disk diffusion method DDM and MICs.

**2 MATERIAL and METHODS**

**Patients and Clinical Specimens:**

 A total of 600 sputum samples were collected from out- and inpatients who suffering from lower respiratory tract infection (LRTI) (pneumonia, COPD) attending to the Chest Unit in Al-Sadder Medical City, Al-Hakeem General Hospital and Clinic Consultive Center for Chest Disease and Al-Zahra'a Hospital for Childbirth and Children in Al-Najaf province during the period from February 2013-Aprile 2014. The patients included both sexes (male and female) and the age range (1-80 years).

**Bacterial Isolates and Culture Conditions:**

Quantitative sputum cultures were made for each specimen according to sputum gram stain for pneumonia infections. Sputum specimens were homogenized with an equal volume of normal saline on a vortex mixer. Blood agar and Chocolate agar were inoculated with 0.1 ml of homogenized specimen and spread on the plates with sterile loope. Plates were incubated in (5-10) % CO2 candle jar at 37 C° for overnight (Wilson, and Martin, 1972). The plates were examined thereafter for bacterial growth and positive plates (α-haemolysis) were then a single pure isolated colony was transferred to trypticase soy agar for the preservation and to submitted the morphological evaluation by Gram staining and carry out other biochemical tests that confirmed the identification of isolates *S. pneumoniae*.

**Identification of Bacteria:**

 The identification of *S. pneumoniae* was achieved according to cellular morphology, culture characters and biochemical reactions that described in Macfaddin, (2000).:-

**Optochin Test:**

 A half plate of 5% sheep blood agar was streaked with an inoculum from a pure isolates of the organism to be tested, then an optochin disc was placed in the center of the inoculum and incubated for 24-48 hrs. at 37 C° in a candle jar, then observation of zones of growth inhibition greater than 14 mm surrounding the disc was considered as positive. This test was used to differentiate *S. pneumoniae* (sensitive) from viridans streptococci (resistance) (Collee, *et al.* 1996).

**Bile Solubility Test:**

 There are two methods for this test, tube method and plate method (Macfaddin, 2000).

**Inulin Fermentation Method:**

 The isolates were inoculated into tubes of phenol red inulin broth and incubated at 37C° for 24hrs. The tubes then examined for the presence of a yellow color indicative of acid formation from the fermentation of inulin and red indicates no inulin fermentation tube (Macfaddin, 2000).

**Identification of Bacteria by STREPTO-SYSTEM 9R Kit:**

 STREPTO-SYSTEM 9R for *S. pneumoniae* identification was used according to the recommendation of company product (Liofilchem, England).

**Vitek–2 for Identification:**

 GP identification card was used for identification of pneumococci (Guido and Pascale, 2005).

**Antibiotics Susceptibility Testing**

**1. Disk Diffusion Method**

 It was performed according to Clinical Laboratory Standard Institute (CLSI, 2014) by using a pure culture of previously identified bacterial organism. The suspension of 5 isolated colonies grown on blood agar plates to 5 ml of tryptic soy broth. This culture was then incubated for 2 hrs to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready-made 0.5 McFarland tube standard. A sterile swab was used to obtain an inoculum from the standardized culture, this inoculum was then swabbed on Muller–Hinton agar plate supplemented 5% horse blood and the antibiotic discs were placed on the surface of this medium and incubated at 37ºC for 24 hrs. Antibiotics inhibition zones were measured and zone size was compared with standard zones from the CLSI (2014), to determine the susceptibility of organism to each antibiotic.

**2. Vitek–2 for Antimicrobial Susceptibility**

 Antimicrobial susceptibility test was also carried out using the Vitek–2 system (bioMérieux, France), by AST-P79 Gram positive susceptibility cards. Antibiotics tested included a cephalosporine (cefotaxime, ceftriaxone), benzylpenicillin, aminopenicillin (amoxicillin), tetracycline, chloramphenicol as well as quinolones (levofloxacin, moxifloxacin), macrolide (erythromycin, telithromycin), linezolid, carbapeneme (ertapenem, meropenem), ofloxacin, sulfonamides (trimethoprim/sulfamethaxol) and glycopeptides (vancomycin).

**Results and Discussion**

**Prevalence of *S.* *pneumoniae* according to risk factors**

 In the current study, the prevalence of *S. pneumoniae* isolatesbased on patients age in different groups were shown in figure (1). The highest one appeared at the (51-60) years age group with 19.7%, followed by others at age groups (>60) years, (31-40) years and (1-10) years with 14%, 11% and 10.8%, respectively. The low isolation frequency was recorded at (11-20) years and (21-30) years in a percent 5.1% and 3.8% respectively, i.e. at the youth period.

 **Figure (1): Prevalence of *S. pneumoniae* according to the age.**

 According to sex, there was no significant difference (P>0.05) between pneumococcus isolates and type of sex. However *S. pneumoniae* isolated from males in a percentage of (54.1%) which was more than females (45.9%) as shown in Figure (2).

**Figure (2): Prevalence of *S. pneumoniae* according to the sex.**

There was no significant variation (P>0.05) between residence and pneumococcal infection (figure 3).

**Figure (3): Prevalence of *S. pneumoniae* according to the residence.**

This can be attributed to the smoking habit which is common in males and the result in figure (4) ensure that, since the incidence of pneumonia in smoker patients (54.1%) have the same percent of male with pneumococcal infection. Smoking has been shown to increase the risk of invasive pneumococcal disease substantially.

**Figure (4): Prevalence of *S. pneumoniae* according to the smoking.**

These results are in agreement with Al-Taaie, (2013) who reported that pneumococci showed a total incidence in the age groups more than 49 years, despite the differences in percent of isolates. Pejcic *et* *al*., (2011) observed pneumococcal pneumonia is much more frequent in elderly people than in younger and middle-aged population. Gupta *et al.,* (2012) reported that pneumococcus is the most single common organism identified in hospitalized elderly patients with CAP. Worldwide the pneumococcus is responsible for more than 14.5 million episodes of IPD annually and up to 11% of all deaths in children (O’Brien *et al.,* 2009). Notably, in individuals >65 years of age the case-fatality rate for IPD can be as high as 30% (Maruyama *et al.,* 2010). Thus pneumococcal infections are a major medical problem for both children and the elderly.

 The high incidence of pneumococcal isolate at (51-60) age group may be due to impaired of immune system, and most elderly are infected with chronic diseases. In age group (31-40), females (8) showed high number of case than males (2), most females during this period may be taken many drug and hormone for pregnancy difficult. So that they may be exposes to infection due to decrease in there immunity. The low immunity and highly exposed to contaminated materials may have a role in increase infection with pneumonia during (1-10) age group.

 Pneumonia remains the leading cause of mortality resulting from infectious disease worldwide. In 2008 alone, it killed nearly 1.6 million children <5 years of age (Black *et al*., 2010). Over 90% of the estimated 1.8 million annual deaths due to acute respiratory infections in children less than 5 years of age occur in developing countries and are mainly due to bacterial infections (Bryce *et al*., 2005). Typical bacterial pathogens causing CAP in children less than 5 years of age include *S. pneumoniae*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae* (Gentile *et al*., 2012).

**Antimicrobial Susceptibility test**

**1. Antibiotic sensitivity test**

**1.1. Disk diffusion method (DDT)**

 Figure (5) and table (1) show the phenotypic susceptibility of 37 *S.**pneumoniae*isolates to (24) commonly used antimicrobial agents by using Kirby-Bauer disk diffusion method (Bauer *et al.,* 1966). The results were interpreted according to the diameter of inhibition zone and compared with stander zones of inhibition determined by CLSI (2014).

 The results of this test showed that *S.**pneumoniae* has a great resistance to most commonly antibiotics used in hospitals, *S.**pneumoniae* showed different susceptibility towards antibiotics used in this study as shown in figure (6). The highest rate of resistance is seen with erythromycin 37/37 (100%), azithromycin 31/37 (83.8%) , clindamycin 31/37 (83.8%) and trimethoprim/sulfamethaxzol 30/37 (81.1%) and moderately resistance to ceftriaxone 25/37 (67.6%), cefotaxime 24/37 (64.9%), chloramphenicol 24/37 (64.9%), tetracycline 22/37 (59.5%) and benzylpenicillin 17/37 (45.9%) whereas is relatively lower resistance toward amoxicillin 13/37 (35.1%), levofloxacin 13/37 (35.1%), amoxicillin-clavulanic acid 12/37 (32.4%), cefoxitin 12/37 (32.4%), meropenem 6/37 (16.2%), ciprofloxacin 6/37 (16.2%), telithromycin 5/37 (13.5%), gentamicin 4/37 (10.8%), clarithromycin 4/37 (10.8%), cefepime 3/37 (8.1%), oxacillin 3/37 (8.1%), rifampicin 3/37 (8.1%) and ertapenem 1/37 (2.7%). Development of antibiotic resistance is often related to the overuse, and misuse of the antibiotics prescribed. Resistance of *S.**pneumoniae* continues to be an important clinical therapeutic problem, such that which can be found in an increasing multidrug resistance in these bacteria. The results of this study showed that *S.**pneumoniae*isolates were found to be a remarkable sensitive to Vancomycin (100%) and Imipenem (100%) as well.

**Figure (5): The resistant rate of *S. pneumoniae* isolates to the (23) antibiotic**

**Table (1): Antibiogram of 37 *S. pneumoniae* isolates**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **R** | **I** | **S** | **Symbol** | **Antibiotics** |
| **(%)** | **No.** | **(%)** | **No.** | **(%)** | **No.** |
| 35.1 | 13 | 0 | 0 | 64.9 | 24 | Ax | Amoxicillin |
| 32.4 | 12 | 10.8 | 4 | 56.8 | 21 | AMC | Amoxicillin-Clavulanic acid |
| 8.1 | 3 | 5.4 | 2 | 86.5 | 32 | FEP | Cefepime |
| 64.9 | 24 | 0 | 0 | 35.1 | 13 | CTX | Cefotaxime |
| 67.6 | 25 | 0 | 0 | 32.4 | 12 | CRO | Ceftriaxone |
| 16.2 | 6 | 0 | 0 | 83.8 | 31 | MEM | Meropenem |
| 2.7 | 1 | 0 | 0 | 97.3 | 36 | ETP | Ertapenem |
| 0 | 0 | 0 | 0 | 100 | 37 | IPM | Imipenem |
| 0 | 0 | 0 | 0 | 100 | 37 | VA | Vancomycin |
| 100 | 37 | 0 | 0 | 0 | 0 | E | Erythromycin |
| 83.8 | 31 | 0 | 0 | 16.2 | 6 | AZM | Azithromycin |
| 10.8 | 4 | 16.2 | 6 | 73 | 27 | CLR | Clarithromycin |
| 13.5 | 5 | 0 | 0 | 86.5 | 32 | TEL | Telithromycin |
| 59.5 | 22 | 2.7 | 1 | 37.8 | 14 | TE | Tetracycline |
| 16.2 | 6 | 10.8 | 4 | 73 | 27 | CIP | Ciprofloxacin |
| 35.1 | 13 | 5.4 | 2 | 59.5 | 22 | LEV | Levofloxacin |
| 8.1 | 3 | 8.1 | 3 | 83.8 | 31 | OX | Oxacillin |
| 81.1 | 30 | 0 | 0 | 18.9 | 7 | SXT | Trimethoprim/ Sulfamethaxzol |
| 64.9 | 24 | 2.7 | 1 | 32.4 | 12 | C | Chloramphenicol |
| 8.1 | 3 | 16.2 | 6 | 75.7 | 28 | RA | Rifampicin |
| 83.8 | 31 | 0 | 0 | 16.2 | 6 | DA | Clindamycin |
| 45.9 | 17 | 35.1 | 13 | 18.9 | 7 | P | Benzylpenicillin |
| 10.8 | 4 | 10.8 | 4 | 78.4 | 29 | CN | Gentamicin |
| 32.4 | 12 | 8.1 | 3 | 43.24 | 16 | FOX | Cefoxitin |

 The resistance rate of *S. pneumoniae* varies with the locality or region studied, is influenced by the frequency and intensity of utilization, and empirical use of the antimicrobial drugs is frequent (Gossens, 2009).

 Flamm *et al.,* (2013) found that *S. pneumoniae* was evaluated for MDR status against PEN, CRO, erythromycin (ERY), tetracycline (TET), trimethoprim/sulfamethoxazole (TMP/SMX), and levofloxacin (LEV). MDR were defined as nonsusceptible (NS) to at least 2 of the above agents. Ceftaroline and levofloxacin exhibited high rates of susceptibility at 100.0 and 98.9%, respectively. Ceftriaxone, cefuroxime and amoxicillin/clavulanate susceptibilities were at 89.2, 71.8 and 83.3%, respectively. There was a high rate of resistance to erythromycin at 42.7%, and resistance to tetracycline, trimethoprim/sulfamethoxazole and clindamycin ranged from 20.324.6%. The results of the present study disagreement with these above study. Since, *S. pneumoniae* isolates in this study exhibited a low rate of susceptibility to levofloxacin and other antibiotics, while show 100% resistance to erythromycin.

 On the other hand, during 2000-2001, it has reported a rise in resistance rates to ﬂuoroquinolones FQ among invasive *S. pneumoniae* reaching up to 3.5% of isolates (Sener *et al.,* 2007). In Lebanon, *S. pneumoniae* isolates have shown increasing resistance to penicillin, macrolides, and other antimicrobial agents, but to our knowledge, none so far have been reported against FQ (Kanj *et al.,* 2007).

Rijal *et al.,* (2010) have been pointed out that 2.17% of *S*. *pneumoniae* isolates were resistant to Erythromycin and Chloramphenicol and 2.17% of isolates were intermediately resistant to Cefotaxime. This result was in imputable with results of the present study in which 100% of isolates appeared resistance to Erythromycin an 64.9% for Chloramphenichole and cefotaxim at else.

The new ﬂuoroquinolones are widely used to treat respiratory tract infections, especially in patients with COPD. Although Domenech *et al.,* (2010) showed a low rate 3.5% of ciproﬂoxacin resistance in pneumonia isolates; it was lower than rates found in a recent study 16.2%. The high consumption of quinolones could explain the higher resistance rate. The differences observed in the rates of susceptibility to ciproﬂoxacin 16.2% and to levoﬂoxacin 35.12% are due to isolates 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 (Fuller and Low, 2005).

 The association trimethoprim/sulphamethoxazole in this study showed a high rate of resistance, 81% full resistance. Compared with a preceding study with strains in Brazil, there were an increased number of resistant strains, similar to the situation in other countries (Riedel *et al.,* 2007) and the combination of trimethoprim/ sulphamethoxazole should not be recommended to treat pneumococcal infections, because of the high rate of resistant strains.

 Levin *et al.,* (1996) observed a rate of tetracycline resistance opposite of that reported in this study 32%. 14% of strains showed intermediate resistance, and 4% of strains showed full resistance. Of these tetracycline-resistant strains, were multi-resistant. These results indicate that the empirical use of tetracycline in pneumococcal infections is limited. Weber *et al.* (2010) observed a rate of chloramphenicol, 3% of the strains were resistant. All isolates showed full resistance to erythromycin.

 The emergence of high-level resistance to antimicrobials is an increasing threat to global health (Levy and Marshall, 2004), and even a small increase in antibiotic-refractory bacterial subpopulations or MIC could herald the emergence of higher-level resistance (Balaban *et al.,* 2004). Therefore, any factor contributing to an increase of antibiotic resistance is critically important. Tran *et al.,* (2011) founded that penicillin-triggered lysis could be partially prevented by heat shock pretreatment makes it clear that *in vivo* stresses, such as inﬂammation, respiratory bursts in phagocytes, and temperature upshift, may induce higher ClpL levels and increase resistance to penicillin.

 The most remarkable ﬁnding of this study is the level of erythromycin resistance (100%) and the result was in accordance with the ﬁndings. Currently, the antibiotic resistance patterns of *S. pneumoniae* isolates vary widely from one country to another within Europe. Studies have shown wide variations of antimicrobial resistance against *S. pneumoniae* among countries. Chloramphenicol and Erythromycin resistance was observed in only 2.17% of the strains without any relationship to Penicillin resistance. These drugs are the most common regimen used in the hospitals of our country, is still a good empirical choice for the treatment of pneumoccocal meningitis.

**1.2. Minimum Inhibitory Concentration (MIC) Test**

 To confirm the results obtained by (DDT) the study used MIC method for testing the susceptibility of the *S. pneumoniae* towards some tested antibiotics in attempt to detect the proper method and giving a real picture for susceptibility of this organism for these antibiotics. The MIC values were based on break point recommended by CLSI, (2014) for estimation of the response. The break point represents the optimum concentration of the drug that can reach the serum and provide high level of therapy. The microorganism was considered sensitive if the estimated MIC were less than the break point. In the present study, sixsteen antibiotics were tested for (MIC) against 37 *S. pneumoniae* isolates by using Vitek-2 Gram positive (GP) antibiotics susceptibility testing (AST) cards (41497) AST-GP74 (Biomer) as shown in table (2).

 The results of antibiogram test with the automated Vitek-2 compact system revealed that 100% and 83.8% of *S. pneumoniae* isolates were resistant to erythroycin and SXT with MIC ≥1 mg/ml and 4/76 mg/ml of these antibiotic respectively, and moderately resistance to cefotaxime 64.9%, ceftriaxone 64.9% and chloramphenicol 64.9% with MIC 4 mg/ml for CTX and CRO at ealse, and MIC 8 mg/ml for C only. All isolates were showed sensitivity in 100% for each Vancomycin and Linzolid with MIC ≤1 mg/ml and ≤2 mg/ml, respectively. *S. pneumoniae* isolates showed high rate of sensitivity to Ertapenem 97.3% with MIC ≤1 mg/ml, Telithromycin 89.2% with MIC ≤1, Meropenem 86.5% with MIC ≤0.25 mg/ml.

**Table (2): MIC values of *S. pneumoniae* isolates toward antibiotics.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **R** | **I** | **S** | **Break point****S≤ I ≥R** | **Symbol** | **Antibiotics** |
| **(%)** | **No.** | **(%)** | **No.** | **(%)** | **No.** |
| 35.1 | 13 | 0 | 0 | 64.9 | 24 | 2 4 8  | AMX | Amoxicillin |
| 32.4 | 12 | 40.5 | 15 | 27.1 | 10 | 2 4 8  | P | Benzylpenicillin |
| 64.9 | 24 | 0 | 0 | 35.1 | 13 | 1 2 4  | CTX | Cefotaxime |
| 64.9 | 24 | 0 | 0 | 35.1 | 13 | 1 2 4  | CRO | Ceftriaxone |
| 64.9 | 24 | 0 | 0 | 35.1 | 13 | 4 - 8 | C | Chloramphenicol |
| 2.7 | 1 | 0 | 0 | 97.3 | 36 | 1 2 4 | ETP | Ertapenem |
| 100 | 37 | 0 | 0 | 0 | 0 | 0.25 0.5 1 | E | Erythromycin |
| 35.1 | 13 | 10.8 | 4 | 54.1 | 20 | 2 4 8  | LEV | Levofloxacin |
| 0 | 0 | 0 | 0 | 100 | 37 | 2 - - | LNZ | Linezolid |
| 13.5 | 5 | 0 | 0 | 86.5 | 32 | 0.25 0.5 1 | MEM | Meropenem |
| 16.2 | 6 | 0 | 0 | 83.8 | 31 | 1 2 4  | MXF | Moxifloxacin |
| 16.2 | 6 | 0 | 0 | 83.8 | 31 | 2 4 8  | OFX | Ofloxacin |
| 10.8 | 4 | 0 | 0 | 89.2 | 33 | 1 2 4  | TEL | Telithromycin |
| 54.1 | 20 | 0 | 0 | 45.9 | 17 | 1 2 4  | TE | Tetracycline |
| 83.8 | 31 | 0 | 0 | 16.2 | 6 | 0.5/9.5 1/19-2/38 4/76 | SXT | Trimethoprim/ Sulfamethaxzol |
| 0 | 0 | 0 | 0 | 100 | 37 | 1 - -  | VA | Vancomycin |

Flamm *et al.,* (2013) found that ceftriaxone (MIC50/90, ≤0.25/2 μg/mL) when tested against *S*. *pneumoniae* isolates in the USA during 2009-2012. There was a high rate of resistance to levofloxacin exhibited high rates of susceptibility at 98.9%. Ceftriaxone and amoxicillin/clavulanate susceptibilities were at 89.2 and 83.3%, respectively. Relatively, there was a high rate of resistance to erythromycin at 42.7%, and resistance to tetracycline, trimethoprim/sulfamethoxazole and clindamycin ranged from 20.3-24.6%. Only 86.5% of *S. pneumoniae* strains were inhibited at a penicillin MIC of ≤2 μg/mL (penicillin parenteral non-meningitis susceptible breakpoint) and 57.3% at ≤0.06 μg/mL.

 Rijal *et al.,* (2010) were found that MICs against penicillin, Chloramphenicol, Erythromycin and Cefotaxime were determined. Whereas penicillin MICs were mostly around 0.016mgl-1 and showed 3 (6.5%) were intermediately resistant, no penicillin resistant strains were isolated. Over the 4 years, erythromycin MICs ranged from 0. 062 to 8mgl-1 resistance was recorded in 1 (2.17%). Chloramphenicol MICs ranged from 1 to 4 mgl-1 overall 1 (2.17%) was considered resistant toward Chloramphenicol whereas the MICs of Cefotaxime ranges from 0.008 mgl-1 to 0.25 mgl-1. Overall only 1 (2.17%) was found intermediately resistant to Cefotaxime in this study period.

 Forty-six strains (72%) were susceptible to penicillin with a minimum inhibitory concentration (MIC) <0.06µg/mL. Among the resistant strains, 20% showed intermediate resistance (between 0.12 and 1µg/mL) and 8% showed full resistance (>2µg/mL). These results showed that the local and national rates of penicillin resistance are stable compared to the results of other investigators, who reported rates of 26% (Zettler *et al.,* 2006), and still can be considered a good option to control pneumococcal infection.

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