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Original Article / Özgün Araştırma

Resistance of *Streptococcus pneumoniae* Strains Isolated from Clinical Samples to Various Antibiotics

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Abstract

Objective: It was aimed to determine the antibiotics resistance rates of *Streptococcus pneumoniae* (*S. pneumoniae)* strains which are important causes of mortality and morbidity with increasing resistance rates.

Methods: Antibiotic susceptibilities of 80 *S. pneumoniae* specimens identified from samples sent from various clinics in our hospital were analyzed retrospectively. Isolated strains were identified by classical methods and BD Phoenix 100 system (Becton Dickinson Diagnostic, USA) and their antimicrobial susceptibility was evaluated by Kirby-Bauer disc diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) document. Penicillin, cefotaxime and meropenem susceptibilities were examined by gradient test; while erythromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin, tetracycline, vancomycin and linezolid resistances were analyzed by disc diffusion method.

Results: A total of 80 isolates including 32 blood samples, 29 respiratory samples, 10 cerebrospinal fluid (CSF), 5 vitreous, 3 pleural fluid and 1 abscess aspiration were included in the study. Penicillin resistance in CSF isolates was 70% and there was no resistance to cefotaxime and meropenem. Of 70 non-CSF isolates, 15 (21.4%) were moderately resistant, 14 (20%) were highly resistant and 41 (58.6%) were susceptible to penicillin. Cefotaxime resistance was detected in 2 (2.9%) of these isolates. Intermediate values were found in 2 (2.9%) isolates and remaining 66 (94.2%) isolates were susceptible to cefotaxime. There was no meropenem resistance in non-CSF isolates. Resistance rates of erythromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin and tetracycline in all isolates were 32.5%, 27.5%, 42.5%, 3.7% ve 30% respectively. There was no resistance to vancomycin and linezolid.

Conclusion: Penicillin resistance is high in *S. pneumoniae* strains and penicillin has no place in empirical treatment of pneumococcal meningitis. Periodic monitoring of infectious agents and antibiotic susceptibilities in certain regions may guide clinicians to initiate empirical treatment.

Keywords: Antibiotic, Resistance, *Streptococcus pneumoniae*

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Klinik Örneklerden İzole Edilen *Streptococcus pneumoniae* Suşlarının Farklı Antibiyotiklere Direnç Profilleri

Öz

Amaç: Artan direnç oranların edeni ile önemli oranlarda mortalite ve morbiditeye neden olan *Streptococcus pneumonia (S. pneumoniae*) suşlarının çeşitli antibiyotiklere direnç oranlarının belirlenmesi amaçlanmıştır.

Yöntemler: Hastanemizdeki çeşitli kliniklerden gönderilen örneklerden tespit edilen 80 adet *S. pneumoniae* örneğinin antibiyotik duyarlılıkları retrospektif olarak incelendi. İzole edilen suşlar klasik yöntemler ve Phoenix 100 sistemi (Becton Dickinson Diagnostic, ABD) ile tanımlandı ve antimikrobiyal duyarlılıkları, Avrupa Antimikrobiyal Duyarlılık Testi (EUCAST) dokümanına göre Kirby-Bauer disk difüzyon yöntemi ile değerlendirildi. Penisilin, sefotaksim ve meropenem duyarlılıkları gradyan testi ile incelendi; eritromisin, klindamisin, trimetoprim-sülfametoksazol, levofloksasin, tetrasiklin, vankomisinve linezolid dirençleri disk difüzyon yöntemiyle analiz edildi.

Bulgular: Çalışmaya 32 kan örneği, 29 solunum örneği, 10 beyin omurilik sıvısı (BOS), 5 vitrözsıvı, 3 plevral sıvı ve 1 apse aspirasyonu dahil toplam 80 izolat dahil edildi. BOS izolatların dapenisilin direnci %70 idi ve sefotaksim ve meropenem için direnç yoktu. BOS dışı 70 izolattan 15'i (%21,4) orta derecede dirençli, 14'i (%20) yüksek dirençli ve 41'i (%58,6) penisiline duyarlı idi. Bu izolatların 2'sinde (%2,9) sefotaksim direnci tespit edildi. Ara değer2 (%2,9) izolatta bulundu ve gerikalan 66 (%94,2) izolat sefotaksime duyarlıydı. BOS dışı izolatlarda meropenem direnci yoktu. Tüm izolatlarda eritromisin, klindamisin, trimetoprim-sulfametoksazol, levofloksasin ve tetrasiklin direnç oranları sırasıyla %32,5, %27,5, %42,5, %3,7 ve %30 idi. Vankomisin velinezolide direnç yoktu.

Sonuç: *S. pneumoniae* suşlarında penisilin direnci yüksektir ve penisilinin pnömokokal menenjitin ampirik tedavisinde yeri yoktur. Belirli bölgelerdeki enfeksiyöz ajanların ve antibiyotik duyarlılıklarının periyodik olarak izlenmesi, klinisyene ampirik tedaviyi başlatmada rehberlik edebilir.

Anahtar kelimeler: Antibiyotik, Direnç, *Streptococcus pneumoniae*.

INTRODUCTION

Streptococcus pneumonia (S. pneumoniae) is still remains an important public health problem[1]. In year 2000, 14.5 million severe pneumococcal diseases and approximately 826 000 deaths were reported in children younger than 5 years of age[2]. Pneumococci are colonized only in humans and are located in the normal flora of the nasopharynx and oropharynx[3,4]. Pneumococcal disease is transmitted through infected droplets and aerosols from infected patients or healthy carriers[1,5].

Many factors may increase the likelihood of pneumococcal colonization: young age (particularly <5 years), old age (> 65 years), and suppression of the immune system are the most important ones[2,6]. Pneumococcal colonization is also affected by chronic conditions such as socioeconomic factors, crowding, alcoholism, and smoking, previous anesthesia history, viral agents causing respiratory infections, chronic obstructive pulmonary disease, corticosteroid therapy and pneumococcal vaccination[6,7]. Although pneumococcal colonization is generally asymptomatic, due to disruption of the immune system, it can often progress and spread to sterile body areas. In this way, it can cause different pneumococcal diseases ranging from less serious infections such as sinusitis, conjunctivitis and acute otitis media to more serious and fatal infections such as invasive pneumococcal diseases (IPD) and community-acquired pneumonia[6]. The first antibiotic-resistant *S. pneumoniae* strain was isolated in Australia in the late 1960s[8]. Soon, the resistance rates to different antimicrobial agents among *S. pneumoniae* strains continued to increase and became a worldwide problem.

In recent years, the presence of macrolides, tetracyclines, cotrimaxazole and chloramphenicol-resistant strains of *S. pneumoniae* in addition to penicillin have

started to cause serious problems in treatment[9]. Since antimicrobial resistance characteristics vary according to geographical regions and countries depending on the widespread use of antibiotics, spread of resistant strains and living conditions, each region should determine and monitor its own resistance status through surveillance studies[10]. The aim of this study was to determine the resistance rates of S. pneumoniae strains isolated in our laboratory to various antibiotics.

METHODS

In this study, 80 *S.pneumoniae* strains isolated from clinical samples taken from patients of various age groups in central microbiology laboratory of our hospital between January 2014 and December 2019 were examined. In the identification of isolated pneumococcal strains, in addition to direct microscopic examination of clinical samples, culture on 5% sheep blood agar medium, macroscopic appearance of bacterial colonies and the presence of alpha hemolysis, Gram stain microscopic examination, catalase test, and optochin sensitivity test were used. Among the colonies, Gram-positive diplococci with alpha hemolysis, and optochin-sensitive colonies were further identified by Phoenix automated bacterial identification system (Becton Dickinson, USA). Sheep blood Mueller Hinton agar was used in the sensitivity test. The medium was incubated at 35 °C, with 5% CO2 for 24 hours. Antimicrobial susceptibilities were determined by Kirby-Bauer disc diffusion method and evaluated according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) document[11]. Penicillin, cefotaxime and meropenem susceptibilities were examined by gradient test (ETEST®, bioMérieux, France) and erythromycin, clindamycin, trimethoprim-sulfamethoxazole (TMP-SXT), levofloxacin, tetracycline, vancomycin and linezolid susceptibilities were examined by disc diffusion (Oxoid ™) method. Penicillin and meropenem breakpoints are different for meningitis and non-meningitis isolates according to EUCAST standards. Minimal inhibitory concentration (MIC) values were studied by gradient test. Strains isolated from meningitis infections were susceptible to penicillin with minimum inhibitory concentration (MIC) ≤ 0.06 µg / ml, and resistant with MIC> 0.06 µg / ml, while strains were susceptible to meropenem with MIC ≤ 0.25 µg / ml and strains with MIC> 0.6 µg / ml were considered resistant. Strains isolated from non-meningitis infections were susceptible to penicillin if MIC ≤ 0.06 µg / ml and resistant to if MIC ≥ 2 µg / ml; for meropenem strains were found to be susceptible if MIC ≤ 2 µg/ml and were resistant if MIC> 2 µg / ml. For cefotaxime strains with MIC ≤ 0.5 µg / ml were considered susceptible and strains with ≥ 2 µg / ml were considered resistant.

'S.pneumoniae ATCC 49619'was used as the standard strain for quality control.

Ethical approval: The study was approved by the local ethics committee (date: 21.01.2020 and number: 16).

RESULTS

A total of 80 isolates including 32 blood samples, 29 respiratory samples,10 cerebrospinal fluid (CSF), 5 vitreous, 4 pleural fluid and 1 abscess aspiration fluid were included in the study. Regarding the participants, 51 (63.7%) of the patients were male and 29 (36.3%) were female. The median age of the men were 41.8 years (range: 1-87) and women were 39.4 years (range: 0-91). Nine (90%) of the CSF isolates were male and one (10%) female, with a mean age of 47.4 years. Eight of these patients were hospitalized in the emergency department and two in the infectious disease clinic. Non-CSF isolates are obtained from patients in pediatric clinic, internal medicine, intensive care unit, infectious disease clinic, ophthalmology clinic,

hematology/oncology and other clinics, with a rate of 32.6%, 17.4%, 15.4%, 11.5%, 7.7%, 7.7%, and 7.7%, respectively.

In our study, it was observed that parenteral penicillin resistance was 70% in 7 CSF isolates and there was no resistance to cefotaxime and meropenem. The total resistance of 70 non-CSF isolates to penicillin was 41.4% (n: 29), moderate and high-level penicillin resistance rates were 21.4% (n: 15) and 20% (n:14), respectively. Cefotaxime resistance was detected in 2 (2.8%) of these isolates. Intermediate values were found in 2 (2.8%) isolates and remaining 66 (94.4%) isolates were susceptible. There was no meropenem resistance in non-CSF isolates. Resistance rates of erythromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin and tetracycline in all isolates were resistance rates of erythromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin and tetracycline in all isolates were 32.5%, 27.5%, 42.5%, 3.7% and 30%, respectively. There was no resistance to vancomycin and linezolid. In this study, the resistance rates of various antibiotics to *S. pneumoniae* strains in CSF and non-CSF isolates are shown in tables I and II.

Table I: The resistance rates of various antibiotics to *S. pneumoniae* strains in CSF samples (n:10)

Antibiotics	Number of resistant strains	Percentage of resistant strains (%)
Penicillin G	7	70
Cefotaxime	0	0
Meropenem	0	0
Erythromycin	4	40
Clindamycin	3	30
TMP-SXT	6	60
Tetracycline	2	20
Levofloxacin	0	0
Vancomycin	0	0
Linezolid	0	0

CSF: Cerebrospinal fluid,TMP-SXT: Trimethoprim-Sulfamethoxazole

Table II. The resistance rates of various antibiotics to *S. pneumoniae* strains in non-CSF samples (n: 70)

Antibiotics	Number of resistant strains	Percentage of resistant strains
Penicillin G*	29	41.4
Cefotaxime**	4	5.8
Meropenem	0	0
Erythromycin	22	31.4
Clindamycin	19	27.1
TMP-SXT	28	40
Tetracycline	22	31.4
Levofloxacin	3	4.2
Vancomycin	0	0
Linezolid	0	0

CSF: Cerebrospinal fluid,TMP-SXT: Trimethoprim-Sulfamethoxazole

** In 14 (20%) of them high level, and in 15 (21.4%) of them moderate resistance; **2 (2.9%) of them were resistant, 2 (2.9%) were having intermediate value in isolate.*

In CSF isolates, the MIC values of antibiotics tested were 0.006 – 32µg/ml forpenicillin,0.008-0.5µg/ ml for cefotaxime, and 0.004-0.25µg/ ml for meropenem. All pneumococcal isolates were susceptible to cefotaxime and meropenem; but 7 of them were resistant to penicillin and only 3 of them was susceptible to penicillin.

In non-CSF isolates, the MIC values of antibiotics tested were 0.002 – 32µg/ml for penicillin, 0.006-4µg/ml for cefotaxime, and 0.002-0.5µg/ ml for meropenem. All pneumococcal isolates were susceptible to meropenem; two were resistant and two were intermediate susceptible to cefotaxime while 14 of them were having high level resistance, 15 of them were having moderate level resistance to penicillin and 41 of them were susceptible to penicillin.

DISCUSSION

S. pneumoniae is a member of the normal flora of the upper respiratory tract. Nasopharyngeal carriage, which varies according to age, season and environmental conditions, is important for the development of infection. It is a bacterium that can spread from the colonized region to various regions and may cause infections. *S. pneumoniae* is also one of the main causes of sepsis and septic shock[12].The main findings of this study were; in CSF isolates very high (70%) resistance rates of *S. pneumoniae* to Penicillin G, followed by TMP-SXT (60%) and in non-CSF isolates, resistance rates of *S. pneumoniae* to Penicillin G was much lower with a 41.4% and followed by TMP-SXT (40%). On the other hand, absence of resistance of *S. pneumoniae* to Vancomycin and Linezolid were hopeful.

Although penicillin is the first choice in the treatment of pneumococcal infections, resistance to penicillin has become increasingly common all over the world. According to the "European Centers for Disease Control and Prevention (ECDC)" data, the highest penicillin resistance was observed in S. pneumoniae strains in Romania (46.7%) and in Spain (27.9%) and the lowest resistance was reported in Netherlands (2.1%) and Belgium (1.3%). Similarly, rates of resistance to erythromycin are also increasing, with Romania (48%) and Slovakia (41%) among the countries having the highest erythromycin resistance. According to this study, *S. pneumoniae* strains have been reported to be 18.8% non-penicillin-susceptible (moderate and high-level resistance) and erythromycin resistance was 16.1%[13]."Centers for Disease Control and Prevention (CDC)"reported that in 2 897 invasive pneumococci isolates, sensitivity of penicillin, cefotaxime, erythromycin, TMP-SXT, tetracycline, levofloxacin and vancomycin were 96%, 97.9%, 70.7%, 82%, 88.7, 99.9%, and 100% according to The Clinical & Laboratory Standards Institute(CLSI) criteria[14].

El Moujab et al. reviewed the national and local data about the pneumococcal epidemiology and resistance in the Middle East countries including Lebanon, Egypt, Saudi Arabia, Kuwait, Turkey, Palestine, United Arab Emirates, Oman, Bahrain, Iran and Jordan for areas related to, national, and made a compilation based on local data[15]. In this study, it was observed that antibiotic susceptibilities of invasive and non-invasive samples differed by country and years in different age groups. For example, the highest penicillin resistance in Saudi Arabia was 66% in clinical invasive isolates under 5 years of age based on national data and it was 90% in clinical invasive isolates under 15 years of age, based on local data. In the same study, in Turkey, between the years 2011-2013, covering all age groups, involving invasive and invasive samples, penicillin resistance was found as 61.9% in the table prepared based on national data. In general, when the above countries are evaluated together, the penicillin resistance is between 9.2 -90%; although different according to countries, the highest and lowest resistances for erythromycin, clindamycin, fluoroquinolone, TMP-SXT, vancomycin and tetracycline is 8.2-77%, 3.4-46%, 0-15.4%, 11.8-100%, 0-8%, and 31-77% %, respectively[15]. In their study with 258 *S. pneumoniae*, Ilki et al. found that, according to the 2008 CLSI criteria, penicillin resistance was 3.5%, medium and high level penicillin resistance was 3.1% and 0.4%, and resistance rates to erythromycin, tetracycline and TMP-SMX were found to be 19%, 26.8% and 49.2%, respectively[16]. In a study of Hasçelik et al. in Turkey, carried out with the participation of 14 different centers, in non-CSF isolates, intermediate and high level penicillin resistance was 21.7% and 16.8%, respectively while the penicillin resistance in CSF isolates was 52.6%, and the cefotaxime resistance was 5.3%, while

moderate sensitivity was found as 18.4%. The researchers shared that the sensitivities of the isolates were also different between the centers[17]. In the study conducted by Öksüz and Gürler, moderate resistance to penicillin G 2% and cefotaxime 3% was found in 100 isolates. Erythromycin resistance was reported in 25% of the strains isolated from invasive samples, 37% of the strains isolated from non-invasive samples at a total of 33%[18]. In another clinical study from our country, 80 clinical isolates recovered from 19 pediatric and 61 adult patients were analyzed, and the author reported that low level resistance to penicillin was determined in only 1 (1%) strain, while high level resistance wasn't present. In that study, TMP-SMX, erythromycin, chloramphenicol and ofloxacin resistance rates were reported as 56%, 27.5%, 9%, and 2.5%, respectively. All strains were found susceptible to vancomycin, linezolid and levofloxacin[19]. Söyletir et al. reported the results of the Survey of Antibiotic Resistance (SOAR) for respiratory tract infection pathogens collected in 2011-13 from Turkey and determined that among 333 isolates of *S. pneumoniae* tested; penicillin, erythromycin and cefuroxime resistance rates were 38%, 51% and 64.6%, respectively[20]. They also reported that, >90% of the isolates were susceptible to amoxicillin/clavulanic acid, ceftriaxone, levofloxacin and high-dose intravenous penicillin.

In a meta-analysis of 16 studies, Hosseini et al. reported the pooled prevalence of *S. pneumoniae* nasopharyngeal carriage as 18% among healthy children and the antibiotic resistance rates were 26%, 30% and 34% to penicillin, erythromycin and tetracycline, respectively[21]. In a recent review, Cherazard et al. reported that there has been a steady decline in susceptibility of *S. pneumoniae* to commonly used beta-lactams due to the genetic structural modification in penicillin-binding proteins[22]. In the same review, the macrolide resistance was between 20% and 40%, clindamycin resistance was about 22%, TMP-SMX resistance was around 35%; while the fluoroquinolone resistance was still low. These data were also compatible with our results.

We sub-grouped the CSF and non-CSF isolates in this study, which was the main power of the study. However, there are some limitations that should be mentioned. First is the low number of isolates and secondly this study was performed in a single center and carrying the handicaps of this condition.

Based on the above data, the sensitivities of pneumococcal strains may be different between countries and even in different centers within the same country. In our study, penicillin resistance was found to be high in CSF isolates. This result shows us that penicillin is not suitable for meningitis. Absence of cefotaxime, meropenem, vancomycin and linezolid resistance in CSF isolates are pleasing. However, the low number of isolates can be seen as a disadvantage of this study. In non-CSF isolates, 5.8% of cefotaxime resistance (resistant and intermediate value) is compatible with that data from Turkey. Erythromycin is also a primary drug used in non-meningitis infections, but it has no role in treatment unless the strains are susceptible in antibiotic susceptibility tests. According to our data, we observed that levofloxacin has a higher percentage of susceptibility than other antimicrobials, and we may think that it is a viable option when antibiotics other than beta-lactam group are needed. In order to limit the spread of resistant pneumococcal strains, antibiotic treatments should be evaluated carefully; health policies should be developed in this direction regarding the prevention of unnecessary antibiotic use and improving vaccination programs.

Ethics Committee Approval: Ethical approval: The study was approved by the local ethics committee (date: 21.01.2020 and number: 16).

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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