**ORIGINAL STUDIES**

University of Novi Sad, Faculty of Medicine, Serbia
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**Susceptibility of Common Bacterial Respiratory Pathogens to Antimicrobial Agents in Outpatients from South Backa District**

**Osetljivost na antibiotike ispitana je disk-difuzionom metodom i metode.**

**Sažetak**

**Uvod.** Akutne infekcije gornjih respiratornih puteva najčešći su razlozi posete pacijenata lekaru opšte prakse. Prekomerna upotreba antibiotika u ovim stanjima izuzetno je rasprostranjena iako su najčešće izazvane virusima. Cilj rada bio je da se ispita zastupljenost i osetljivost na antibakterijske lekove najčešće izolovanih bakterijskih uzročnika infekcija gornjih respiratornih puteva i utvrdi da li su dobijeni rezultati na ispitivanom uzorku u skladu sa preporukama postojećeg nacionalnog vodiča za terapiju infekcija gornjih respiratornih puteva. **Materijal i metode.** Ispitivanjem je obuhvaćeno 945 izolata iz briseva grla i nosa vanbošných pacijenata u periodu od 1. do 31.3.2008. godine kao i 330 izolata u istom periodu 2013. godine u Južnobackom okrugu. Izolacija i identifikacija sojeva ispitana je disk-difuzionom metodom prema preporukama Clinical and Laboratory Standards Institute. **Rezultati.** Nađene su bakterije Strepotoccus pyogenes, Streptococcus pneumoniae, Branhamella catarrhalis i Haemophilus influenzae. U oba ispitivana perioda osetljivost Streptococcus pyogenes, Branhamella catarrhalis i Haemophilus influenzae na ispitivane antibiotike nije se bitno menjala. U 2008. godini nisu izolovani sojevi Staphylococcus aureus rezistentni na meticilin, dok je u 2013. godini rezistencija zabeležena kod 5,93% izolata. Osetljivost Staphylococcus pneumoniae na erythromycin i clindamycin u 2013. godini bila je niža u odnosu na 2008. godinu. **Zaključak.** Rezultati našeg istraživanja slazu se s preporukama nacionalnog vodiča o primeni prirodnih penicilina kao lekova prvog izbora u terapiji tonzilofaringitisa. Za terapiju rinosinusitis može se preporučiti primena amoksicilina sa klavulanskim kiselinom, a kao lek drugog izbora cefalosporini druge generacije. **Ključne reči:** Infekcije respiratornog trakta; Bakterijske infekcije; Testovi mikrobane osetljivosti; Antibakterijski lekovi; Nacionalni vodići; Bakterijska otpornost na lekove; Faringitis; Prekomerna upotreba lekova

**Summary**

**Introduction.** Acute infections of the upper respiratory tract are the most common reasons why patients visit general practitioners. Overuse of antibiotics in treatment of these conditions is extremely common practice although these infections are most frequently caused by viruses. The aim of this study was to determine the distribution and susceptibility of common pathogens to antimicrobial agents that cause infections of the upper respiratory tract in outpatients and to determine whether the results obtained from the examined sample were in accordance with the recommendations of the current National Guideline. **Material and Methods.** The study included 945 strains isolated from the throat and nasal swabs from January 1st to March 31st, 2008, as well as from 330 strains isolated from January 1st to March 31st, 2013 in South Backa District, Serbia. Susceptibility tests were performed by the standard disc diffusion method and according to the criteria recommended by the Clinical and Laboratory Standards Institute. **Results.** The most commonly isolated strains were Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, Branhamella catarrhalis, and Haemophilus influenzae. Susceptibility of Streptococcus pyogenes, Branhamella catarrhalis and Haemophilus influenzae to examined antibiotics did not substantially change over the two study periods. None of the isolates of Staphylococcus aureus demonstrated resistance to meticillin in 2008, while the percentage of resistant strains was 5.93% in 2013. Susceptibility rates of Staphylococcus pneumoniae isolates to erythromycin and clindamycin were lower in 2013 than in 2008. **Conclusion.** The investigation results follow the recommendations of the National Guideline for the usage of natural penicillin in the treatment of tonsillopharyngitis. Amoxicillin/clavulanic acid is recommended for the treatment of rhinosinusitis, and second generation cephalosporins are the second choice. **Key words:** Respiratory Tract Infections; Bacterial Infections; Microbial Sensitivity Tests; Anti-Bacterial Agents; Practice Guidelines; Drug Resistance, Bacterial; Pharyngitis; Inappropriate Prescribing
Introduction

Acute infections of the upper respiratory tract are the most common reasons for seeing general practitioners. These infections account for 30-50% of sick leaves in adults and 60-80% of absence from school in schoolchildren [1].

Overuse of antibiotics in treatment of these conditions is extremely common practice, although these infections are most frequently caused by viruses. Antibiotics treatment is prescribed to 70% of patients with sore throat, 85-98% of patients with rhinosinusitis and 60% of patients with common cold [2,3]. This inadequate administration of antibiotics has resulted in increasing resistance of the most frequent bacterial causes of upper respiratory tract infections to antibiotics throughout the world over the last decades. This especially refers to Streptococcus pneumoniae which is becoming resistant to antibiotics most commonly used for treatment of respiratory infections [4,5].

Distribution of respiratory pathogens and degree of their resistance is the information of a great significance for the medical professionals in their everyday practice since it helps them choose the proper antibiotic drugs. Therefore, several national [5,6] and international [4,7,8] surveillance programs were conducted in last two decades. The objective of these programs was to monitor worldwide resistance of the most common respiratory pathogens in outpatients (Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis) and it has been verified as the widespread problem in antimicrobial resistance in many countries in Europe. An increase in antimicrobial resistance has been reported in European countries with high levels of overall antimicrobial use [4].

A systematic surveillance of pathogens that cause upper respiratory tract infections has not been conducted in Serbia, including the region of Vojvodina, so far [9].

Swabs are taken only occasionally; thus, the respiratory infection treatment in outpatients typically involves administration of antibacterial drugs which are expected to be effective in these cases. A surveillance of resistance of the most common pathogens causing upper respiratory infection in outpatients was conducted on the territory of South Backa District in 2000 and 2002 [10,11]. Within the surveillance period, an increase in antimicrobial resistance of these pathogens was reported.

The aim of this study was to determine the distribution and susceptibility to antimicrobial agents of common pathogens causing infections of the upper respiratory tract in outpatients, as well as to determine whether the results obtained from the examined sample were in accordance with the recommendations of the current National Guideline "The choice and use of antibiotics in general practice" of the Ministry of Health of Serbia, in the cases of upper respiratory tract infections - sinusitis and tonsillitis.

Material and methods

Potentially positive pathogens were isolated in 954 cases and in 330 during the period from January 1st to March 31st, 2008 and from January 1st to March 31st, 2013, respectively.

The study included 1284 isolates from the throat and nasal swabs in outpatients of all ages with the symptoms of upper respiratory tract infections at the Department of Clinical Bacteriology, Center of Microbiology, Institute of Public Health of Vojvodina during the study period.

Identification of bacteria was done on the basis of morphological, cultural, biochemical and serological characteristics using standard methods [12].

Susceptibility tests were performed by the standard disc diffusion method and according to the criteria recommended by the National Committee of Clinical and Laboratory Standards (CLSI) [13].

Susceptibility to the following antimicrobial drugs was determined: penicillin, ampicillin, amoxicillin/clavulanic acid, cefaclor, erythromycin, azithromycin, co-trimoxazole, clindamycin, fusidic acid, ofloxacin, ciprofloxacin, and levofloxacin. Isolates with intermediate susceptibility were classified as resistant.

Susceptibility of Streptococcus pneumoniae to penicillin was determined by the oxacillin disk (1 μg). The minimum inhibitory concentration (MIC) for penicillin was tested in 2013 for oxacillin resistant strains.

Cefotaxime discs (30 μg) were used to test staphylococcal isolates for methicillin-resistance according to the criteria recommended by the CLSI. Strains resistant to cefotaxime were considered resistant to methicillin as well as to all beta-lactam antibiotics and their combination with inhibitors of beta lactamase.

Isolates of Haemophilus influenzae, Moraxella catarrhalis and isolates of Staphylococcus aureus susceptible to penicillin were tested for the production of beta-lactamase using nitrocefin test.

The discs manufactured by BIO-RAD, France were used in the study.

Results

Bacteria that were most frequently isolated from the outpatients’ throat and nasal swabs during the three-month evaluation period in 2008 were Strepto-
coccus pyogenes (37.63%) (359/954), Staphylococcus aureus (29.25%) (279/954), Streptococcus pneumoniae (20.55%) (196/954), Haemophilus influenzae (7.86%) (75/954), and Branhamella catarrhalis (2.41%) (23/954), whereas Staphylococcus aureus (40.91%) (135/330), Streptococcus pyogenes (34.85%) (115/330), Branhamella catarrhalis (9.70%) (32/330), Streptococcus pneumoniae (9.39%) (31/330), and Haemophilus influenzae (5.15%) (17/330) were the most frequently isolated bacteria during the investigated period in 2013 (Table 1).

Susceptibility of Streptococcus pyogenes to antibiotics most commonly used in the treatment of streptococcal infections is shown in Table 2. All isolates were susceptible to antibiotics. There was no resistance to penicillin in either period. Only two isolates (0.56%) of Staphylococcus pyogenes showed resistance to erythromycin in 2008, while there were three isolates (6.09%) resistant to this antibiotic in 2013. All isolates were susceptible to clindamycin in 2008; however, three isolates showed resistance in 2013.

All isolates of Staphylococcus aureus were susceptible to methicillin in 2008, while in 2013 eight isolates were resistant to methicillin. In penicillin-sensitive isolates, the production of beta-lactamases was not proved. Susceptibility to other examined antibiotics was lower in 2013 compared to 2008.

Out of 196 Streptococcus pneumoniae, 116 (59.48%) were resistant to oxacillin in 2008. In 2013, 14 (45.16%) of 31 isolates Streptococcus pneumoniae showed resistance to oxacillin. Susceptibility of isolates of Streptococcus pneumoniae to other antibiotics in 2013 was lower than in 2008, with the exception of co-trimoxazole (67.74% vs. 39.80%).

Isolates of Haemophilus influenzae and Branhamella catarrhalis did not show a significant change in susceptibility in 2013 compared to 2008.

Discussion

Due to a great number and variety of respiratory infections, monitoring the resistance of their causes is of great significance for both microbiologists and clinical professionals.

Rapid development of bacterial resistance to antibiotics (beta-lactamase positive Branhamella catarrhalis as well as Haemophilus influenzae, penicillin resistant pneumococcus) and appearance of other multiresistant bacteria, make the current empirical treatment of these infections more complicated in many countries and increase the risk of potential complications as well [4,14,15,16].

Isolates of Streptococcus pyogenes in South Backa District have preserved high susceptibility to erythromycin and clindamycin, which is a very good result, considering the fact that resistance to erythromycin is getting higher worldwide [17,18]. In Europe, a high level of resistance to erythromycin has been reported in Italy (30-40%), Portugal (24%), Spain (21%), and France (13%) [19]. Increased resistance of Streptococcus pyogenes to erythromycin in 40% of isolates was reported in Finland in late 1980s, which was related to substantially increased prescribing and use of erythromycin in treatment of upper respiratory tract infections. However, a decreased use of erythromycin in this country led to a substantial reduction in the resistance of Streptococcus pyogenes isolates [20]. Recent research indicates that the reduced use of long-acting macrolides (claritromycin, roxithromycin, azithromycin) significantly affects decreased bacterial resistance to erythromycin. Thus, in Northern Italy, the reduction of azithromycin use resulted in a considerably decreased resistance of Streptococcus pyogenes to erythromycin from 33.3% in 2001 to 0.2% in 2008 [21]. In addition to macrolides use of 3.55 Defined Daily Dose (DDD)/1000 inhabitants/day (which is in accordance with the European countries with medium high consumption), the percentage of Streptococcus pyogenes resistance to erythromycin has been extremely low (0.56%) on the territory of South Backa. A similar situation has been reported in Slovakia, where, in addition to macrolides use similar to the one in our district (3 DDD/1000 inhabitants/day), the level of resistance...
was the lowest among the countries of Central and Eastern Europe (less than 10%) [19]. The resistance of *Streptococcus pyogenes* to erythromycin was 6.09% in 2013, which is still a low resistance.

According to the National Guideline “Choice and Use of Antibiotics in General Practice” of the Ministry of Health of Serbia in 2004 [22], the use of natural penicillin or first generation cephalosporins has been recommended as an empirical treatment of the first choice in the cases of tonsillopharyngitis caused by *Streptococcus pyogenes*. Namely, despite the 70-year-long, widespread and very frequently uncontrolled use of penicillin, *Streptococcus pyogenes* has still remained 100 per cent susceptible to this antibiotic worldwide [6,7,8], which has also been confirmed in our research. Testing the resistance of the most common cause of tonsillopharyngitis, *Streptococcus pyogenes*, on the territory of South Backa has shown high susceptibility to natural penicillin, which means that the National Guideline is fully applicable in South Backa District.

Fast development of *Streptococcus pneumoniae* resistance to penicillin, macrolides and cephalosporines substantially affected the efficiency of treatment of streptococcus respiratory infections in recent decades. The results of analysis of *Streptococcus pneumoniae* resistance to antibacterial drugs in fifteen European countries indicate that there are significant differences among the countries - North European countries (Norway, Sweden, Denmark and the Netherlands) report a much lower degree of resistance than South and East European countries (Greece, Italy, France and the Slovak Republic). The rate of resistance to penicillin varied from 0% in Denmark to 57.1% in Greece. The rate of resistance to macrolides ranged from 6.9% in Norway to 57.1% in Greece, and the percentage of multiresistant isolates was again highest in Greece, 42.9% [4]. Furthermore, a moderate increase of resistance to penicillin, macroli-

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**Table 2. Susceptibility of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Branchamella catarrhalis* isolated from nose and throat swabs of outpatients in the South Backa District from January 2013 to March 2013, expressed as the total number of analysed strains and the percent of sensitive strains identified from nose and throat swabs.**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pyogenes</th>
<th>Branhamella catarrhalis</th>
<th>Streptococcus pneumoniae</th>
<th>Haemophilus influenzae</th>
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<tbody>
<tr>
<td></td>
<td>year/godi-</td>
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<td>year/godi-</td>
<td>year/godi-</td>
<td>year/godi-</td>
</tr>
<tr>
<td>penicillin</td>
<td>279 2.87</td>
<td>135 6.77</td>
<td>359 100 115 100.0</td>
<td>23 0.00</td>
<td>32 0.00</td>
</tr>
<tr>
<td>ampicillin</td>
<td>23 0.00</td>
<td>32 0.00</td>
<td>0.0</td>
<td>75 93.33</td>
<td>17 82.35</td>
</tr>
<tr>
<td>amoxicillin/clavulanic acid</td>
<td>23 100 32 100</td>
<td></td>
<td></td>
<td>75 100 17 94.12</td>
<td></td>
</tr>
<tr>
<td>methicillin</td>
<td>279 100 135 94.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cefaclor</td>
<td>279 94.98 135 90.37</td>
<td>359 99.44 115 93.91</td>
<td>23 95.7 32 93.75 196 75</td>
<td>31 51.61</td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>75 100 17 94.12</td>
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<tr>
<td>co-trimoxazole</td>
<td>279 99.64 135 98.51</td>
<td>23 60.9 32 93.75 196 75</td>
<td>31 67.74 39.80 17 76.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clindamycin</td>
<td>279 98.56 135 91.91</td>
<td>359 100 115 96.52</td>
<td>196 88.78 31 61.29</td>
<td></td>
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<tr>
<td>fusidic acid</td>
<td>196 100</td>
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<td>ofloxacin</td>
<td>32 100</td>
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<td>ciprofloxacin</td>
<td>31 100</td>
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<td>levofloxacin</td>
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</tbody>
</table>

n – total number of analyzed strains; % – percentage of sensitive strains
Streptococcus pneumoniae could not be specified reliably. In 2013, the resistance of the MIC of penicillin for oxacillin resistant isolates in 2008, the resistance of these isolates to penicillin cannot be specified reliably. In 2013, the resistance of Streptococcus pneumoniae to penicillin was 45.16%.

Isolates of Streptococcus pneumoniae exhibited lower susceptibility to erythromycin in both periods in comparison to the previous research (the resistance was only 10.1% in 2002). Susceptibility to co-trimoxazole was lower not only than the one found in the previous research but also in comparison with the resistance of pneumococcus in Poland, the country with the highest rate of pneumococcal resistance to this antibiotic (48.2%) in Europe, whereas susceptibility was higher in 2013 (67.74%) [4]. Susceptibility of pneumococcus to fluoroquinolones has been maintained for years in South Backa District, as well as in most European countries [4,16].

Furthermore, the results of the study in fifteen European countries indicated a significant association between the levels of antimicrobial use and the rates of antimicrobial resistance in Streptococcus pneumoniae [4]. Thus, for example, in France, the resistance of Streptococcus pneumoniae to erythromycin was 50.1%, while the overall use of macrolides was 5 DDD/1000 inhabitants/day, whereas the resistance of the same bacteria to erythromycin was 11.3% in the Netherlands, while the use of macrolides was 2 DDD/1000 inhabitants/day.

The results of our research match these results. The resistance of Streptococcus pneumoniae to erythromycin in South Backa District was 25% in 2008, while the overall use of macrolides in our area was not especially high (3.55 DDD/1000 inhabitants/day) [23], which is similar to the European countries with medium high consumption, according to annual report of European Surveillance of Antbiotics Consumption - ESAC [24]. A high resistance of Streptococcus pneumoniae to co-trimoxazole (60.2%) in 2008 can be explained in terms of higher co-trimoxazole consumption in South Backa District (1.86 DDD/1000 inhabitants/day) than the one in Finland (1.43 DDD/1000 inhabitants/day). In fact, Finland is the country with the highest outpatient consumption of this antibiotic in Europe, the rate of Streptococcus pneumoniae resistance to co-trimoxazole being 22% there. Higher susceptibility of Streptococcus pneumoniae to co-trimoxazole was found in 2013 in South Backa District compared to 2008, but because of the small number of isolates a valid conclusion cannot be reached. High susceptibility of pneumococcus isolates to fluoroquinolones has been reported despite a relatively high use of these antibiotics in our District (1.57 DDD/1000 inhabitants/day), which is in accordance with the countries in Europe with medium high consumption of fluoroquinones – Croatia (1.44 DDD/1000 inhabitants/day), Hungary (1.75 DDD/1000 inhabitants/day) [24].

Since all the isolates were susceptible to ofloxacin (as a part of fluoroquinone screening) in our investigation conducted in 2008, fluoroquinones, recommended for the treatment of respiratory infections such as moxifloxacin and levofloxacin, are expected to be effective in treatment of these infections. In 2013, isolates of Streptococcus pneumoniae were susceptible to levofloxacin (100%).

All isolates of Staphylococcus aureus obtained from the outpatients’ nose swabs in South Backa District were susceptible to methicillin. According to the available reports, there are no specific data on prevalence of methicillin resistant staphylococcus isolated from the nose swabs of outpatients in Serbia. In Cuprija, where Staphylococcus aureus was isolated from different swabs (nose, wound, eye, ear, and skin) in outpatients, the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) isolates was 17.7% [25]. In Nis, the prevalence of clinical MRSA isolates (isolates were also obtained from different swabs) was even higher - 35.31% [26]. However, not even in clinical isolates is MRSA present in such a high percentage in South Backa District as it is in other parts of Serbia, which explains their high susceptibility to methicillin. Thus, according to data from 2005, MRSA was present in 41% clinical isolates at Orthopedic Hospital in Banjica, Belgrade; in 44% at Clinical Centre Kragujevac; 49% at Military Medical Academy, Belgrade; 55% at Clinical Centre Nis; up to 81% at Clinical Centre of Serbia, in Belgrade [27]. The frequency of MRSA strains in clinical specimens obtained in hospitalized patients in 2007 at Clinical Centre of Vojvodina was only 7.5% [28].

Isolates of Haemophilus influenzae proved to be highly susceptible to all the antibiotics tested in both investigated periods. Higher susceptibility to ampicillin, amoxicillin/clavulanic acid, and cephalor was reported in comparison to the previous research (2002) in South Backa District.

High prevalence of susceptible isolates of Hae- mophilus influenzae is also reported in Italy (ampicillin 87%, amoxicillin/clavulanic acid 99.6%, cephalor 97.9% [29], whereas in England the percentage of ampicillin resistant isolates was 17.4% [30] and in the USA it was as high as 29% [13]. High susceptibility to ciprofloxacin and azithromycin is in accordance with the previous research in our District, as well as with the results of other studies [29].

All Branhamella catarrhalis isolates produced beta-lactamases which were detected using nitrocefin test, whereas they showed high susceptibility to other antibiotics in either of the periods. According to the National Guideline, Haemophi- lus influenzae, Streptococcus pneumoniae and
Branchamella catharralis are listed as the most common causes of acute sinusitis, and amoxicillin is recommended to be used as empirical treatment of primary choice. In South Backa District, the most common pathogens isolated from nose swabs were Staphylococcus aureus, Streptococcus pneumoniae and Haemophilus influenzae.

Since we do not have any data on susceptibility of Streptococcus pneumoniae to amoxicillin, what we can conclude based on susceptibility of Staphylococcus aureus and Haemophilus influenzae is that amoxicillin cannot be recommended as an antibiotic of first choice in South Backa District due to the high resistance of Staphylococcus aureus. This drug can be recommended for treatment of acute rhinosinusitis because of high susceptibility of Staphylococcus aureus to methicillin as well as of susceptibility of Staphylococcus aureus to methicillin. A second-generation cephalosporin can be recommended as its substitute.

**Conclusion**

The results show that infections of the upper respiratory tract are most frequently caused by Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae, Branchamella catharralis, and Haemophilus influenzae in South Backa District. The investigation results are in accordance with the recommendations of the National Guideline for the usage of natural penicillin in the treatment of tonsilopharyngitis.

Amoxicillin/clavulanic acid can be recommended to treat rhinosinusitis and second generation cephalosporins can be the second choice treatment.

A regular surveillance of the antimicrobial resistance patterns is very valuable not only at the international but also at national levels since these data are of great importance for the empirical use of antibiotics in areas where resistance testing is performed.

**References**


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