Introduction

Streptococcus pneumoniae (S. pneumoniae) is the cause of respiratory tract infections, otitis media, sinusitis, bacteriemia, and meningitis. Children, old people, persons with functional or anatomic asplenia and immunocompromised adults represent the most sensitive population for the development of infections caused by this bacterium.

Penicillin used to be the treatment of choice for pneumococcal infections for very long. However, the information about an ever increasing resistance...
to penicillin has caused the changes in the empirical therapeutic approach in community-acquired respiratory tract infections [1, 2]. A previous study has demonstrated that the resistance to penicillin is detected in 65% of invasive isolates obtained from pediatric patients [3]. Pneumococcal isolates demonstrating a high level of resistance to cephalosporins (minimal inhibitory concentration, (MICs ≥ 4 µg/ml)) are rare; nevertheless, they have been reported in some countries [4].

Macrolides have frequently been used and when overused, the resistance of pneumococci to these antimicrobial agents has been reported. A more recent study of the resistance of respiratory isolates of S. pneumoniae obtained from pediatric patients performed in Serbia (the territory of Belgrade) has shown the resistance to macrolides in 36.6% of the cases [5].

The data on regional or local susceptibility patterns can be very significant in the selection of empirical therapy. Limited data are available about the susceptibility of respiratory isolates in hospitalized children. Therefore, this study was aimed at examining the susceptibility pattern of pneumococcal isolates obtained from the respiratory tract of children hospitalized in the Clinical Center of Niš, south-eastern Serbia.

### Material and Methods

The study sample consisted of isolates of S. pneumoniae obtained from hospitalized children. Pneumococcal isolates were obtained from the tracheal aspirates of 190 pediatric patients admitted with respiratory tract infections. Duplicate isolates from the same patient were excluded from the analysis. The aspirates were collected in the period from January 2012 to July 2014. The patients were between 1 month and 14 years of age (median age being 3.31 years). The children were divided into three age groups: 0-1 year; 1-2 years; and ≥ 4 μg/ml)) are rare; nevertheless, they have been reported in some countries [4].

The macrolide resistance phenotype was detected by means of the double disc diffusion test, using the erythromycin and clindamycin discs placed 15 mm apart from each other. Isolates resistant to erythromycin and showing a circular zone around clindamycin were defined as the M phenotype; isolates showing a D-shaped zone of inhibition around the disc of clindamycin were defined as the MLS inducible phenotype; isolates with a circular zone of inhibition whose diameter was smaller than the diameter in the sensitive isolates around both erythromycin and clindamycin or without the zone of inhibition were defined as the constitutive (macrolide-lincosamide-streptogramin) MLS phenotype.

S. pneumoniae ATCC 49619 strain was used for the purpose of quality control.

### Results

A total of 190 isolates of S. pneumoniae were included in the study. The seasonal distribution of respiratory isolates of S. pneumoniae is given in Graph 1.

The isolates were collected from 113 (59.47%) boys and 77 (40.53%) girls. Regarding the age group distribution, 62 (32.63%) isolates were obtained from the children in the group from 0 to 1 year, 39 (20.52%) and 89 (46.84%) were taken from the children between 1 to 2 years and 2 to 14 years of age, respectively.

A total of 176 (92.63%) isolates were resistant to oxacillin (Table 1). For all isolates, the MICs to amoxicillin and ceftriaxone were determined in order to interpret the susceptibility to beta lactam

### Table 1. Resistance of S. pneumoniae to oxacillin

<table>
<thead>
<tr>
<th>Oxacllin resistant/Rezistentni na oksacilin</th>
<th>Age group/Starosne grupe</th>
<th>No of isolates (%)/Br. izolata (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No %/Ukupno Br. %</td>
<td>0 - 1 year/godine</td>
<td>1 - 2 years/godine</td>
</tr>
<tr>
<td>176</td>
<td>92.63</td>
<td>58 (32.95)</td>
</tr>
</tbody>
</table>
antibiotics. All tested isolates were susceptible to amoxicillin and ceftriaxone. For amoxicillin, minimal inhibitory concentrations inhibiting 50% of isolates (MIC$_{50}$) and 90% of isolates (MIC$_{90}$) were 0.50 μg/ml and 1.0 μg/ml, respectively (ranging from 0.016 μg/ml to 2.0 μg/ml). For ceftriaxone, MIC$_{50}$ and MIC$_{90}$ were 0.25 μg/ml and 0.50 μg/ml, respectively (ranging from 0.016 μg/ml to 1.0 μg/ml). Out of all tested isolates, 84 (44.21%) had an amoxicillin MIC ≤ 0.50 μg/ml and 144 (75.79%) MIC ≤ 1.0 μg/ml. Fifty-four (28.42%) isolates had a ceftriaxone MIC ≤ 0.25 μg/ml and 124 (65.26%) a MIC ≤ 0.50 μg/ml (Graph 2).

The susceptibility to other tested non-beta-lactam antibiotics is presented in Table 2. Out of all tested isolates, 41 (21.6%) were susceptible to erythromycin and 56 (29.47%) to clindamycin. A low rate of susceptibility to erythromycin was detected in all age groups (14.51%; 25.64%; 24.72%). There were two different phenotypes of macrolide resistance among 149 isolates of S. pneumoniae: the M phenotype was detected in 15 (10.07%) isolates and constitutive MLS resistance to erythromycin and clindamycin) in 134 (89.93%) isolates. There were 54.21% and 36.02% of isolates susceptible to tetracycline and cotrimoxazole, respectively. The susceptibility to tested antibiotics did not reveal significant differences among age groups. All tested isolates were susceptible to ofloxacin and rifampicin.

Discussion

S. pneumoniae is an organism which can be a colonizer in healthy children, but it is also one of the most common causes of respiratory infections. In case of severe respiratory tract infection, children often have to be hospitalized. The resistance of S. pneumoniae to antimicrobial agents could delay adequate treatment, which could lead to a higher risk for development of an invasive infection [7].

Detection of S. pneumoniae isolates resistant to penicillin in the 1960s, as well as the growth of resistance rates with time, caused an ever decreasing use of penicillin. Amoxicillin and cephalosporins are increasingly used in the therapy of pneumococcal infections, in addition to antibiotics from other classes [4].

The Alexander Project is the surveillance study that examined the susceptibility of bacteria causing community-acquired respiratory tract infections. The data from this study demonstrated that 95.1% of pneumococcal isolates collected in 26 countries were susceptible to amoxicillin. Only 11.5% of isolates resistant to penicillin were resistant to amoxicillin as well [8]. Moreover, orally administered amoxicillin exhibited a better pharmacokinetic profile than orally administered penicillin [9]. According to these findings, amoxicillin could be an appropriate agent for the treatment of respiratory tract infection. Since the resistance to antimicrobial agents shows geographical variations, it is essential to know the susceptibility of the etiological agent present in the local hospital. Monitoring of the resistance trends makes it possible to introduce adequate initial therapy.

In this study, we evaluated the susceptibility of respiratory isolates of S. pneumoniae isolated from hospitalized children. These are the first data on the susceptibility of non-invasive S. pneumoniae in hospitalized patients. The susceptibility to the following commonly used antibiotics was evaluated: two beta lactam antibiotics (amoxicillin and ceftriaxone), erythromycin, clindamycin, tetracycline, ofloxacin, cotrimoxazole and rifampicin.

All tested isolates were sensitive according to the CLSI breakpoints for amoxicillin for non-invasive S. pneumoniae ($S \leq 2 \mu g/ml$ to $R 8 \geq \mu g/ml$). The data from the study performed by Lismond et al., which evaluated the sensitivity of respiratory isolates of S. pneumoniae obtained from children and adults, have shown that only 3.2% of isolates are non-susceptible to amoxicillin. However, MIC$_{50}$ and MIC$_{90}$ in the isolates evaluated in this study were 0.06 μg/ml and 0.125 μg/ml, respectively, which was significantly lower than minimal inhibitory concentrations detected in our study. Similarly, Lismond et al. demonstrated that MIC$_{50}$ and MIC$_{90}$ for ceftriaxone were 0.03 μg/ml and 0.125 μg/ml, respectively, while MIC$_{50}$ and MIC$_{90}$ were 0.25 μg/ml and 0.50 μg/ml, respectively for the isolates in our study [10].

The previous study showed the antimicrobial susceptibility of the total of 2279 invasive and non-invasive isolates of S. pneumoniae collected in eight European countries. For isolates tested in this study, MIC$_{50}$ and MIC$_{90}$ to amoxicillin were 0.016 μg/ml and 1.0 μg/ml, respectively, and to cefotaxime 0.03 μg/ml and 1.0 μg/ml, respectively. The lowest values of MIC$_{50}$ and MIC$_{90}$ for amoxicillin, being 0.016 μg/ml and 0.03 μg/ml, respectively were detected in isolates obtained in Austria, Belgium and Switzerland [11].
Table 2. Susceptibility of *S. pneumoniae* to other tested non beta-lactam antibiotics by age groups

<table>
<thead>
<tr>
<th>Antibiotic agents</th>
<th>No. of isolates</th>
<th>Total susceptibility</th>
<th>Susceptibility by age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Br. izolata</em></td>
<td><em>Ukupno osetljivi</em></td>
<td><em>Osetljivost po starosnim grupama</em></td>
</tr>
<tr>
<td>Erythromycin/Eritromycin</td>
<td>190</td>
<td>41</td>
<td>21.6</td>
</tr>
<tr>
<td>Clindamycin/Klindamicin</td>
<td>190</td>
<td>56</td>
<td>29.47</td>
</tr>
<tr>
<td>Tetracycline/Tetraciklin</td>
<td>190</td>
<td>103</td>
<td>54.21</td>
</tr>
<tr>
<td>Ofloxacin/Ofloksacin</td>
<td>190</td>
<td>190</td>
<td>100</td>
</tr>
<tr>
<td>Cotrimoxazole/Kotrimoksazol</td>
<td>161</td>
<td>58</td>
<td>36.02</td>
</tr>
<tr>
<td>Rifampicin/Rifampin</td>
<td>29</td>
<td>29</td>
<td>100</td>
</tr>
</tbody>
</table>

MIC<sub>90</sub> and MIC<sub>99</sub> for pediatric isolates collected from sterile body sites in China were higher than in our study. Ma et al. demonstrated that MIC<sub>90</sub> and MIC<sub>99</sub> for amoxicillin-clavulanic acid were 0.75 μg/ml and 2.0 μg/ml, respectively, and for ceftriaxone 1.0 μg/ml and 2.0 μg/ml, respectively. In non-meningeal isolates, the resistance to ceftriaxone was detected in 3.8% [12]. A rather good sensitivity of *S. pneumoniae* to amoxicillin suggests that this antibiotic can be used as an initial therapy of respiratory tract infections. However, a study performed in France has suggested that strains may appear with high-level resistance to amoxicillin as the result of selective pressure [4].

Macrolides are antibacterial agents frequently used for the treatment of respiratory tract infections in children. Moreover, since it is difficult to differentiate the pneumococcal infections of the respiratory tract from the infections caused by atypical organisms, macrolides are often used to treat these infections in pediatric outpatients. Due to simple administration, azithromycin (once a day) is commonly used as an empirical therapy of infections of the respiratory tract in children [13]. The resistance of pneumococci to these antimicrobial agents has been reported together with overuse of macrolides [1].

Horvat et al. showed increased resistance of pneumococcal isolates obtained from outpatients of all ages, to erythromycin in the period from 2008 to 2013 [14]. The susceptibility of *S. pneumoniae* to macrolides in children has already been evaluated in Serbia, and this study included the isolates collected from various body sites. A high rate of resistance to macrolides was observed, ranging from 22.2% in 2004 to 44.9% in 2009. Macrolide resistant pneumococcal isolates showed M phenotype less frequently (27.3%) compared to constitutive MLS phenotype (72.7%) [5]. We detected a much higher rate of resistance to erythromycin and clindamycin in our isolates than in a recent study, that was 78.4% and 70.5% respectively. In our study, M phenotype was detected in only 10.07% of isolates. The majority of tested isolates in this study showed the constitutive MLS phenotype. The detected resistance rates to erythromycin and clindamycin in the studied age groups were similar. There were several possible explanations for this finding: first, our study took place a couple of years later (from January 2012 to July 2014); second, in the present study we evaluated the susceptibility of isolates obtained from the children in another part of the country, south-eastern Serbia; and third, the isolates originated only from aspirates.

A high rate of resistance to macrolides has been detected in some Asian countries, such as China (96.6%), Japan (87.9%), and Taiwan (91.6%) [12, 15, 16]. In contrast to those findings, the data from the studies in India have shown that the resistance of pneumococcal isolates to erythromycin was very low. The resistance of invasive pneumococcal isolates to erythromycin was only 4.2% in India, whereas there were no pneumococcal isolates from children with severe pneumonia that showed resistance [17, 18].

Reinert et al. have reported the susceptibility to clarithromycin of pneumococcal isolates collected in 31 centers of eight European countries and the resistance in 28% of isolates. The resistance rates showed a degree of variability among the countries. Lower resistance rates were detected in Austria, Germany and Portugal (10.0%; 10.6%; and 10.3%, respectively), in Belgium it was 23.7%; in Switzerland 17.3%, while the highest resistance rates were detected in Italy, Spain and France (35.5%; 43.6%; and 46.1%, respectively). In macrolide resistant isolates of *S. pneumoniae*, the constitutive MLS phenotype was more common than M phenotype [11].

In our study, resistance rates to cotrimoxazole and tetracycline (63.7% and 45.8%, respectively) were lower than those to erythromycin and there were no isolates resistant to ofloxacin and rifampicin. In the study of Reinert et al., susceptibility testing of *S. pneumoniae* to twelve antibiotics was performed and the highest rate of resistance was detected to cotrimoxazole in isolates collected in Austria (11.3%), Germany (14.5%), Italy (41.1%), Portugal (24.1%), Spain (66.5%) and Switzerland (28.9%) [11].

Reynolds et al. have reported that the largest increase of treatment costs was observed for the patients hospitalized with pneumococcal isolates resistant to...
The authors estimated that 32% of pediatric outpatients under 18 years of age treated with erythromycin for pneumococcal pneumonia had to be hospitalized due to inadequate treatment. In order to reduce the impact of macrolide treatment failure, the Pediatric Infectious Disease Society has suggested the use of amoxicillin in the treatment of community-acquired pneumonia, while macrolides should be administered only in case of infection with atypical microorganisms [19].

**Conclusion**

The susceptibility of non-invasive pneumococcal isolates obtained from hospitalized children was reported in this study. The high rate of resistance of *S. pneumoniae* isolates detected in our study indicated that macrolides should not be recommended as an empirical therapy of pneumococcal respiratory tract infection in our territory. Opposite to this finding, the susceptibility of all tested isolates to amoxicillin indicated that this antibiotic could be the therapy of choice in pediatric practice. Further research is warranted in order to monitor the trends of sensitivity of *S. pneumoniae*. It would also be valuable if we could ascertain whether the hospitalization of pediatric outpatients with pneumococcal respiratory tract infection is the consequence of treatment failure.

**References**
