

Short Communication

Prevalence of Macrolide Resistance in *Streptococcus pyogenes* Collected in Serbia

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SUMMARY: The purpose of our study was to determine the prevalence of macrolide resistance in 3,188 pharyngeal *Streptococcus pyogenes* isolates collected at the Institute of Public Health of Serbia during the period 2006–2008. The disk diffusion tests were used to determine the susceptibility of the isolates. Two hundred and sixteen *S. pyogenes* isolates (6.8%) were resistant to erythromycin, with 9 isolates coresistant to tetracycline: 181 isolates harbored *mefA* gene, 19 *ermB* gene, 11 *ermA*(TR) gene, 5 *ermB* and *mefA* genes, and 7 *tetM* gene. Although the prevalence of macrolide resistance in pharyngeal *S. pyogenes* isolates is low in Serbia, monitoring of the emergence of resistance is advisable.

Streptococcus pyogenes is an important etiological agent that causes a broad spectrum of infections (1). Penicillin is the drug of first choice for the treatment of infections caused by this organism (2). Macrolides are recommended as alternative treatments for patients who are allergic to penicillin. Unfortunately, the worldwide development of macrolide resistance, with wide variations according to country (2–8), may occasionally limit the use of these antibiotics. Moreover, a concomitant increase in resistance to macrolides and tetracyclines has been observed among pathogenic and commensal streptococci, mostly because their major resistance determinants are carried on the same mobile element (9). Therefore, the emergence and spread of resistance to macrolides and tetracyclines in *S. pyogenes* constitute an important problem in the management of streptococcal infections.

S. pyogenes can exhibit macrolide resistance through two main mechanisms: posttranscriptional target site modification and macrolide efflux (2). Target site modification is mediated by the *ermB* and *ermA*(TR) genes leading to macrolide, lincosamide, and streptogramin B (MLS_B) resistance phenotype and can be expressed constitutively or inducibly (4). Active efflux pump mechanism is mediated by the *mefA* gene leading to M resistance phenotype (erythromycin resistance and clindamycin susceptibility). The efflux mechanism selectively pumps 14- and 15-membered macrolides out of the cell, but not 16-membered macrolides or lincosamides. The genus *Streptococcus* can carry *tetK*, *tetL*, *tetM*, *tetO*, *tetQ*, and *tetT* genes (10). Mobile genetic elements carrying *tetM* or *tetO* sometimes harbor genes encoding macrolide resistance (11). Selection for resistance to one antibiotic can influence the evolution of resistance to another drug in multiresistant bacteria (12).

The purpose of our study was to determine the prevalence as well as phenotypic and genotypic characterization of macrolide resistance in *S. pyogenes* pharyngeal isolates collected at the Institute of Public Health of Serbia (IPHS) during the period 2006–2008.

A total of 3,188 nonduplicate pharyngeal *S. pyogenes* isolates were studied. Strains were identified by standard laboratory methods.

Disk diffusion assay, according to the recommendations of the Clinical and Laboratory Standards Institute (13), showed that 216 (6.8%) *S. pyogenes* isolates were resistant to erythromycin. There were no erythromycin-intermediate isolates found. All 216 erythromycin-resistant isolates were susceptible to penicillin, ampicillin, cefotaxime, ceftriaxone, vancomycin, and chloramphenicol. Nine of them showed coresistance to tetracycline with no tetracycline-intermediate isolates.

The phenotype of macrolide resistance was determined by the double-disk test with erythromycin (15 µg) and clindamycin (2 µg) disks (separated by 12 mm) as described previously (14). According to the double-disk test, 181 *S. pyogenes* isolates showed the M resistance phenotype, 20 the inducible MLS_B (iMLS_B) resistance phenotype, and 15 the constitutive MLS_B (cMLS_B) resistance phenotype.

All macrolide- and tetracycline-resistant isolates were screened for the causative resistance genes by polymerase chain reaction (PCR) amplification, using primers and conditions as previously described (15). One hundred and eighty-one isolates harbored *mefA* gene, 19 *ermB* gene (11 with the cMLS_B phenotype and 8 with the iMLS_B phenotype), 11 *ermA*(TR) gene, and 5 isolates harbored *ermB* and *mefA* genes simultaneously (4 with the cMLS_B phenotype and 1 with the iMLS_B phenotype) (Table 1).

Seven of 9 macrolide and tetracycline-coresistant *S. pyogenes* isolates showed iMLS_B and 2 cMLS_B resistance phenotype: 6 isolates harbored *ermB* gene (4 with the iMLS_B and 2 with the cMLS_B phenotype), while 3 isolates harbored *ermA*(TR) gene (all with the iMLS_B phenotype). The presence of *tetM* gene was detected in 7 of 9 isolates (Table 2).

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Table 1. Distribution of macrolide resistance phenotypes and erythromycin resistance genes among 216 pharyngeal *S. pyogenes* isolates collected at the Institute of Public Health of Serbia, 2006–2008

Macrolide resistance phenotype	No. of strains tested	No. of strains with:		
		<i>ermB</i>	<i>ermA(TR)</i>	<i>mefA</i>
cMLS _B ¹⁾	15	15		4
iMLS _B ²⁾	20	9	11	1
M ³⁾	181			181

¹⁾: cMLS_B, constitutive resistance to macrolides-lincosamides-streptogramin B (MLS_B).

²⁾: iMLS_B, inducible resistance to MLS_B.

³⁾: M, erythromycin resistance and clindamycin susceptibility.

Table 2. Distribution of macrolide resistance phenotypes and macrolide- and tetracycline-resistance genes among 9 pharyngeal *S. pyogenes* isolates collected at the Institute of Public Health of Serbia, 2006–2008

<i>S. pyogenes</i> isolate	Macrolide resistance phenotype	Macrolide resistance gene		Tetracycline resistance gene
		<i>ermB</i>	<i>ermA(TR)</i>	<i>tetM</i>
<i>S. p.</i> 1	iMLS _B	+		+
<i>S. p.</i> 2	iMLS _B		+	+
<i>S. p.</i> 3	iMLS _B		+	+
<i>S. p.</i> 4	cMLS _B	+		+
<i>S. p.</i> 5	iMLS _B	+		+
<i>S. p.</i> 6	iMLS _B		+	
<i>S. p.</i> 7	iMLS _B	+		
<i>S. p.</i> 8	iMLS _B	+		+
<i>S. p.</i> 9	cMLS _B	+		+

S. pyogenes has remained fully susceptible to penicillin for decades, despite the continued use of this agent as the first-line antibiotic in the treatment of infections caused by this pathogen (16). This is also confirmed by the present results *in vitro*. In our study the rate of macrolide resistance of *S. pyogenes* isolates was 6.8%. Our results correspond with the data published from France (6.2%) (17), Austria and Hungary (4.7 and 3.7%, respectively) (18). Higher rates of macrolide resistance of *S. pyogenes* isolates were found in some other countries, such as Germany (13.3%) (19), Greece (19.3%) (4), and Spain (23.5%) (2).

Our findings indicate that among the erythromycin-resistant pharyngeal *S. pyogenes* strains isolated in Serbia over the last few years, the M phenotype was detected in 83.8% of the strains, the iMLS_B phenotype in 9.3% of the strains, and the cMLS_B phenotype in 6.9% of the strains. The M phenotype was found to be predominant among the erythromycin-resistant *S. pyogenes* isolates in various countries, such as Greece (4), Austria and Hungary (18), Germany (19), Spain (2), and Argentina (8). An equal distribution of the M and iMLS_B phenotypes was reported from the European section of Turkey (15), while iMLS_B and cMLS_B phenotypes were predominant in Korea (7).

Macrolides appear to remain a useful and adequate remedy for the treatment of pharyngeal *S. pyogenes* infections in Serbia. Since changes of resistance rates and their prevailing mechanisms can occur rapidly, con-

comitant monitoring of the antibiotic consumption and emergence of resistance is advisable.

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