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The prevalence of resistance to macrolides and lincosamides among community and hospital acquired Staphylococci and Streptococci isolates in southeast Serbia

Учесталост резистенције на макролиде и линкозамиде код амбулантних и болничких изолата стафилокока и стрептокока у југоисточној Србији

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The prevalence of resistance to macrolides and lincosamides among community and hospital acquired Staphylococci and Streptococci isolates in southeast Serbia

Учесталост резистенције на макролиде и линкозамиде код амбулантних и болничких изолата стафилокока и стрептокока у југоисточној Србији

SUMMARY

Introduction/Objective The increasing resistance to macrolides and lincosamides among staphylococci and streptococci is becoming a global problem.

The aim of this study was to investigate the prevalence of macrolide-lincosamide-streptogramin (MLS) resistance phenotypes in staphylococcal and streptococcal isolates in southeast Serbia.

Methods The MLS phenotypes were determined by the double-disk diffusion method in 2121 of inpatient and outpatient staphylococcal and streptococcal isolates collected during a 1-year period at the Center for Microbiology.

Results The methicillin-resistant staphylococci isolates were significantly more resistant to penicillin, erythromycin, clindamycin, gentamicin and ciprofloxacin (100%, 100%, 29.2%, 65.6%, and 53.1%, respectively) than methicillin-sensitive (93.6%, 64.9%, 12.0%, 28.9%, and 11.7%, respectively). The inducible clindamycin resistance phenotype was dominant in *S. aureus* and coagulase-negative staphylococci isolates. *S. pneumoniae*, *S. pyogenes*, and *S. agalactiae* isolates showed very high resistance to erythromycin (77.8%, 46.2%, and 32.4%, respectively). All staphylococci and streptococci isolates were sensitive to vancomycin and linezolid, and all beta-haemolytic streptococci isolates to penicillin and ceftrixone.

Conclusion The phenotypic triage of staphylococci is necessary in order to separate inducible resistant and truly clindamycin sensitive isolates. Macrolides should not be recommended for empirical therapy of streptococcal infections. Penicillins remain the drug of choice for treatment of streptococcal infections in our local area.

Keywords: staphylococci; streptococci; MLS resistance phenotypes; inducible clindamycin resistance

САЖЕТАК

Увод/Циљ Растућа резистенција на макролиде и линкозамиде код стафилокока и стрептокока је постала глобални проблем.

Циљ ове студије је био да истражи учесталост макролид-линкозамид-стрептограмин (МЛС) фенотипова резистенције код изолата стафилокока и стрептокока у југоисточној Србији.

Методе МЛС фенотипови били су утврђени дупли-диск дифузионом методом на 2121 болничком и амбулантном изолату стафилокока и стрептокока прикупљеном током једногодишњег периода у Центру за микробиологију.

Резултати Изолати метицилин-резистентних стафилокока били су резистентнији на пеницилин, еритромицин, клиндамицин, гентамицин и ципрофлоксацин (100%, 100%, 29,2%, 65,6% и 53,1%, респективно) него метицилин-осетљиви (93,6%, 64,9%, 12,0%, 28,9% и 11,7%, респективно). Индуцибилни клиндамицин резистентни фенотип је био доминантан код изолата *S. aureus* и коагулаза-негативних стафилокока. Изолати *S. pneumoniae*, *S. pyogenes* и *S. agalactiae* показали су веома високу резистенцију на еритромицин (77,8%, 46,2% и 32,4%, респективно). Сви изолати стафилокока и стрепрокока били су сензитивни на ванкомицин и линезолид, а сви изолати бета-хемолитичких стрептокока на пеницилин и цефтриаксон.

Закључак Фенотипска тријажа стафилокока је неопходна да би се одвојили индуцибилнло резистентни од стварно клиндамицин осетљивих изолата. Макролиди се не препоручују за емпиријску терапију стрептококних инфекција. Пеницилин остаје лек избора за третман стафилококних инфекција у нашем округу.

Кључне речи: стафилококе; стрептококе; МЛС фенотипови резистенције; индуцибилна клиндамицин резистенција

INTRODUCTION

Inpatient *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* infections was the biggest problem in pre-antibiotic era [1]. Today, when large number of antibiotics are available, we are faced again with problem of treatment of infections caused by penicillin-resistant pneumococci, methicillin- and vancomycin-resistant strains of *S. aureus* and coagulase negative staphylococci (CNS) [1].

S. aureus cause a variety of infections, ranging from mild skin infections to fatal bacteremia: osteomyelitis, pneumonia, arthritis, staphylococcal scalded skin syndrome, endocarditis, myocarditis, pericarditis, and bacteremia [2, 3]. The most common CNS infections are nosocomial bacteremia related to central venous catheter, endocarditis in patients with artificial heart valves, infections from intravenous catheter insertion site, and postoperative infections in ophthalmic surgery [2]. *S. pneumoniae* bacteria can cause serious invasive infections, such as meningitis, bacteremia, and pneumonia, as well as non-invasive infections such as sinusitis and acute middle ear infections [4]. *S. agalactiae* causes serious infections in newborns and pregnant women, acute and chronic respiratory infections, endocarditis, sepsis, meningitis, and pyelonephritis [5, 6]. *S. pyogenes* causes uncomplicated upper respiratory tract and skin infections, but also severe life-threatening infections, which being very common in developing countries [7].

Macrolide and lincosamide antibiotics are often used for the treatment of staphylococci and streptococci infections. Therapeutic use of macrolide-lincosamide-streptogramin group B (MLSb) antibiotics can cause inducible macrolide-lincosamide-streptogramin group B (iMLSb) resistance and subsequent clinical failure of therapy, especially in staphylococcal infections. The iMLSb resistance phenotype leads to clindamycin treatment failure due to rapid *in vitro* conversion of inducible to constitutive macrolide-lincosamide-streptogramin group B (cMLSb) resistance phenotype.

A simple way to detect iMLSb resistant strains is double-disk diffusion method (D-test). Without the D-test, all clinical isolates with iMLSb resistance would be erroneously interpreted as clindamycin susceptible causing inappropriate antibiotic therapy.

The aim of this study was to determine and compare the prevalence of MLS resistance in staphylococcal and streptococcal isolates from inpatient and outpatient clinical samples in southeast Serbia. To determine observed MLS resistance phenotypes D-test were used.

METHODS

We analyzed 2121 clinical isolates of staphylococci and streptococci, collected during a 1-year period (October 2012 - October 2013) at the Center for Microbiology of the Public Health Institute in Vranje, Serbia, including 865 isolates from the nasal and throat swabs, 810 from the purulent discharge, 442 from the genital secretions, and 4 isolates from the urine. Multiple specimens from the same patient were avoided. The following clinical species were considered: *S. aureus*, CNS, *S. pneumoniae*, *S. agalactiae*, and *S. pyogenes*. The local Ethics Committee approved the study according to the Declaration of Helsinki (No. 01-5072/2013). The authors declare that informed consent was not required.

Bacterial identification

S. aureus was identified using Gram-stain, catalase test (positive), the mannitol salt agar (Chapman medium), and the tube coagulase test. The staphylococcal strains, which turn the color of the medium from red to yellow and produce free coagulase were identified as a *S. aureus*, else were identified as CNS [2]. *S. pneumoniae* was identified using Gram stain, catalase (negative), and optochin test (BioRad, USA). The slide agglutination test was used as confirmatory identification of *S. pneumoniae* (Slidex pneumo-kit; bioMérieux, France) [8]. *S. agalactiae* was identified using Gram stain, catalase test (negative), CAMP test, and rapid latex agglutination test (Streptex-Slidex® Strepto Plus-BioMérieux, France) [8]. The identification of *S. pyogenes* was performed using Gram stain, catalase test (negative), the susceptibility test to bacitracin (0.04 UI, Taxo A, BBL Microbiology system), and rapid latex agglutination test (Streptex-Slidex® Strepto Plus-BioMérieux, Marcy l'Etoile, France) [8].

Antibiotic susceptibility testing

The antibiotic susceptibility test was performed by the standard disk diffusion method using Muller Hinton agar according to the CLSI guidelines [9]. The following antibiotic discs were used: Erythromycin 15 µg, Clindamycin 2 µg, Gentamicin 10 µg, Ciprofoxacin 5 µg, Penicillin G 10 µg, Ceftriaxone 30 µg, Cefoxitin 30 µg, Vancomycin 30 µg, Linezolid 30 µg (Bioanalyse®, Ankara, Turkey). Methicillin resistance in staphylococci was determined by cefoxitin disk diffusion method (30 µg) [9]. Penicillin-susceptible Staphylococcus isolates were further tested for beta-lactamase production using a nitrocefin disk test (Bioanalyse®, Ankara, Turkey) [2]. Reference strains *S. pneumoniae* ATCC 49619 and *S. agalactiae* ATCC 12403 were used for quality control (QC). QC of erythromycin and clindamycin disks was performed by reference *S. aureus* ATCC 25923 strain according to a standard disk diffusion QC procedure [9]. In addition, QC was also performed with laboratory owns strains of *S. aureus* and *S. pyogenes* which show both results of positive and negative D-test.

Determination of resistance phenotypes

MLSb resistance phenotypes were determined by the D-test. Erythromycin (15µg) and clindamycin (2µg) disks were placed at an edge-to-edge distance of 12 mm on inoculated Mueller-Hinton agar. The following MLS resistance phenotypes were detected: erythromycin-sensitive and clindamycin-sensitive (Er/Cli S), cMLSb which were resistant to erythromycin and clindamycin, iMLSb which were determined by placing erythromycin and clindamycin disks in adjacent positions resulting in a D-shaped zone around the clindamycin disk, susceptible to clindamycin (without blunting zone) and resistant to erythromycin (M/MSb), and resistant to clindamycin and sensitive to erythromycin (LSa/b).

RESULTS

The overall antimicrobial resistance of tested isolates is presented in Table 1, except for vancomycin, linezolid, and ceftriaxone because resistance to vancomycin and linezolid among staphylococci and streptococci, and resistance to ceftriaxone among streptococci were not detected.

Staphylococci showed the highest resistance rate to penicillin, while the lowest showed *S. pyogenes* and *S. agalactiae* isolates (Table 1). The highest resistance rate to erythromycin showed MRSA (86.2%, 112/130 community- and 87.5%, 28/32 hospital-acquired) and MRCNS (87.8%, 43/49 community- and 100%, 22/22 hospital-acquired) isolates, while the lowest showed *S. agalactiae*. The highest resistance rates to clindamycin were among community-associated strains of *S. pneumoniae* (38.2%, 21/55) and MRSA (29.2%, 38/130), while the lowest were among community-associated strains of MSSA and MSCNS. The highest resistance rate to gentamicin showed *S. agalactiae* (72.7%, 101/139 community- and 72.7%, 8/11 hospital-associated) and MRSA (65.6%, 21/32 hospital-acquired) isolates, while the lowest showed MSSA and MSCNS isolates. The highest resistance rate to ciprofloxacin showed MRSA (40.8%, 53/130 community- and 53.1%, 17/32 hospital-acquired) and MRCNS (34.7%, 17/49 community- and 40.9%, 9/22 hospital-acquired) isolates, while the lowest resistance rate showed *S. pneumoniae* and MSSA isolates (Table 1).

Table 1. Antimicrobial resistance rates among community- and hospital-acquired stpahylococci and streptococci isolates.

Bacteria	Cefoxitin		Penicillin		Erythromycin		Clindamycin		Gentamicin		Ciprofloxacin	
	Comm. n/N (%)	Hosp. n/N (%)	Comm. n/N (%)	Hosp. n/N (%)	Comm. n/N (%)	Hosp. n/N (%)	Comm. n/N (%)	Hosp. n/N (%)	Comm. n/N (%)	Hosp. n/N (%)	Comm. n/N (%)	Hosp. n/N (%)
S. aureus	130/784 (16.6)	32/160 (20.0)	723/784 (92.2)	142/160 (88.8)	464/784 (59.2)	94/160 (58.8)	68/784 (8.7)	10/160 (6.3)	159/784 (20.3)	56/160 (35.0)	96/784 (12.2)	23/160 (14.4)
MRSA	130/130 (100.0)	32/32 (100.0)	130/130 (100.0)	32/32 (100.0)	112/130 (86.2)	28/32 (87.5)	38/130 (29.2)	8/32 (25.0)	58/130 (44.6)	21/32 (65.6)	53/130 (40.8)	17/32 (53.1)
MSSA	0/654 (0.0)	0/128 (0.0)	593/654 (90.7)	110/128 (85.9)	352/654 (53.8)	66/128 (51.6)	30/654 (4.6)	2/128 (1.6)	100/654 (15.3)	37/128 (28.9)	40/654 (6.1)	5/128 (3.9)
CNS	49/583 (8.4)	22/116 (19.0)	527/583 (90.4)	110/116 (94.8)	343/583 (58.8)	83/116 (71.6)	74/583 (12.7)	11/116 (9.5)	112/583 (19.2)	29/116 (25.0)	58/583 (9.9)	19/116 (16.4)
MRCNS	49/49 (100.0)	22/22 (100.0)	49/49 (100.0)	22/22 (100.0)	43/49 (87.8)	22/22 (100.0)	10/49 (20.4)	1/22 (4.5)	28/49 (57.1)	9/22 (40.9)	17/49 (34.7)	9/22 (40.9)
MSCNS	0/534 (0.0)	0/94 (0.0)	478/534 (89.5)	88/94 (93.6)	300/534 (56.2)	61/94 (64.9)	64/534 (12.0)	10/94 (10.6)	82/534 (15.4)	20/94 (21.3)	41/534 (7.7)	11/94 (11.7)
S. pneumoniae	14/55 (25.5)	7/9 (77.8)	5/55 (9.1)	1/9 (11.1)	35/55 (63.6)	7/9 (77.8)	21/55 (38.2)	2/9 (22.2)	24/55 (43.6)	3/9 (33.3)	0/55 (0.0)	0/9 (0.0)
S. agalactiae	-	-	0/139 (0.0)	0/11 (0.0)	45/139 (32.4)	2/11 (18.2)	30/139 (21.6)	1/11 (9.1)	101/139 (72.7)	8/11 (72.7)	45/139 (32.4)	4/11 (36.4)
S. pyogenes	-	-	0/238 (0.0)	0/26 (0.0)	104/238 (43.7)	12/26 (46.2)	40/238 (16.8)	7/26 (26.9)	79/238 (33.2)	10/26 (38.5)	51/238 (21.4)	6/26 (23.1)

MRSA - Methicillin-resistant *S. aureus*; MSSA - Methicillin-susceptible *S. aureus*; CNS - Coagulase negative staphylococci; MRCNS - Methicillin-resistant coagulase negative staphylococci; MSCNS - Methicillin-susceptible coagulase negative staphylococci

Comparison between hospital- and community-associated isolates showed significant ($p < 0.05$) higher resistance rate to gentamicin in hospital- than community-associated *S. aureus*, MSSA, and MRSA isolates (Table 1). MRSA compared to MSSA hospital- and community-acquired isolates showed significant ($p<0.05$) higher resistance rate to all observed antibiotics. CNS isolates showed significant ($p<0.05$) higher resistance rate to cefoxitin and erythromycin in hospital- than community-associated isolates. MRCNS compared to MSCNS community-acquired isolates showed significant ($p<0.05$) higher resistance rate to penicillin and gentamicin. MRCNS compared to MSCNS community- and hospital-acquired isolates showed significant (p<0.05) higher resistance to cefoxitin, erythromycin, and ciprofloxacin. Comparison between *S. pneumoniae* isolates showed significant (p<0.05) higher resistance rate to cefoxitin in hospital- than community-associated isolates. Significant differences (p<0.05) were between *S. pneumoniae* and *S. agalactiae* to penicillin, clindamycin, gentamicin, and ciprofloxacin (in community-acquired isolates), and to erythromycin (in community- and hospital-acquired isolates); between *S. pneumoniae* and *S. pyogenes* to penicillin, erythromycin, and clindamycin (in community-acquired isolates); between *S. agalactiae* and *S. pyogenes* to penicillin, gentamicin, and ciprofloxacin (in community-acquired isolates) (Table 1).

The iMLSB was the most prevalent phenotype among methicillin-resistant and methicillin-susceptible staphylococci except among hospital-acquired MSCNS strains, where M/MSb resistance phenotype was dominant (Table 2). The cMLSb phenotype was the most prevalent in MRSA strains (27.7%, 36/130 from outpatient and 21.9%, 7/32 inpatient specimens). LSa/b phenotype was the rarest among all of MLS resistance phenotypes and most common in MRSA strains from inpatient samples, and MSCNS and MSSA strains from outpatient samples.

Table 2. The frequency of MLS resistance phenotypes among community- and hospital-acquired stpahylococci isolates.

Phenotypes	MRSA			MSSA			MRCNS			MSCNS		
	Comm. n (%)	Hosp. n (%)	*p*	Comm. n (%)	Hosp. n (%)	*p*	Comm. n (%)	Hosp. n (%)	*p*	Comm. n (%)	Hosp. n (%)	*p*
Er/Cli S	16 (12.3)	3 (9.4)	0.768	299 (45.7)	62 (48.4)	0.628	6 (12.2)	0 (0.0)	0.167	225 (42.1)	32 (34.0)	0.172
cMLSb	36 (27.7)	7 (21.9)	0.656	27 (4.1)	2 (1.6)	0.205	10 (20.4)	1 (4.5)	0.154	55 (10.3)	9 (9.6)	1.00
M/MSb	16 (12.3)	10 (31.3)	0.014	91 (13.9)	24 (18.8)	0.172	13 (26.5)	10 (45.5)	0.169	98 (18.4)	27 (28.7)	0.024
iMLSb	60 (46.2)	11 (34.4)	0.242	234 (35.8)	40 (31.3)	0.362	20 (40.8)	11 (50.0)	0.605	147 (27.5)	25 (26.6)	0.900
LSa/b	2 (1.5)	1 (3.1)	0.485	3 (0.5)	0 (0.0)	1.00	0 (0.0)	0 (0.0)	1.00	9 (1.7)	1 (1.1)	1.00
Total	130 (100.0)	32 (100.0)		654 (100.0)	128 (100.0)		49 (100.0)	22 (100.0)		534 (100.0)	94 (100.0)	

MRSA - Methicillin-resistant *S. aureus*; MSSA - Methicillin-susceptible *S. aureus*; MRCNS - Methicillin-resistant coagulase negative staphylococci; MSCNS - Methicillin-susceptible coagulase negative staphylococci; Er/Kli S - Susceptibility to erythromycin and clindamycin; cMLSb - Constitutive resistance to Macrolide-Lincozamide-Streptogramin B; M/MSb - Resistance to Macrolide/Macrolide-Streptogramin B; iMLSb - Inducible resistance to Macrolide-Lincozamide-Streptogramin B; LSa/b - Resistance to Lincozamide-Streptogramin A/Streptogramin B

Comparison between inpatient and outpatient isolates showed significant (p<0.05) difference in MRSA and MSCNS isolates with M/MSb phenotype (Table 2). Comparison between MRSA and MSSA isolates showed significant (p<0.05) difference among community-acquired isolates in frequency of Er/Cli S, cMLSb, and iMLSb phenotypes, and among hospital-acquired isolates in frequency of Er/Cli S and cMLSb phenotypes. Comparison between MRCNS and MSCNS isolates showed significant (p<0.05) difference among community-acquired isolates in prevalence of Er/Cli S, and among hospital-acquired isolates in prevalence of Er/Cli S and iMLSb phenotypes (Table 2).

The cMLSb was the most prevalent phenotype among *S. pneumoniae* from outpatient isolates, among *S. agalactiae* from inpatient and outpatient isolates, and among *S. pyogenes* from inpatient isolates (Table 3). The M/MSb was the most prevalent phenotype among *S. pneumoniae* from inpatient isolates, and among *S. pyogenes* from outpatient isolates.

Table 3. The frequency of MLS resistance phenotypes among community- and hospital-acquired streptococci isolates.

Phenotypes	*S. pneumoniae*			*S. agalactiae*			*S. pyogenes*		
	Comm. n (%)	Hosp. n (%)	*p*	Comm. n (%)	Hosp. n (%)	*p*	Comm. n (%)	Hosp. n (%)	*p*
Er/Cli S	20 (36.4)	2 (22.2)	0.706	85 (61.2)	9 (81.8)	0.211	134 (56.3)	14 (53.8)	0.837
cMLSb	21 (38.2)	2 (22.2)	0.469	21 (15.1)	1 (9.1)	1.00	40 (16.8)	7 (26.9)	0.276
M/MSb	9 (16.4)	3 (33.3)	0.351	18 (12.9)	1 (9.1)	1.00	45 (18.9)	5 (19.2)	1.00
iMLSb	5 (9.1)	2 (22.2)	0.253	6 (4.3)	0 (0.0)	1.00	19 (8.0)	0 (0.0)	0.232
LSa/b	0 (0.0)	0 (0.0)	1.00	9 (6.5)	0 (0.0)	1.00	0 (0.0)	0 (0.0)	1.00
Total	55 (100.0)	9 (100.0)		139 (100.0)	11 (100.0)		238 (100.0)	26 (100.0)	

Er/Kli S - Susceptibility to erythromycin and clindamycin; cMLSb - Constitutive resistance to Macrolide-Lincozamide-Streptogramin B; M/MSb - Resistance to Macrolide/Macrolide-Streptogramin B; iMLSb - Inducible resistance to Macrolide-Lincozamide-Streptogramin B; LSa/b - Resistance to Lincozamide-Streptogramin A/Streptogramin B

There was no significant (p>0.05) difference between community- and hospital-acquired streptococci isolates in frequency of MLS resistance phenotypes (Table 3). Comparison between *S. pneumoniae* and *S. agalactiae* showed significant (p<0.05) difference among community-acquired isolates in frequency of Er/Cli S and cMLSb phenotypes, and among hospital-acquired isolates in frequency of Er/Cli S. Comparison between *S. pneumoniae* and *S. pyogenes* showed significant (p<0.05) difference among community-acquired isolates in frequency of Er/Cli S and cMLSb phenotypes (Table 3).

DISCUSSION

Development of antimicrobial resistance in staphylococci and streptococci include the emergence of multidrug-resistant bacteria. Initially, MRSA strains mainly caused hospital infections [10]. However, since about a decade ago, the number of community-acquired MRSA strains has significantly increased in a number of countries [10].

All of our staphylococcal and streptococcal isolates were susceptible to vancomycin and linezolid, and all of beta-haemolytic streptococcal isolates were susceptible to penicillin and ceftriaxone, similar to other researchers [11-13].

In our study, 20.0% (32/160) of hospital-associated and 16.6% (130/784) of community-associated *S. aureus* isolates were resistant to methicillin, with no significant difference in prevalence between hospital and community MRSA strains. The prevalence of hospital-associated MRSA strains in Belgium, Bulgaria and France based on 2015 surveillance data were similar to ours, whereas in Romania, Malta, Portugal, Cyprus and Greece were much higher (over 30%) [3]. We have found that 8.4% (49/583) in community-acquired and 19.0% (22/116) of hospital-acquired MRCNS isolates, while Považan et al. have found 62.2% (122) MRCNS isolates from hemocultures sampled from inpatients of the Institute for Pulmonary Diseases of Vojvodina [14]. In our study, all of MRSA and MRCNS isolates were resistant to penicillin which was in accordance with global report of antimicrobial susceptibility testing [10]. More than half of Staphylococcus isolates in our study were resistant to erythromycin, similarly with global macrolide resistance rate in staphylococci [15]. We have found that more than 85% MRSA and MRCNS isolates have shown significantly higher resistance to erythromycin than the MSSA and MSCNS isolates (about 55%). Similar data have been reported in other regions of Serbia and Greece [14, 16]. We have found high prevalence of resistance to clindamycin, gentamicin, and ciprofloxacin among community- and hospital-associated MRSA and MRCNS isolates, and low among MSSA and MSCNS isolates, similarly with other studies [11, 17]. We didn't find significant difference between community- and hospital-acquired *S. aureus* isolates in resistance to all antimicrobial agents, except to gentamicin (for both MRSA and MSSA isolates). Also, among our CNS isolates, there were significant more inpatient isolates resistant to cefoxitin and erythromycin than outpatient isolates. Both hospital- and community-acquired MRSA showed higher resistance rates to all tested antimicrobial agents than MSSA isolates, and MRCNS showed higher resistance rates to all antibiotics than MSCNS isolates (except inpatient isolates to clindamycin), as well as in study by Kim and others [17]. However, Považan and others [14] found extremely higher resistance rates to clindamycin, gentamicin, and ciprofloxacin among their hospital-acquired MRCNS strains (more than 70%) in relation to ours.

Generally, the iMLSb was the most frequent phenotype among methicillin-resistant (about 40%) and methicillin-susceptible staphylococci (about 30%) except outpatient MSCNS isolates, where the M/MSb phenotype was dominant (28.7%), similar to studies from different geographic locations [11,18]. In Europe, there was a high prevalence (more than 80%) of the cMLSb phenotype in MRSA, whereas the iMLSb was dominant in MSSA isolates[15,16]. In our study, there were no significant difference of prevalence of MLS phenotypes between inpatient and outpatient staphylococci isolates, except for M/MSb phenotype which was significantly prevalent in inpatient than outpatient MRSA and MSCNS isolates. Among all of MLS phenotypes, the rarest LSa/b was found in MRSA, MSSA, and MSCNS isolates, as well as in France and Czech Republic [19,20]. One of MSSA isolates was different from others LSa/b phenotypes by channel of sensitivity between clindamycin and erythromycin disc, and it looked like "keyhole". In South Korea similar novel phenotype has been described in 46 of *S. agalactiae* isolates [5].

There were not significant differences between our community- and hospital-associated *S. pneumoniae* isolates in their resistance to antibiotics. Only the small percentage of our *S. pneumoniae* isolates showed resistance to penicillin (9.1%, 5/5 community- and 11.1%, 1/9 hospital-acquired), while Mladenović-Antić and others discovered higher resistance to penicillin (27%) in hospital-acquired pneumococci isolates in the first decade of this century in Nišava region, Serbia [21]. In our region, we discover a very high resistance rate to erythromycin in *S. pneumoniae* (63.6%, 35/55 community- and 77.8%, 7/9 hospital-acquired isolates), which was in accordance with findings by Dinić and others (78.4% and 65.6%, respectively) [22]. However, Hadnađev and Mijač and others found lower rate of resistance to erythromycin in *S. pneumoniae* (36% and 45%, respectively) in their studies in Serbia [4, 23]. Some parts of Malta and Romania had similar prevalence rate of macrolide resistance among *S. pneumoniae* in 2012 and 2015 to ours findings. Wide inter-country variations in the emergence of macrolide-resistant *S. pneumoniae* were recorded across Europe, with prevalence ranging from 0% to 74% in period from 2012 to 2015 [3]. Also, a very high resistance rate to clindamycin among our community-associated strains of *S. pneumoniae* (38.2%, 21/55) was detected, while neither one of our *S. pneumoniae* isolates showed resistance to ciprofloxacin, which was similar to other researches from Serbia [22, 24].

In our region, cMLSb phenotype was the most prevalent (38.2%) of all *S. pneumoniae* isolates from outpatient samples, whereas the M/MSb (33.3%) was dominant among hospital-acquired isolates. Different from our findings, the dominant MLS resistance phenotype was cMLSb among hospital isolates of *S. pneumoniae* in Nišava district and central and northern parts of Serbia [22, 23, 25], but authors from Italy [26] yielded results similar to our findings.

There were no *S. agalactiae* isolates resistant to penicillin and ceftriaxone as well as in Italy [13]. Our *S. agalactiae* isolates showed relatively high resistance rates to erythromycin (32.4%, 45/139 community- and 18.2%, 2/11 hospital-acquired) and clindamycin (21.6%, 30/139 community- and 9.1%, 1/11 hospital-acquired). In Italy [13], the same resistance to erythromycin (19%) was observed among *S. agalactiae* isolates as well as in Spain [6], but the resistance to clindamycin was significantly higher (53%). There was a similarity between our region and regions of the United States regarding resistance rate to erythromycin among *S. agalactiae* isolates (ranged from 38% to 41.9%) [27]. In our area were found very high resistance rate to gentamicin (about 70%) and ciprofloxacin (about 30%) among both community- and hospital-associated *S. agalactiae* isolates.

The cMLSb resistance phenotype was dominant among *S. agalactiae* community-acquired strains, whereas the same proportions of cMLSb and M/MSb were found as the commonest resistance phenotype among hospital-acquired *S. agalactiae* isolates, consistent with other studies [13, 27]. We detected a small percentage of rare LSa/b resistance phenotype (6.5%, 9/139) among community-acquired *S. agalactiae* isolates, similar with other study [13]. Resistance rate to macrolides and lincosamides in *S. agalactiae* has been steadily increased, although it varies greatly between regions [13].

We didn't find resistant strain to penicillin among *S. pyogenes* isolates, so it would be remained the first-line antibiotic in the treatment of *S. pyogenes* infections [28]. The very high resistance rates to erythromycin among ours *S. pyogenes* (43.7%, 104/238 community- and 46.2%, 12/26 hospital-acquired) isolates were found, while the reported resistance rate to erythromycin among community-acquired *S. pyogenes* isolates in Serbia from 2004 to 2009 was only 19% [4]. Resistance rates to erythromycin, clindamycin, gentamicin and ciprofloxacin among our *S. pyogenes* isolates were higher than in other parts of Serbia and some European countries [7, 29]. The very high resistance to erythromycin among our *S. pyogenes* isolates can be explained by uncontrolled and excessive consumption of total macrolide and long-acting macrolides (i.e. azithromycin) and other antibiotics in Southeast Serbia.

Dominance of M/MSb phenotype among community-acquired *S. pyogenes* isolates observed in our study, correspond well to results of many other studies [25, 28, 29]. Also, cMLSb was the most common resistance phenotype among our hospital-associated *S. pyogenes* isolates. However, MLS phenotype is increasingly reported in Europe [7].

In general, the resistance rates to macrolides and lincosamides showed wide variations in bacterial species and geographical region. These variations were mostly developed because of differences in antimicrobial use, infection prevention, and infection control practices in different regions. Monitoring the frequency of staphylococcal and streptococcal resistance to macrolides and lincosamides and various mechanisms of resistance at the local level is essential for determining empirical therapy. Physicians should consider local and regional resistance patterns when they choose an appropriate medication for the treatment of both inpatient and outpatient staphylococcal and streptococcal infections.

CONCLUSION

This study is the first extensive report on macrolide and lincosamide resistance of common hospital- and community-associated staphylococcal and streptococcal isolates in Southeast Serbia. Our results indicated that there was a significant prevalence of the iMLSb resistance phenotype in all inpatient and outpatient staphylococcal isolates and phenotypic triaging of all staphylococci is necessary in order to distinguish between inducible resistant and truly clindamycin susceptible isolate. The methicillin-resistant inpatient and outpatient staphylococci isolates were significantly more resistant to penicillin, erythromycin, clindamycin, gentamicin and ciprofloxacin than methicillin-sensitive. Our findings also indicate a very high resistance to macrolides in both inpatient and outpatient *S. pneumoniae*, *S. pyogenes*, and *S. agalactiae* isolates, that reached 77.8%, 46.2% and 32.4%, respectively, so these antibiotics should not be recommended for empirical therapy of infection caused by these bacteria. Penicillins remain the drugs of choice for treatment of streptococcal infections in our local area. Because of constant changes of resistance rates to antibiotics, survey of the antibiotic usage and development of resistance is recommended.

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