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Original Article

Prevalence of inducible clindamycin resistance among community-associated staphylococcal isolates in central Serbia

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Abstract

The emergence of resistance to most antimicrobial agents in staphylococci indicates the need for new effective agents in the treatment of staphylococcal infections. Clindamycin is considered to be one safe, effective and less costly agent. We analysed 482 staphylococcal isolates. Detection of inducible clindamycin resistance was performed by the D-test, while the presence of methylases genes: *erm* (A), *erm* (B) and *erm* (C), as well as, macrolide efflux gene *mef* was determined by polymerase chain reaction. Inducible clindamycin resistance phenotype was significantly higher in *Staphylococcus aureus* (*S. aureus*) strains then in coagulase-negative staphylococci (CNS). Among analysed *S. aureus* isolates, the predominance of the *erm* (C) gene, followed by the *erm* (A) gene were detected. These results indicate that the D-test should be routinely performed on each staphylococcal isolates.

Key words: *Coagulase-negative staphylococci, inducible clindamycin resistance, Staphylococcus aureus*

Introduction

The increasing prevalence of resistance to most antimicrobial agents in staphylococci, especially spread of resistant strains in the community, signify the need for new effective agents to treat staphylococcal infections. Widespread use of macrolide–lincosamide–streptogramin (MLS) antibiotics, has led to an increase in a number of staphylococci acquiring cross-resistance to MLS antibiotics.[1] This cross-resistance to MLS antibiotic (MLS resistance) in staphylococci, is generally attributable to one of two mechanisms.

First is an active efflux, due to energy-dependent pump, which expels antimicrobial agents from the bacterial cell. Efflux mechanism is encoded by *mrs (A)* gene and confers resistance to macrolides and type B streptogramin, but clindamycin remains active (MS_B resistance). Second mechanism is modification of the drug-binding site on the bacterial ribosome, mediated by ribosomal methylases, which leads to the reduced binding of MLS antibiotics. Ribosomal methylases are encoded by *erm* genes (*erm* (A) or *erm* (C) in staphylococci) and results in resistance to macrolides, lincosamides and type B streptogramin (MLS_B resistance).[2,3]

Phenotypic expression of this resistance can be constitutive ($cMLS_B$ resistance phenotype) and inducible ($iMLS_B$ resistance phenotype). In inducible expression, the bacteria produce inactive methylase mRNA that is unable to translate in to ribosomal methylase, but becomes active only in the presence of a strong inducer, such as erythromycin. By contrast, in constitutive expression, active methylase mRNA is produced in continuity, and in the absence of an inducer. *In vitro*, staphylococcal isolates with $cMLS_B$ phenotypes are resistant to all MLS_B antibiotics, whereas those with $iMLS_B$ phenotypes demonstrate resistance to macrolides, while appearing susceptible to lincosamides and type B streptogramin.[3,4]

To detect $iMLS_B$ resistant strains, there are special disk-approximation test that involve the placement of an erythromycin disk in close proximity to a disk containing clindamycin. As the erythromycin diffuse through the agar, resistance to the clindamycin is induced, giving a D shape to the zone of clindamycin disk.[4]

Published data of inducible clindamycin resistance among pathogen staphylococcal isolates in Serbia, to the best of our knowledge, are missing. Because of that, the present study was aimed, to investigate MS_B and MLS_B resistance and to record the current trend in regard to the prevalence and distribution of inducible

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clindamycin resistance among community-associated (CA) *Staphylococcus aureus* (*S. aureus*) and coagulase-negative staphylococci (CNS) in region of central Serbia.

Materials and Methods

Bacterial isolates

We analysed 482 staphylococcal isolates, collected from the Public Health Institute, Kragujevac. The Public Health Institute in Kragujevac provides microbiology laboratory services to primary health care centre (e.g. general practitioner, preventative care) in an outpatient setting. Staphylococcal isolates were labelled as community-associated staphylococcal isolates if the source patient had none of the following risk factors: a history of hospitalisation or surgery within one year prior to the date of specimen collection, haemodialysis and residence in long-term care facility. Staphylococcal isolates were collected from different patient samples (pus, wound swab, aspirates, eye swab, sputum, nasal and throat swab). Strains were identified using conventional bacteriological methods, and their susceptibility testing was first performed by the standard disk diffusion method on Mueller–Hinton agar (MHA), according to the standards of Clinical and Laboratory Standards Institute (CLSI).[5] Each erythromycin-resistant clindamycin susceptible isolates were further tested for inducible resistance with D-test, as described in the CLSI recommendations. Isolates with the MS_B resistance, demonstrated circular clindamycin zone, while isolates with the $iMLS_B$ phenotype, demonstrated a flattening of the clindamycin zone. Isolates resistant to both erythromycin and clindamycin confers to $cMLS_B$ resistance phenotype.

Detection of resistance genes

Polymerase chain reaction (PCR) was performed on 20 *S. aureus* strains displaying MLS_B resistance phenotype. Genomic DNA was extracted from staphylococcal overnight cultures using a QIAamp DNA Mini Kit (Qiagen) following protocol for isolation of genomic DNA from Gram-positive bacteria. Presence of erythromycin resistance methylase *erm* genes *erm* (A), *erm* (B) and *erm* (C), as well as macrolide efflux gene *mef* was determined by PCR using the primer pairs as described by Lim *et al.*,[6] All primers were synthesised by Invitrogen. Each reaction was carried out in a final volume of 50 µl using 2 µl of template, 1 µl of each primer (100 µM), 25 µl of Maxima® Hot Start Green PCR Master Mix (Fermentas) and 21 µl of PCR grade water. PCR conditions consisted of a denaturation step (4 min at 94°C), followed by 35 cycles at 94°C for 30 s of denaturation, 30 s of annealing at 52°C, 1 min of elongation at 72°C and a final extension step (5 min at 72°C). Amplified products were detected by gel electrophoresis on E-Gel iBase (Invitrogen) in 2% (w/v) agarose gels (E-Gel® 2%, Invitrogen) and visualised in Gel Doc XR system (Bio-Rad). The size of the PCR products was determined by comparison with a 100-1000 bp molecular weight marker O'GeneRuler™ 100 bp DNA Ladder (Fermentas). Positive control strains used in this study were: *Escherichia coli* NM522 (*erm*A), *Bacillus subtilis* 168 (*erm*B), *Bacillus subtilis* BD170 (*erm*C), *Streptococcus pyogenes* 02C1064 (*mef*).

Data were analysed using Chi-squared test ($\chi 2$) and the probability level of 0.05 was considered to be statistically significant.

Results

Our study included 482 staphylococcal isolates, of which 395 (82%) were *S. aureus* and 87 (18%) were CNS. Of the 482 staphylococcal isolates, 191 (40%) were resistant to erythromycin (ERY-R).

In order to accurate insight into the possibility of misinterpretation of staphylococcal resistance to clindamycin, we performed D-test and found that majority of ERY-R *S. aureus* isolates, display $iMLS_B$ phenotypes, while most of ERY-R CNS, show MS_B phenotypes [Table 1]. Truly susceptible isolates (MS_B phenotype) were significantly higher in CNS strains then in *S. aureus* strains ($\chi 2 = 13.19$; $P < 0.001$). Falsely susceptible isolates ($iMLS_B$ phenotype) were significantly higher in *S. aureus* strains then in CNS strains ($\chi 2 = 16.52$; $P < 0.001$). However, no statistically significant difference of truly resistant isolates ($cMLS_B$ phenotype) was observed between *S. aureus* and CNS isolates ($\chi 2 = 0.20$; $P = 0.655$).

The predominant genes associated with macrolide resistance among *S. aureus* were the *erm* (C) and the *erm* (A), which were detected in 55% (11/20) and 40% (8/20) of the isolates, respectively [Table 2]. The *erm* (C) determinant was found in six (60%) $cMLS_B$ *S. aureus* isolates and one of these strains also contained *erm* (A). Similarly, half of $iMLS_B$ strains harboured *erm* (C) gene. The distribution of *erm* (A) gene in $cMLS_B$

Table 1: Distribution of the MS_B, $iMLS_B$ and $cMLS_B$ resistance phenotypes among erythromycin resistant *S. aureus* and CNS isolates

	n (%)		
	ERY-R; *S. aureus*	ERY-R; CNS	Total
MS_B	31 (24)	32 (52)	63 (33)
$iMSL_B$	64 (50)	11 (18)	75 (39)
$cMLS_B$	34 (26)	19 (30)	53 (28)
Total	129 (100)	62 (100)	191 (100)

*ERY-R: Erythromycin resistant, MS_B: Macrolides and type B streptogramin resistance, $iMSL_B$: Inducible macrolides, lincosamides and type B streptogramin resistance, $cMLS_B$: Constitutive macrolides, lincosamides and type B streptogramin resistance, CNS: Coagulase-negative staphylococci

Table 2: Distribution of the *erm* and *mef* genes among 20 MLS_B *S. aureus* isolates

	n (%)		
	$cMLS_B$ *S. aureus*	$iMLS_B$ *S. aureus*	Total
erm (A)	3 (30)	4 (40)	7 (35)
erm (B)	0 (0)	0 (0)	0 (0)
erm (C)	5 (50)	5 (50)	10 (50)
mef	0 (0)	0 (0)	0 (0)
erm (A)+ *erm* (C)	1 (10)	0 (0)	1 (5)
Not detected	1 (10)	1 (10)	2 (10)
Total	10 (100)	10 (100)	20 (100)

$iMLS_B$: inducible macrolides, lincosamides and type B streptogramin resistance, $cMLS_B$: constitutive macrolides, lincosamides and type B streptogramin resistance, MLS_B: macrolides, lincosamides and type B streptogramin resistance

and $iMLS_B$ *S. aureus* isolates was identical since both phenotypes carried this gene in 40% of the cases. Two of the MLS_B isolates did not harbour any of the resistance genes. Among tested isolates *erm* (B) and *mef* genes were not detected [Figure 1].

Discussion

One of the major concerns with regard to the therapeutic use of clindamycin in staphylococcal infection is the possible presence of inducible resistance to clindamycin, and subsequent clinical failure of therapy.[7]

Simple laboratory testing, (i.e. erythromycin-clindamycin 'D-zone' test) can separate strains that have the genetic potential to become resistant during therapy from strains that are truly susceptible to clindamycin. Without the double-disk test, all staphylococcal isolates with $iMLS_B$ would have been mistakenly interpreted as clindamycin-susceptible. On the other side, to categorically consider all erythromycin resistant staphylococci as clindamycin resistant would deny potentially safe and effective therapy for patients infected with isolates that carry only the export mechanism.[8]

For instance, in our study we had 24% truthfully (MS_B) and 50% untruly ($iMLS_B$) clindamycin susceptible *S. aureus* isolates, resulting in an underestimated clindamycin resistance rate of 26% ($cMLS_B$) instead of 76% ($iMLS_B$ and $cMLS_B$) among *S. aureus* and similarly, 30% instead 48% among CNS [Table 1].

Also, our results support the fact that *erm* (C) and *erm* (A) genes predominated within erythromycin-resistant *S. aureus* isolates with MLS_B phenotype.[9,10] Yet, whereas, some of previous reports demonstrated prevalence of *erm* (A) genes,[11] other, similar to our study, showed that the *erm* (C) gene was more common than *erm* (A) in MLS_B *S. aureus* strains.[12]

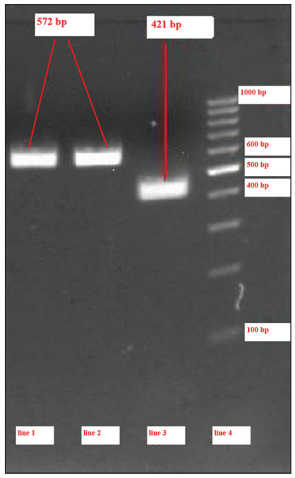

Figure 1: PCR of *S. aureus* isolates. Lines 1 and 2: Amplified product (572 bp) of *erm* (C) gene. Line 3: Amplified product (421 bp) of *erm* (A) gene. Line 4: Molecular weight marker

For these reasons it is necessary to examine the prevalence of $iMLS_B$, especially in a setting where methicillin resistance leads physicians to prescribed different macrolides or lincosamides empirically.[13] This prevalence may change over time with the emergence of strains with different sensitivity patterns, so periodic surveys should be performed if testing is not routine.

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