Inducible clindamycin resistance in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* of inpatient, outpatient and healthy carriers in Bosnia and Herzegovina

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**ABSTRACT**

**Aim** To investigate the iMLSB prevalence in 142 methicillin-susceptive (MSSA) and 48 methicillin-resistant (MRSA) in-patient (65), outpatient (75), and healthy carrier (150) *Staphylococcus aureus* isolates in Zenica-Doboj Canton, Bosnia and Herzegovina.

**Methods** Disk diffusion testing by placing clindamycin (CLI) and erythromycin (ERY) disks 15 mm apart (edge to edge) on a Mueller-Hinton agar, as per CLSI guideline was performed. Two distinct induction phenotypes labelled as D and D+, and three non-induction phenotypes designated as Neg, R (constitutive, cMLSB), and S (susceptible). Methicillin-resistance was confirmed by the presence of mecA gene by PCR. The genetic characterization was performed using spa-typing and the algorithm based upon repeat patterns (BURP).

**Results** iMLSB was detected in six (2.1%) isolates, of which five (3.5%) (two outpatients and three carriers) were MSSA, and one (2.1%) (outpatient) MRSA. One of them, D+ phenotype (iMLSB) was obtained from a carrier (MSSA). None of the inpatients had iMLSB. HD phenotype was not detected. One (MRSA) isolate has shown negative phenotype. Two strains with iMLSB originated from skin and soft tissue (MRSA) and eye infection (MSSA) belonged to the same MLST CC8, with different spa-types (t451 and t008, respectively). R phenotype (cMLSB) was detected in two (inpatient) isolates (0.7%).

**Conclusion** D test identified 2% of wrongly reported isolates as clindamycin sensitive. Despite low prevalence of *S. aureus* with iMLSB, it is a significant finding that they were mostly MSSA, and all were isolated from outpatients or carriers. D-test becomes an imperative part of routine antimicrobial susceptibility test for all *S. aureus* isolates.

**Key words:** macrolide-lincosamide-streptogramin B resistance, cMLSb, iMLSb
INTRODUCTION

The importance of Staphylococcus aureus (S. aureus) as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections in hospital as well as in community settings, is its extraordinary potential to develop antimicrobial resistance (1). Resistance to beta-lactam antibiotics is coded by the mecA gene which is situated on the mobile genetic element staphylococcal cassette chromosome mec (SCCmec) (2,3). The increasing prevalence of methillin resistant S. aureus (MRSA) together with its resistance to many other antibiotics apart from beta-lactam antibiotics is an increasing problem which additionally complicates the therapy and management of these infections (2,4,5). Clindamycin is considered to be one of the alternative agents with good in vitro and in vivo activity (6).

Erythromycin and clindamycin represent two distinct classes of antimicrobial agents (macrolide and lincosamide, respectively) that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells (7). It is well known that macrolide resistance in staphylococci is caused in two ways: either by ribosomal modification mediated by 23S rRNA methylases encoded by the erm genes (7), or by active efflux pump encoded by the msrA gene (8). The target site modification mechanism, macrolide-lincosamide-streptogramin B (MLSb) resistance, results in resistance to erythromycin, clindamycin, and streptogramin B (5). This mechanism can be constitutive (cMLSb), where the rRNA methylase is always produced, or inducible (iMLSb), where methylase is produced only in the presence of an inducing agent (5). Erythromycin is an effective inducer, but clindamycin is a weak inducer (5). In vitro, S. aureus isolates with cMLSb resistance are resistant to both erythromycin and clindamycin, and isolates with iMLSb resistance are resistant to erythromycin, but susceptible to clindamycin. In these strains in vivo therapy with clindamycin may select for constitutive erm mutants (5), which may lead to clinical failure (9). The isolates with msrA-mediated efflux also appear erythromycin resistant and clindamycin susceptible by in vitro tests; however, such isolates do not typically become clindamycin resistant during the therapy (5).

An in vitro induction (disk diffusion, D-test) test can distinguish staphylococci that have inducible erm-mediated resistance from those with msrA-mediated resistance (9,10). For erythromycin-resistant isolates, induction tests can help laboratories to determine whether results for clindamycin should be reported as susceptible (when the induction test is negative) or as resistant (when the induction test is positive) (11). This test might be performed either as part of a standard disk diffusion procedure or on an inoculum check agar plate (12). In the Steward et al. study several different D-zone phenotypes that had not been previously described in the literature were noted (11).

D-test could detect strains with constitutive resistance which are resistant to all macrolides (14-, 15- and 16-membered rings), lincosamides and streptogramin B, while inducibly-resistant strains are resistant only to 14- and 15-membered-ring macrolides (13). While broth dilution susceptibility tests fail to detect inducible resistance, this phenotype can be detected by the double-disk diffusion test, which is an induction test using closely positioned erythromycin and clindamycin disks (13).

In the present study S. aureus isolates were tested with erythromycin and clindamycin by D-test to find out the prevalence of inducible clindamycin resistance in MSSA (methicillin sensitive S. aureus) and MRSA originated from the in-patients, outpatients, and carriers (healthy food handlers) in Zenica-Doboj Canton, Bosnia and Herzegovina.

MATERIAL AND METHODS

Sixty five and 75 consecutive, non-duplicate S. aureus strains isolated from various clinical specimens in various hospital departments, including outpatient department, respectively, of the Cantonal Hospital Zenica (849 bed tertiary level hospital admitting about 25,000 patients/year, with 240,000 patient-days) in the period December 2009–May 2010 were included in the analysis. The total number of 150 consecutive, non-duplicate S. aureus strains isolated from nasal swabs of healthy food handlers admitted to the Laboratory for Sanitary and Clinical Microbiology of Cantonal Public Health Institute Zenica in the period September 2007 – December 2009 were included in the study as well. Population covered by these institutions is 331,229 in Zenica-Doboj Canton (ten municipalities), Bosnia and Herzegovina.

S. aureus isolates were identified according to standard microbiological methods (14) using...
sheep blood (5%) agar plate (Columbia agar base, Oxoid, Basingstoke, UK). All plates were incubated at 35°C ambient air for 24h. Isolates suspected of being *S. aureus* from sheep blood agar were first checked for catalase and Gram stain, if it was necessary. All *S. aureus* isolates were confirmed by coagulase latex agglutination test (Oxoid). *S. aureus* isolates were tested for oxacillin and cefoxitin sensitivity/resistance by disk-diffusion method at Mueller-Hinton (MH) agar (Oxoid) (growth zone inhibition around 1 µg and 30 µg oxacillin and cefoxitin disk, respectively) in accordance with CLSI (Clinical Laboratory Standards) (12). Isolates were stored at −70°C.

All *S. aureus* isolates were analyzed for the presence of the *S. aureus*-specific *femA* gene, and MRSA-specific *mecA* gene using a multiplex real-time PCR assay (15).

The disc diffusion method using Mueller-Hinton agar (Oxoid, Besingstoke, UK) was used to test *S. aureus* against 11 antimicrobials (Oxoid) in accordance with CLSI (12): erythromycin (15 µg), clindamycin (2 µg), imipenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), vancomycin (30 µg), amikacin, (30 µg), rifampicin, RIF (5 µg); mupirocin (200 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol, CHL (30 µg). Multidrug resistance (MDR) was defined as resistance to three or more groups of antibiotics (beta-lactam drugs were excluded).

All erythromycin-resistant and erythromycin-sensitive *Staphylococcus* strains were further tested by D-test for the presence of inducible clindamycin resistance. The isolates were subcultured to trypticase soy agar plates containing 5% defibrinated sheep blood (Oxoid). On Mueller Hinton agar, standard recommendations for inoculum preparation and inoculation were followed (0.5 McFarland standard): erythromycin disc (2 µg) was placed at distance of 15 mm (edge to edge) from clindamycin disk (15 µg) (12). Following overnight incubation at 37°C, the interpretation was done according to Steward et al (11): D phenotype (inducible MLS, ERY R, CLI S (Blunted, D-shaped clear zone around CLI disk proximal to the ERY disk) (Figure 1A);

D+ phenotype (inducible MLS, ERY R, CLI S (Blunted, D-shaped zone around CLI disk proximal to the ERY disk and small colonies growing to CLI disk in otherwise clear zone) (Figure 1B);

Neg phenotype (MS, ERY R, CLI S (clear zone around CLI disk) (Figure 1C);

R phenotype (constitutive MS): no hazy zone, growth up to CLI and ERY disks;

S phenotype (no resistance) ERY R, CLI S (clear, susceptible zone diameters)
**HD phenotype** (constitutive MLSB) ERY R, CLI R (two zones of growth appear around the CLI disk: one zone is a light, hazy growth extending from the CLI disk to the second zone where the growth is much heavier; the inner, hazy zone is blunted proximal to the ERY disk as in phenotype D; R phenotype (constitutive MSB): no hazy zone, growth up to CLI and ERY disks (Figure 1D); S phenotype (no resistance) ERY R, CLI S (clear, susceptible zone diameters) (Figure 1E).

The applied susceptibility criteria were according to CLSI (12).

Staphylococcus aureus ATCC 25923 control strain was used.

Real-time amplification of the *spa* locus, followed by sequencing, was performed as described before (16). The *spa* types were clustered into *spa* CCs using the algorithm based upon repeat pattern (BURP) with the Ridom Staph Type, version 1.5, software package (http://www.ridom.de) (17). The default settings recommended by the manufacturer were used. Since it has been shown that *spa* typing, together with the algorithm BURP, yields results consistent with typing results obtained by multilocus sequence typing (MLST) (17-19), the associated CCs, as determined with MLST (multilocus sequence typing) were allocated through the Ridom SpaServer (http://spaserver.ridom.de).

**RESULTS**

During the period December 2009–May 2010 140 consecutive, non-duplicate *S. aureus* strains obtained from clinical material of 65 in-patients and 75 outpatients, 31 (47.7%) and 14 (18.7%), of which were MRSA, respectively, were analyzed for inducible resistance to clindamycin. One hundred and fifty non-duplicate *S. aureus* obtained from the nasal swab of healthy food handlers (carriers) in the period September 2007–December 2009, of which 3 (2.0%) were MRSA analyzed for inducible resistance to clindamycin as well. Clinical isolates were obtained from various clinical specimens of various hospital departments including outpatients department.

D-test detected inducible clindamycin resistance (iMLS<sub>B</sub>) in six (out of 290, 2.1%) *S. aureus* isolates, five (two outpatients and three carriers) MSSA, and one (outpatient) MRSA (from skin and soft tissue infection, SSTI). One of them showing D+ phenotype (iMLS<sub>B</sub>) belonged to MSSA carrier. None of the in-patient MSSA/MRSA had iMLS<sub>B</sub>. Inducible clindamycin resistance (iMLS<sub>B</sub>) was detected in one (MRSA) (out of 48, 2.1%; out of 290 all *S. aureus* isolates, 0.3%) has shown negative phenotype (Table 1).

<table>
<thead>
<tr>
<th>Setting</th>
<th>No (%) of isolates with disk-diffusion phenotype</th>
<th>MRSA/MRSA</th>
<th>D</th>
<th>D+</th>
<th>Neg</th>
<th>HD</th>
<th>R</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-patients</td>
<td>(n=65)</td>
<td>31 (47.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>96.9</td>
</tr>
<tr>
<td>MSSA (n=34)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>MRSA (n=31)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>64.5</td>
<td></td>
</tr>
<tr>
<td>Outpatients</td>
<td>(n=75)</td>
<td>14 (18.7%)</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>MSSA (n=61)</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>MRSA (n=14)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>(n=150)</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>MSSA (n=147)</td>
<td></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>MRSA (n=3)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total (n=290)</td>
<td></td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>251</td>
<td></td>
</tr>
<tr>
<td>MSSA (n=243)</td>
<td></td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>86.6</td>
<td></td>
</tr>
<tr>
<td>MRSA (n=47)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>86.6</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Distribution of *S. aureus* phenotypes by disk-diffusion induction test**

**Table 2. Characteristics of *S. aureus* isolates according to clindamycin inducible test**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Protocol No</th>
<th>Isolate origin</th>
<th>Zone inhibition (mm)</th>
<th>Phenotype</th>
<th>MRSA/MRSA</th>
<th>spa-type</th>
<th>spa-CC (MLST CC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In-patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35991</td>
<td>throat</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>R</td>
<td>MRSA</td>
<td>t1179</td>
<td>singleton</td>
</tr>
<tr>
<td>11678</td>
<td>surgical wound</td>
<td>0 (0)</td>
<td>1 (15)</td>
<td>R</td>
<td>MRSA</td>
<td>t016</td>
<td>192 (22)</td>
</tr>
<tr>
<td><strong>Outpatients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20733</td>
<td>SSTI</td>
<td>S (30)</td>
<td>R (12)</td>
<td>NEG</td>
<td>MRSA</td>
<td>Non-typable</td>
<td></td>
</tr>
<tr>
<td>33005</td>
<td>SSTI</td>
<td>S (25)</td>
<td>R (0)</td>
<td>D</td>
<td>MRSA</td>
<td>t451</td>
<td>008/024 (8)</td>
</tr>
<tr>
<td>84729</td>
<td>eye</td>
<td>S (25)</td>
<td>R (0)</td>
<td>D</td>
<td>MRSA</td>
<td>t088</td>
<td>008/024 (8)</td>
</tr>
<tr>
<td>6120</td>
<td>nose</td>
<td>S (25)</td>
<td>R (0)</td>
<td>D</td>
<td>MRSA</td>
<td>t084</td>
<td>084 (15)</td>
</tr>
<tr>
<td><strong>Healthy food handlers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22870</td>
<td>nose</td>
<td>S</td>
<td>R</td>
<td>D</td>
<td>MSSA</td>
<td>t192</td>
<td>192 (22)</td>
</tr>
<tr>
<td>3759</td>
<td>nose</td>
<td>S</td>
<td>R</td>
<td>D</td>
<td>MSSA</td>
<td>t026</td>
<td>excluded</td>
</tr>
<tr>
<td>38071</td>
<td>nose</td>
<td>S</td>
<td>R</td>
<td>D+</td>
<td>MSSA</td>
<td>t1107</td>
<td>002 (5)</td>
</tr>
</tbody>
</table>

CLI, clindamycin; ERY, erythromycin; MSSA, methicillin-sensitive Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; CC, clonal complex; SSTI, skin and soft tissue infection; I, intermediate; R, resistant; S, susceptible.
The strains with iMLS B were mostly obtained from the nose, in three cases from carriers and in one case from outpatient clinical sample; two strains with iMLSB originated from SSTI (MRSA) and eye infection (MSSA) belonging to the same MLST CC (clonal complex) 8, but they had different spa-types (Table 2). R phenotype (cMLSB) was detected in two (inpatient) isolates (out of 290 of all S. aureus isolates, 0.7%). One of them (MRSA) has shown intermediate zone inhibition to both erythromycin and clindamycin, and it was sensitive to all antibiotics (excluding beta-lactam antibiotics) (isolated from throat, belonged to spa-type t1179). The other one (MSSA) has shown intermediate zone inhibition to erythromycin but it was resistant to clindamycin, as well as to trimethoprim-sulfamethoxazole (isolated from surgical wound, it belonged to spa-type t016) (Table 2).

No resistance to vancomycin, mupirocin or rifampicin was detected. There were 30 and 62 (46.8% and 96.9%) in – and outpatient MSSA isolates, respectively, sensitive to all antibiotics tested (excluding beta-lactams), and corresponding rates for MRSA were seven and four (22.6% and 28.6%), respectively. There were 127 (70.9%) MSSA and one (of the three) MRSA carriers’ isolates sensitive to all tested antibiotics. None of MSSA isolates from in- and outpatients were resistant to more than three antibiotics. Only one MRSA in both in- and outpatients (2.6% and 7.1%, respectively) was resistant to more than three antibiotics. Thirteen (7.3%) MSSA carriers’ isolates have shown resistance to more than three antibiotics, but none of MRSA isolates (Table 3).

**DISCUSSION**

Among S. aureus isolates from our collection the prevalence of inducible clindamycin resistance of 2.0% was much lower comparing to other studies, which noted inducible clindamycin resistance of 14.5% (20), 17% (21), 21% (22), or 23.2% (23). Constitutive clindamycin resistance of 0.7% was also lower than in other studies in which 3.6% (20), and 26% (22) cMLSB were noticed. Mostly, inducible clindamycin resistance is higher than constitutive (20,21,23). Low prevalence of both cMLSB and iMLS B found in this study was comparable to the study of Angel et al. where no constitutive clindamycin resistance was found, but 23.2% of inducible clindamycin resistance (23). Fokas et al. have reported higher cMLSB resistance of 60%, than iMLS B resistance, of 35% (24). The prevalence of inducible resistance to clindamycin greatly varied in different geographical regions (11,21,24,25).

Some authors have pointed out differences between MRSA and MSSA according to clindamycin inducible resistance phenotypes. In most reports MRSA has shown higher prevalence of cMLS B than iMLS A phenotypes in comparison to MSSA (21,25,26), but there are some reports with opposite findings (21,27). The results of this study have shown only two (hospital) isolates, one MSSA and one MRSA, with cMLS B phenotype, while five (of six) MSSA isolates have shown iMLS A phenotype. Only one MRSA had negative phenotype, while in the Gupta et al. study 16% of MRSA with this phenotype were found (28).

These differences in the prevalence of clindamycin inducible resistance phenotypes are probably a consequence of various prescribing habits of physicians and frequencies of particular antibiotic usage. S. aureus from this collection have shown low prevalence of resistance to all antibiotics tested (except to gentamycin), as well as very low prevalence of multidrug resistance.

### Table 3. Antimicrobial resistance of S. aureus isolates

<table>
<thead>
<tr>
<th>Setting</th>
<th>MRSA/MSSA</th>
<th>IMP</th>
<th>ERY</th>
<th>VAN</th>
<th>GEN</th>
<th>AMK</th>
<th>CIP</th>
<th>CLI</th>
<th>SXT</th>
<th>CHL</th>
<th>RIF</th>
<th>MUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inpatients MSSA</td>
<td>14.3</td>
<td>6.9</td>
<td>0</td>
<td>75.9</td>
<td>0</td>
<td>0</td>
<td>3.2</td>
<td>3.4</td>
<td>18.6</td>
<td>7.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (68)</td>
<td>5.6</td>
<td>2.9</td>
<td>0</td>
<td>38.2</td>
<td>0</td>
<td>1.5</td>
<td>2.9</td>
<td>10.3</td>
<td>5.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Outpatients MRSA</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>62.5</td>
<td>0</td>
<td>7.1</td>
<td>0</td>
<td>33.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (79)</td>
<td>9.4</td>
<td>0</td>
<td>0</td>
<td>12.5</td>
<td>0</td>
<td>2.0</td>
<td>1.3</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Food handlers MSSA</td>
<td>0.5</td>
<td>1.9</td>
<td>0</td>
<td>6.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MRSA (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>66.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (189)</td>
<td>0.6</td>
<td>1.6</td>
<td>0</td>
<td>7.9</td>
<td>0</td>
<td>22.0</td>
<td>12.5</td>
<td>1.9</td>
<td>4.0</td>
<td>5.1</td>
<td>0</td>
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</tr>
</tbody>
</table>

*IMP (10 µg); erythromycin, ERY (15 µg); vancomycin, VAN (30 µg); gentamicin, GEN (10 µg), amikacin, AMK (30 µg); ciprofloxacin, CIP (5 µg); clindamycin, CLI (2 µg); trimethoprim-sulfamethoxazole, SXT (25 µg); chloramphenicol, CHL (30 µg); rifampicin, RIF (5 µg); MUP, mupirocin (200 µg); MSSA, methicillin-sensitive Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus;
Some authors reported higher prevalence of inducible clindamycin resistance in MRSA of hospitalized patients with bloodstream or wound infections (21). Although only six isolates from our study showed inducible clindamycin resistance, it is a significant finding that none were from hospitalized patients, yet three of them were obtained from healthy food handlers (from nose samples), as well as five of them were MSSA (only one was MRSA). These isolates had different spa-types, all of them obtained from nose, eye or SSTI colonization/infection. Similarly to these results, Gupta et al. have reported higher incidence of iMLS \(_m\) from community dwellers than from in-patients (28) which might also be attributed to local prescribing policy (21).

In this study, D-test wrongly identified 2% of isolates as clindamycin sensitive, which is much lower than in the study of Debdas et al (25) where 23% of isolates were wrongly reported. In this study, the CLI and ERY disks were placed 15 mm apart, because Steward et al. study has shown that the tests are much more difficult to interpret when the disks are placed more than 20 mm apart (11). The two phenotypes by D- induction test were obtained in this study, each of them had distinct blunting of the inhibition zone proximal to the ERY disk, one with a clear zone of inhibition (D phenotype) and the other one with small colonies within the zone of inhibition (D′ phenotype), as it was previously described by Steward et al (11). Although there is no clinical significance to differentiate between these two phenotypes, it is very important for microbiological laboratories to recognize both phenotypes because these two phenotypes correlate with the presence of \(ermA\) and \(ermC\) genes, respectively (10,11), and because of the therapeutic implications of using clindamycin for the treatment of patients with inducible clindamycin-resistant \(S.\ aureus\) isolates (9). However, differentiating D- from D′- phenotypes could also provide information to help characterize isolates for epidemiologic studies in healthcare and community settings (11). The D-zone test should not be set on strains that are already known to be resistant to both ERY and CLI (11). Since the iMLS \(_m\) resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic locations, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of \(S.\ aureus\). Failure to identify iMLS \(_m\) resistance may lead to clinical failure of clindamycin therapy. Conversely, labelling all erythromycin-resistant \(S.\ aureus\) as clindamycin-resistant prevents the use of clindamycin in infections caused by truly clindamycin-sensitive staphylococcal isolates.

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**TRANSPARENCY DECLARATION**

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REFERENCES

Inducibilna clindamicin rezistencija osjetljivih na meticilin i rezistentnih na meticilin izolata Staphylococcus aureus kod bolničkih i vanbolničkih pacijenata i kliconoša u Bosni i Hercegovini

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SAŽETAK

Cilj Istražiti inducibilnu (iMLS_B) prevalenciju kod 142 osjetljiva na meticilin (MSSA) i 48 na meticilin rezistentna (MRSA) izolata Staphylococcus aureus kod bolničkih (65) i vanbolničkih pacijenata (75), te kod zdravih kliconoša (150), u Zeničko-dobojskom kantonu (Bosna i Hercegovina).

Metode Izolati su testirani disk-difuzijskom metodom; klindamicin (CLI) i eritromicin (ERY) diskovi postavljeni su na udaljenost od 15 mm na Mueller-Hintonov agar, u skladu s CLSI-om. Dva različita inducionalna fenotipa označeni su kao D i D+, a tri neindukcijska fenotipa označena su kao Neg (negativni), R (konstitutivni, cMLSB) i S (osjetljiv). Rezistencija na meticilin potvrđena je prisutnošću meca gena pomoću PCR-a. Genetička karakterizacija urađena je pomoću spa-tipizacije i BURP-a (algorithm based upon repeat patterns).

Rezultati iMLS_B je detektirana u šest (2.1%) izolata, od kojih su pet (3.5%) (dva vanbolnička i tri kliconoše) bili MSSA, i jedan (2.1%) (vanbolnički) MRSA; jedan je pripadao D+ fenotipu (iMLS_B) (kliconoša, MSSA). iMLS_B nije detektiran kod bolničkih izolata. HD fenotip nije detektiran. Jedan (MRSA) izolat pokazao je negativan fenotip. Dva izolata s iMLS_B, dobijeni iz infekcije kože i mekih tkiva (MRSA), te infekcije oka (MSSA), pripadali su istom MLST CC8, ali s različitim spa-tipovima (t451 i t008). R fenotip (cMLS_B) je detektiran kod dva (bolnička) izolata (0.7%).

Zaključak D-testom je detektirano 2% pogrešno određena izolata kao osjetljive na clindamicin. Usporedbi s niskom prevalencijom iMLS_B S. aureus, od važnosti je da su svi bili MSSA, i izolirani su kod vanbolničkih pacijenata i kliconoša. D-test je važan dio rutinskog testiranja izolata S. aureus na antibiotike.

Ključne riječi: makrolid-linkozamid-streptogramin B rezistencija, cMLS_B, iMLS_B