A Study on Class I Integrons and Antimicrobial Resistance among Clinical Staphylococci Isolates from a Turkish Hospital

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Bacterial isolates

One hundred clinical staphylococcal isolates, 50 methicillin-resistant S. aureus (MRSA) and 50 methicillin-resistant coagulase negative staphylococci (MRCoNS), isolated from various clinical samples at Hospital of Ondokuz Mayis University Faculty of Medicine, a 1150-bed training and research hospital located at Black Sea region, between April 2010 and June 2010 were included in the study. S.

Materials and Methods

Bacterial isolates

One hundred clinical staphylococcal isolates, 50 methicillin-resistant S. aureus (MRSA) and 50 methicillin-resistant coagulase negative staphylococci (MRCoNS), isolated from various clinical samples at Hospital of Ondokuz Mayis University Faculty of Medicine, a 1150-bed training and research hospital located at Black Sea region, between April 2010 and June 2010 were included in the study. S.
*Staphylococcus aureus* ATCC 43300 and *E. coli* K8 strains were used as the positive control strains.

**Identification of MRS isolates**

Identification of the isolates was performed by using BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD USA). Methicillin resistance was determined by disc diffusion method according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) [15]. 30 μg cefoxitin discs (BD Diagnostic Systems, Sparks, MD USA) were used. Results were evaluated according to the breakpoints of CLSI [16].

**DNA extraction from MRS isolates**

MRS isolates and positive control strains were subcultured from stock solutions onto agar plates. DNA extraction was performed from isolated colonies by using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA) according to the instructions of the manufacturer.

**Confirmation of identification of MRS isolates by multiplex PCR method**

MRS isolates were investigated for the carriage of staphylococci specific 16S rRNA, nuc and mecA genes. Primers used for multiplex PCR are listed in the Table 1 [17-19]. Multiplex PCR was performed in a 25 μl volume with 1X PCR buffer, 3 mM MgCl2, 200 μM dNTP mix, 2.5U Taq DNA polymerase, 5pmol each of the 16SrRNA and nuc primers, and 12.5pmol mecA primer with 1 μl of template DNA. Thermocycling conditions were as follows: initial denaturation for 2 min at 94°C, 25 cycles of denaturation at 94°C for 15s, primer annealing at 55°C for 30s and 72°C for 90s, and a final extension at 72°C for 10 min. PCR products were analyzed on 1% agarose gel.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was performed by disc diffusion method according to CLSI guideline [15]. Antibiotics tested included erythromycin, clindamycin, trimethoprim-sulfamethoxazole, teicoplanin, tetracycline, linezolid, gentamicin, ciprofloxacin, quinupristin-dalfopristin and chloramphenicol. Results were evaluated according to the breakpoints of CLSI for *S. aureus* and coagulase negative staphylococci [16].

**Investigation of class 1 integrons**

The presence of class 1 integrons in MRS isolates was investigated by PCR. The isolates were screened for intI1 gene. Primers used in the PCR are listed in the Table 1 [20]. PCR was performed in a 25 μl volume with 1X PCR buffer, 1.5mM MgCl2, 200 μM dNTP mix, 0.6U Taq DNA polymerase, 12.5pmol Int-1U and Int-1D primers and 2 μl of template DNA. Thermocycling conditions were as follows: initial denaturation for 5 min at 94°C, 35 cycles of denaturation at 94°C for 30s, primer annealing at 55°C for 30s and 72°C for 90s, and a final extension at 72°C for 7min. PCR products were analyzed on 1% agarose gel.

**Results**

The results of antimicrobial susceptibility testing of MRS isolates are shown in the Table 2. In the multiplex PCR, all MRSA isolates were positive for 16S rRNA, nuc and mecA genes (Figure 1), and all MRCoNS isolates were positive for 16S rRNA and mecA genes, and negative for nuc gene (Figure 2). One hundred MRS isolates were screened by PCR to evaluate the presence of intI1. No positivity was detected for all the isolates tested (Figure 3).

**Table 1: Primers used for the confirmation of MRS by multiplex PCR and for the detection of IntI1 gene region**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MecA-1</td>
<td>AAA ATC GAT GGT AAA GGT TGG C</td>
<td>533</td>
<td>17</td>
</tr>
<tr>
<td>MecA-2</td>
<td>AGT TCT GCA GTA CCG GAT TGG C</td>
<td>533</td>
<td>17</td>
</tr>
<tr>
<td>Nuc-1</td>
<td>GCG ATT GAT GGT GAT ACG GTT</td>
<td>270</td>
<td>18</td>
</tr>
<tr>
<td>Nuc-2</td>
<td>AGC CAA GCC TGG ACG AAC TAA AGC</td>
<td>270</td>
<td>18</td>
</tr>
<tr>
<td>Staph756F</td>
<td>AAC TCT GTT ATT AGG GAA GAA CA</td>
<td>756</td>
<td>19</td>
</tr>
<tr>
<td>Staph756R</td>
<td>CCA CCT TCC TCC GGT TTG TCA CC</td>
<td>756</td>
<td>19</td>
</tr>
<tr>
<td>Int-1U</td>
<td>GTT CGG TCA AGG TTC TG</td>
<td>923</td>
<td>20</td>
</tr>
<tr>
<td>Int-1D</td>
<td>GCC AAC TTT CAG CAC ATG</td>
<td>923</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table 2: Results of the antimicrobial susceptibility testing of 100 MRS isolates**

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Number of isolates resistant to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
</tr>
<tr>
<td>MRSA (n=50)</td>
<td>43</td>
</tr>
<tr>
<td>MRCoNS (n=50)</td>
<td>46</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>89</td>
</tr>
</tbody>
</table>

E, erythromycin; CM, clindamycin; CIP, ciprofloxacin; GN, gentamicin; TE, tetracycline; SXT, trimethoprim-sulfamethoxazole; C, chloramphenicol; LNZ, linezolid; TEC, teicoplanin; QD, quinupristin-dalfopristin

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Discussion

Although multidrug-resistant (MDR) gram-negative bacteria have been a prominent problem for a long time, multidrug resistance in gram-positive bacteria is increasingly significant in the last two decades [10]. The role of class 1 integrons is well documented in the spread of antibiotic resistance genes in gram-negative bacteria [5]. Class 1 integron positive gram-negative bacteria includes *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Burkholderia*, *Campylobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Unsellable*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Stenotrophomonas* and *Vibrio* [21]. Also, the presence of class 1 integrons has been shown in various gram-negative bacteria in our country, Turkey [22-24].

In gram-positive bacteria, there are a small number of studies about integrons. The presence of class 1 integrons in gram-positive bacteria was firstly shown in *Corynebacterium glutamicum*, *Enterococcus faecalis* and various gram-positive bacteria including staphylococci isolated from poultry litter [25-27], but the first comprehensive study with clinical strains was conducted in China. Shi et al. screened 46 gram-positive strains isolated from various clinical samples in a local hospital in Guangzhou, China, for the presence of class 1 integrons [10]. The investigators detected class 1 integrons in all of the strains examined. The class 1 integron positive strains included MRSA, MRCoNS and *E. faecalis*. In another study from China, Xu et al. investigated the presence of class 1 integrons in nosocomial MRSA strains [11]. They detected class 1 integrons in 53% of the clinical strains. In another study by the same researchers, 53 clinical MRCoNS strains were screened for the presence of class 1 integrons [12]. They showed the presence of class 1 integrons in 30 MRCoNS strains. Gene cassettes such as dfrA12-orfF-aadA2 and dfrA17-aadA5, which had been already shown in gram-negative strains, were detected in the class 1 integron positive strains [10-12]. In our study, we could not detect the existence of class 1 integron in clinical MRS isolates.

In another study by Xu et al. compared integron positive MRSA strains with integron negative strains. They showed class 1 integron positive MRSA had only additional resistance to spectinomycin and streptomycin which are not commonly used for antibacterial treatment today [13]. In a previous study, Xu et al. stated that indiscriminate use of existing antibiotics resulted in antibiotic selective pressure and was reflected by the prevalence of integrons [11]. Clinical use of spectinomycin and streptomycin which causes so much selective pressure is questionable. In another study, statistically significant differences in resistance to erythromycin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole were found between class 1 integron positive and negative MRS strains [28]. In only MRSA strains, this was true for gentamicin, tetracycline, and trimethoprim-sulfamethoxazole [14], but the relation of resistance to erythromycin and tetracycline to gene cassettes could not be shown, and the mechanism of resistance to these drugs in both integron positive and negative strains was not examined. In our study, the resistance patterns of the integron negative MRS isolates were quite different from the strains from China. In our study, all integron negative MRSA isolates were resistant to gentamicin and tetracycline and susceptible to trimethoprim-sulfamethoxazole.

Conclusions

Although the role of class 1 integrons is well documented in the spread of antibiotic resistance genes in gram-negative bacteria, much less is known about gram-positive bacteria. To our knowledge, this study was the first study regarding the presence of integrons in clinical staphylococcal isolates. Further studies are needed, especially in Western countries, to determine the significance of integrons in gram-
positive bacteria and their role in DNA transfer between gram-positive and gram-negative bacteria.

Competing interests
The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

Author's contributions
AKG (Akif Koray Guney) performed the experiments, analyzed the results and prepared the manuscript. TB provided technical assistance and suggestion. BD supervised the work and corrected the manuscript for publication. All authors read and approved the final manuscript.

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References