Original Research Article

Mechanisms of Resistance to Erythromycin among Viridans Group Streptococci (VGS) Isolated from Blood Cultures

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A B S T R A C T

The aim of this work was to evaluate erythromycin resistance mechanisms of viridans group streptococci (VGS) isolates obtained from blood cultures. Fifty VGS isolates were obtained from patients suffering from infective endocarditis as suggested by their primary physicians. The macrolide resistance mechanisms of erythromycin resistant isolates were studied by double disc test and real time PCR. Fifteen (30%) VGS isolates resistant to erythromycin were distributed genetically as follows: \textit{ermB} (53.3%), \textit{ermB+mefA/E} (13.3%) (Those isolates containing \textit{ermB} whether alone or combined with \textit{mefA/E} account for MLS\textsubscript{B} phenotype), \textit{mefA/E} (26.6%) (account for M phenotype) and only phenotypically (6.8%) in rank of order. From our study, we concluded that constitutive MLS\textsubscript{B} phenotype associated with \textit{ermB} was the predominant mechanism of macrolide resistance among our erythromycin resistant VGS isolates and M phenotype was associated with \textit{mefA/E}.

Keywords

VGS, Blood cultures, Erythromycin resistance, MLS\textsubscript{B} phenotype, M phenotype, \textit{ermB}, \textit{mefA/E}

Introduction

Viridans group streptococci (VGS) are part of the oral flora and cause infections such as infective endocarditis, septicemia and neutropenia in immunocompetent and immunocompromised patients. VGS have accounted for 25 to 30% of bacteremic episodes among patients with malignancies and are the most common cause of early bloodstream infections among hematopoetic stem cell transplantation recipients (Ergin et al., 2011).

Resistance to macrolides and other antibiotics among blood cultures of VGS is a major concern and could compromise currently available prophylactic and therapeutic regimens (Nandhakumar et al., 2008). It has been shown that VGS can be important reservoirs of resistance genes for the more pathogenic streptococci like \textit{Streptococcus pneumoniae} and \textit{Streptococcus pyogenes} (Brenciani et al., 2014).

There are three different resistance mechanisms causing macrolide resistance in VGS isolates, the first is the target site modification, mediated by the erythromycin
resistance methylases, encoded by the ermA or ermB genes, conferring resistance to macrolide, lincosamide and streptogramin B antibiotics (MLS\(_B\) phenotype). Expression of MLS\(_B\) resistance can be constitutive (cMLS\(_B\)) or inducible (iMLS\(_B\)). The second is the active drug efflux mechanism, mediated by a membrane-bound efflux protein encoded by mefA gene conferring resistance to 14- and 15-membered macrolides only (M phenotype). Finally, the third mechanism of resistance is a mutation in the streptococcal 23S rRNA or ribosomal protein genes, leading to resistance to macrolide or streptogramin B antibiotics (MS phenotype) (Ergin et al., 2006).

The aim of this work was to evaluate erythromycin resistance mechanisms of viridans group streptococci (VGS) isolates obtained from blood cultures.

**Materials and Methods**

The present study was conducted at the Central Microbiology Laboratory, Clinical Pathology Department, Ain Shams University, Cairo, Egypt during the period from January 2014 to October 2014.

**Samples selection and susceptibility testing**

The study was conducted on 50 blood culture specimens with positive signals obtained from patients suffering from infective endocarditis as suggested by their primary physicians. VGS blood culture isolates were identified by colony morphology, gram stain, catalase test and optochin test. Susceptibility to erythromycin was determined using a double disc test with erythromycin (15μg) discs on Mueller-Hinton agar plates containing 5% sheep blood (CLSI, 2009).

**Real - time PCR for detection of erythromycin resistance genes**

**DNA Extraction**

**Cell lysis for Gram-positive bacteria**

One ml of cultured cells was transferred into a 1.5 ml microtube. The tubes were centrifuged at 15,000 g for 1 min to harvest the cells and the supernatant was discarded. The cell pellets were resuspended in 300 μl of cell resuspension solution. Two μl of lysozyme solution was added to each tube and mixed well by inverting. The tubes were incubated at 37°C for 60 minutes with occasional inverting then centrifuged at 15,000 g for 1 min and the supernatant discarded. Then the pellets were resuspended in 300 μl of cell lysis solution.

**RNase treatment**

1.5 μl of RNase Solution was added and mixed by inverting. The tubes were incubated at 37°C for 15–30 min and cooled on ice for 1 min.

**Protein precipitation**

100 μl of Protein Precipitation Solution was added and vortexed vigorously for 20-30 sec, then centrifuged at 15,000 g for 5 min.

**DNA precipitation**

The supernatants were transferred to clean 1.5 ml microtubes each containing 300 μl Isopropanol >99%. The samples were mixed by inverting gently for 1 min. then centrifuged at 15,000 g for 1 min (DNA
should be visible as a small white pellet). The supernatant was discarded and the tube was drained briefly on cleaned absorbent papers. 500 µl 80% Ethanol was added to each tube and inverted several times to wash the DNA pellets then were centrifuged at 15,000 g for 1 min. The ethanol carefully discarded. The tubes were left to dry in air at room temperature for 10–15 min.

**DNA hydration**

50-100 µl of DNA Hydration Solution was added to the dried DNA pellet in each tube. The DNA was hydrated by incubation at 65°C for 60 min. The samples were stored at -20°C till use.

**DNA amplification and detection**

The primers used for *ermB* and *mefA/E* genes and the thermal cycling conditions according to (Ergin et al., 2011).

The amplification program was followed immediately by a melt program consisting of 1 minute at 95°C, 30 sec at 55°C then again to 95°C for 30 sec.

The greenish horizontal line in the graph is the threshold line at which the fluorescence begins to be detected (The point at which the amplification plot crosses the threshold is the cycle threshold=Ct) as shown in figure 1 & 2 (Mulderw, 2009). The T_m (temperature at which 50% of DNA is single stranded) of samples which were identical or close to that of positive control were considered the gene of target as shown in figure 3 & 4.

**Results and Discussion**

In our study, 35 (70%) were sensitive to erythromycin, while 15 (30%) were resistant to erythromycin as shown in table 1, 66.7% (n.10) constitutive MLS_B phenotype as shown in table 2 and 33.3% (n. 5) M phenotype as shown in table 3. Erythromycin resistant VGS isolates were distributed genetically as follows: *ermB* (53.3%), *mefA/E* (26.6%), *ermB+mefA/E* (13.3%) and only phenotypically (6.8%), one isolate did not carry any of macrolide resistance genes studied here as shown in table 4.

Our results agreed with Ergin et al. (2011) who studied 50 VGS blood cultures in which the percentage of erythromycin resistance was 36% (n. 18) and 7 were intermediate resistant (14%), 64% (n. 16) were cMLS_B phenotype and 36% (n. 9) were M phenotype. The percentage genotypic carriage of each gene was: *ermB*, 56% (n. 14); *mefA/E*, 28% (n. 7); *ermB+mefA/E*, 8% (n. 2). Two of the isolates did not carry any of macrolide resistance genes studied there.

Uh et al. (2004) studied on 106 VGS blood cultures with rate of non-susceptibility to erythromycin 33.9%, 22 (61.1%) of 36 erythromycin non-susceptible isolates expressed constitutive resistance to macrolide–lincosamide–streptogramin B antibiotics (a constitutive MLS_B phenotype); 13 isolates (36.1%) expressed an M phenotype; and 1 isolate, a Streptococcus bovis isolate, had an inducible MLS_B resistance phenotype. *ErmB* was found in isolates with constitutive/inducible MLS_B phenotypes, and *mefA* in isolates with the M phenotype. In 3 isolates (2 isolates with a constitutive MLS_B phenotype and in 1 isolate with the M phenotype), none of *ermA*, *erm B*, *erm C* or *mef A* was detected by PCR.

Achour et al. (2004) studied on a total of 169 *S. mitis* isolates were recovered from 66 neutropenic patients at the Tunisian Bone Marrow Transplant Centre. Of these, 120 (70%) were resistant to erythromycin, 58
strains (48.5%) of isolates had an MLS\textsubscript{B} phenotype and 57 (47.5%) displayed the M phenotype.

Rodriguez-Avial et al. (2005) reported that 45.6% (n=79) of VGS were resistant to erythromycin, of the 79 erythromycin-resistant strains, 61% displayed constitutive macrolide – lincosamide - streptogramin B (cMLS\textsubscript{B}) resistance, 35% displayed the M phenotype, and 4% displayed the inducible MLSB (iMLS\textsubscript{B}) phenotype.

Elia and colleagues (2009) stated that 18.7% (78 out of 416) of VGS were resistant to erythromycin and the leading resistance gene was \textit{erm} \textsubscript{B} (74.3% of the isolates), but disagreed in the following order of these genes; \textit{ermB+mefA} combination (17.9%) and \textit{mefA} alone (7.7%).

In contrast to our study Zolezzi et al. (2004) from Spain reported that the M phenotype was predominant (59.38%) among their erythromycin-resistant VGS isolates from oropharynx samples, and they found the \textit{mefA/E} gene in all the strains with M phenotype and \textit{mefE} was the predominant subclass (95.36%).

From our study, we concluded that constitutive MLS\textsubscript{B} phenotype associated with \textit{ermB} was the predominant mechanism of macrolide resistance among our erythromycin resistant VGS isolates and M phenotype was associated with \textit{mefA/E}.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Distribution of erythromycin resistance among all isolates</th>
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<tbody>
<tr>
<td><strong>Erythromycin Resistance</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>Erythromycin Resistance</td>
<td>Count</td>
</tr>
<tr>
<td></td>
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<td>Total</td>
<td>Count</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of MLS\textsubscript{B} phenotype resistance among all isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLS\textsubscript{B} phenotype</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td></td>
<td>Count</td>
</tr>
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<td></td>
<td>%</td>
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<tr>
<td>Total</td>
<td>Count</td>
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Table 3: Distribution of M phenotype resistance among erythromycin resistant VGS isolates

<table>
<thead>
<tr>
<th>M phenotype</th>
<th>Count</th>
<th>Total</th>
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<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 4: Distribution of patterns of resistance by PCR among erythromycin resistant VGS isolates

<table>
<thead>
<tr>
<th>Resistance by PCR</th>
<th>Count</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><strong>ermB</strong></em></td>
<td>8</td>
<td>53.3%</td>
</tr>
<tr>
<td><em><strong>ermB + mef A/E</strong></em></td>
<td>2</td>
<td>13.3%</td>
</tr>
<tr>
<td><em><strong>mef A/E</strong></em></td>
<td>4</td>
<td>26.6%</td>
</tr>
<tr>
<td>No (<em><strong>ermB or mefA/E</strong></em></td>
<td>1</td>
<td>6.8%</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 1: Results of Syber Green real-time PCR in amplification plot with cycle’s number on x axis and florescence on y axis (ermB gene)
**Figure 2** Results of Syber Green real-time PCR in amplification plot with cycle’s number on x axis and florescence on y axis (*mefA/E* gene)

![Amplification Plots](image)

**Figure 3** Results of melting curve, average $T_m = 76.75–77.3^\circ C$ (*ermB*)

![Dissociation Curve](image)

**Figure 4** Results of melting curve, average $T_m = 75.75–77.55^\circ C$ (*mefA/E*)

![Dissociation Curve](image)
References


