Original article

Identification of inducible clindamycin resistance gene in *Streptococcus agalactiae* isolates colonizing pregnant women in Suez Canal University Hospitals

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**ABSTRACT**

**Background:** *Streptococcus agalactiae* is classified as group B *Streptococcus* (GBS) according to Lancefield classification, GBS resides as one of commensals of both genitourinary and gastrointestinal tracts of humans. GBS affects mainly immunocompromised people particularly neonates. An increased rates of macrolides and lincosamides resistance have been noted, which is associated with expression of *erm* genes, resulting in erythromycin and inducible clindamycin resistance. **Aim:** To limit Group B *Streptococcus* transmission to neonates and reduce neonatal morbidity and mortality rates in Suez Canal University Hospitals. **Methods:** 204 clinical vaginal swabs were collected from pregnant women admitted to obstetrics and gynaecology department in Suez Canal University hospitals. Swabs were incubated for 24 hours in lim’s selective broth and then for isolation on Colombia blood agar, then identification by colony morphology, Gram staining and biochemical reactions were done. Antimicrobial susceptibility testing of the isolates was performed to penicillin G, ampicillin, vancomycin, levofloxacin, cefotaxime, erythromycin and clindamycin by disk diffusion method and D test was then performed. Conventional PCR was used to detect *ermA* gene. **Results:** Out of 204 specimens, 54 *S. agalactiae* isolates were isolated with prevalence of 26.47%. All *S. agalactiae* isolates were sensitive to penicillin G (100%). Out of the 33 erythromycin-resistant isolates, 5 isolates showed inducible phenotype, and 4 of them had *ermA* gene (80%). **Conclusions:** In this study, the prevalence of *S. agalactiae* was remarkable and antibiogram is mandatory to detect the inducible resistance phenotype. Further studies are needed to provide a comprehensive data about *S. agalactiae* sensitivity profile to antibiotics in other healthcare facilities to reduce neonatal morbidity and mortality.

**Introduction**

*Streptococcus agalactiae* resides as part of normal flora of the genitourinary and gastrointestinal tracts of humans [1].

Group B *streptococci* disease in neonates is classified into early onset disease that occurs during first 6 days of life, while any other GBS disease that appears after this to up to 3 months of life is classified as late onset disease. In neonates, most early onset infections occurs either through ascending infection or during passage through birth canal, in contrast, late onset infections occur via hospital or community acquired means [1].

Risk factors for infections of neonates include preterm delivery before 37 weeks, prolonged labor more than 18 h, fever of at least 38°C during delivery, prior neonatal history of GBS infection, and Diabetes [2].

Intrapartum antibiotic prophylaxis is indicated for all pregnant females with positive
screening culture for GBS and is routinely obtained at 34 - 37 weeks of gestation or for pregnant women who have a previous or current history of GBS infection [3].

An increased prevalence of macrolide and lincosamide resistant *S. agalactiae* strains has been noted in last decades. Clindamycin resistant strains have caused over 40% of GBS infections, such resistance is correlated with presence of *erm* genes which are erythromycin resistance methylase genes [4]. The current study aims to identify *Streptococcus agalactiae* colonizing pregnant women at Suez Canal University Hospitals and to assess prevalence of *ermA* gene in those isolates.

**Methods**

**Study population**

This is cross sectional descriptive study that was held at Suez Canal University Hospitals (SCUHs) in Ismailia, Egypt, from August 2022 to February 2023. Two hundred and four clinical specimens were collected from pregnant women in SCUHs. All pregnant women were > 18 years at 34 to 37 weeks of gestation. Ethics committee of Faculty of Medicine, Suez Canal University had approved the study in July 2022.

**Specimen collection and processing**

Low vaginal-rectal swabs were collected under aseptic precautions and processed at microbiology laboratory at Faculty of Medicine, Suez Canal University, for isolation and identification of *S. agalactiae*. Specimen processing included inoculation in selective enrichment Lim’s broth, direct smearing and microscopic examination using Gram staining. *S. agalactiae* isolation was done on Colombia blood agar containing colistin (10 µg/ml) and nalidixic acid (15 µg/ml). *S. agalactiae* isolates were phenotypically identified as beta hemolytic colonies on blood agar plates, negative catalase reaction, positive arrow shaped hemolysis on CAMP test, and positive hydrolysis of sodium Hippurate.

**Antibiotic susceptibility testing**

Antibiotic sensitivity testing of isolated *S. agalactiae* was done on modified Muller Hinton agar containing 5% defibrinated sheep blood. The used antibiotic disks were penicillin (10 units), ampicillin (10µg), vancomycin (30µg), levofloxacin (5µg), cefotaxime (30µg), erythromycin (15µg), and clindamycin (2µg). Inhibition zones were interpreted according to CLSI 2022 [5].

**Phenotypic detection of clindamycin resistance (D test)**

Erythromycin resistant isolates were subjected to D test on modified Muller Hinton agar plate by using clindamycin disk (2 µg) and erythromycin disk (15 µg) separated by 15 - 26 mm. Blunting of the inhibition zone adjacent to the erythromycin disk (D shape) was considered as inducible clindamycin resistance (iMLSB) (D test positive).

Isolates, which were clindamycin sensitive and erythromycin resistant, with no apparent blunting were considered as M phenotype (D test negative). While, isolates which were resistant to both clindamycin and erythromycin, were considered as constitutive clindamycin resistance (cMLSB).

**Genotypic detection of *ermA* gene**

Erythromycin resistant *S. agalactiae* isolates were screened for *ermA* resistance gene by conventional polymerase chain reaction (PCR). DNA amplification will be performed using specific primer for detection of *ermA* gene (Table 1) [6].

**Quality control**

Reference strain *S. agalactiae* ATCC number 12386 was used as a control strain.

Reference strain *S. aureus* ATCC number 25923 was used as a control strain.

**Statistical analysis**

Statistical analysis was done with SPSS-25 software (SPSS Inc., Chicago, IL, USA). Data was analyzed and presented as numbers and percentages using graphs and tables with the confidence interval (CI) at 95%. p value of 0.05 was used as the statistical significance limit.

**Results**

This study included 204 vaginal swabs collected from pregnant women > 18 years from 34 to 37 weeks gestation from gynaecology and obstetrics department in SCUHs. Out of the 204 collected specimens, 54 (26.47%) were colonized with *S. agalactiae*.

Participants’ ages ranged from 18 to 44 years with a mean age of 27.5 ±7.43 years, gravidity ranged from 1-7 times with a mean of 3.15 ±1.45,while gestational age of participants ranged from 34-37 weeks with a mean of 35.14 ±1.16 weeks.

There was no association between age, gestational age or gravidity and GBS colonization.
among pregnant participants, as calculated p value was 0.178, 0.164 and 0.386 for age, gravidity and gestational age respectively. (p is <0.05) (Table 2).

All isolates showed β-hemolytic colonies with characteristic ring-like narrow zones and all GBS colonies were large, mucoid and grayish white (Figure 1).

Suspected GBS colonies were all Beta-hemolytic, negative for catalase test, positive for CAMP test producing arrow head shaped hemolysis (Figure 2) and Bacitracin resistant, but varied in response to Hippurate hydrolysis test as 88.9% of isolates were Hippurate hydrolysis positive.

All the GBS isolates were sensitive to penicillin G (100%), while susceptibility to vancomycin was 96.3%, to levofoxacin was 87%, and to ampicillin was 85.2%, isolates exhibited varied susceptibility patterns to other antibiotics (Table 3).

Out of the 33 erythromycin-resistant isolates, five isolates (15.1%) showed inducible macrolide-lincosamide-streptogramin B phenotype (iMLS) (Figure 3), 18 isolates (54.5%) showed constitutive macrolide-lincosamide-streptogramin B phenotype (cMLS), while 10 isolates (30.3%) showed M phenotype (macrolide resistant but clindamycin and streptogramin B susceptible).

All the 33 erythromycin resistant isolates using primary selective enrichment broth cultures underwent PCR using a primer for the *ermA* gene.

Four specimens (12.1%) were positive for *ermA* gene using the *ermA* PCR assay. Positive specimens showed specific bands of approximately 190 bp in size (Figure 4).

A total of 5 specimens (15.1%) of the 33 erythromycin resistant ones were found to be positive for inducible clindamycin resistance (iMLS) by phenotypic disk diffusion D-test method (Figure 3). Out of the 5 D-test positive specimens, *ermA* PCR assay detected 4 as positive (80%) (Figure 4).

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequences</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ermA</em> gene</td>
<td>5'-AAGCGGTAAACCCCATCTGA-3' &lt;br&gt; 5'-TTCGCAAATCCCTTCTCAAC-3'</td>
<td>190 bp</td>
</tr>
</tbody>
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### Table 1. *ermA* gene primers sequences and amplicon size

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</tbody>
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### Table 2. Means of age, gravidity and gestational age in GBS (n=54) and non-GBS (n=150) groups

<table>
<thead>
<tr>
<th></th>
<th>GBS Mean</th>
<th>SD</th>
<th>Non GBS Mean</th>
<th>SD</th>
<th>T</th>
<th>P value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.69</td>
<td>7.84</td>
<td>27.1</td>
<td>7.26</td>
<td>1.35</td>
<td>0.178</td>
<td>NS*</td>
</tr>
<tr>
<td>Gestational age</td>
<td>35.26</td>
<td>1.25</td>
<td>35.1</td>
<td>1.13</td>
<td>0.87</td>
<td>0.386</td>
<td>NS*</td>
</tr>
<tr>
<td>Gravidity</td>
<td>3.38</td>
<td>1.89</td>
<td>3.06</td>
<td>1.25</td>
<td>1.39</td>
<td>0.164</td>
<td>NS*</td>
</tr>
</tbody>
</table>

### Table 3. Antibiotic susceptibility profile among GBS isolates (n=54)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant</th>
<th></th>
<th>Susceptible</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0</td>
<td>0</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>14.8</td>
<td>46</td>
<td>85.2</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>7</td>
<td>13</td>
<td>47</td>
<td>87</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2</td>
<td>3.7</td>
<td>52</td>
<td>96.3</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>37</td>
<td>68.5</td>
<td>17</td>
<td>31.5</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>41</td>
<td>76</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>33</td>
<td>61.1</td>
<td>21</td>
<td>38.9</td>
</tr>
</tbody>
</table>


**Figure 1.** β-hemolytic colonies of GBS with characteristic ring-like zones on sheep blood agar

**Figure 2.** CAMP test (Positive = arrow head hemolysis)

**Figure 3.** Inducible clindamycin resistant GBS (Positive D Test).
Figure 4. Distribution of \textit{ermA} gene (190 bp amplicon)

Lane L is a 100 bp DNA ladder, while lane 1 and lane 2 are negative and positive control respectively, lanes 3, 4, 10, 16 are \textit{ermA} gene positive.

Discussion

The study revealed that 54 women were found to be colonized with GBS with a prevalence rate of 26.47%.

These results were concordant with an Egyptian study performed in Alexandria university by Sadaka et al. [7] The prevalence rate was 25.9% for GBS, similarly, another study performed at Ain Shams university by El Masry et al. [8] reported a similar prevalence rate of 25.9%.

Also, other two studies carried out in Egypt by El Shahawy et al. and Tash et al. showed an agreement with present study [9,10]. Other countries also showed agreement with our study such as Da Rocha et al. [11] and Morita et al. [12] which reported prevalence rates of (28.2%, and 22.4%) respectively.

Colonization rate differences among studies may be due to several factors, such as different specimen sites (vaginal, rectal, or both), varying sample sizes, hygiene practices, antibiotic use, religion and culture beliefs, and different isolation techniques [13].

There was no association between demographic data as participants’ age, gestational age and GBS colonization among pregnant participants, similar results were interpreted by El Masry et al. in Ain Shams university [8].

On the other hand, other studies showed the opposite as in a study in Egypt by Fouad et al. [14] showed that GBS colonization is directly proportional to maternal age and parity. Thus, older pregnant women and multigravidae are at higher risk for GBS colonization. This agrees with the results by Shabayek et al. in respect to maternal age and parity [15]. also, Zakerifar et al., showed significant relationship between gestational age and GBS colonization [16].

Previous results were explained by Patras et al. as recent reports had described a reduction of vaginal \textit{Lactobacilli} in GBS colonized pregnant women which lower the pH through producing lactic acid and help to protect from various microbial pathogens [2].

In current study, susceptibility testing revealed that all the GBS isolates were susceptible to penicillin G 100%, while susceptibility to vancomycin was 96.3%, to levofloxacin was 87%, and to ampicillin was 85.2%, isolates exhibited varied susceptibility patterns to other antibiotics.

Macrolides and lincosamides are structurally different but have similar mode of action [7].

In pathogenic bacteria, \textit{erm} genes cause ribosomal methylation of 23S rRNA, which is part of the large 50S ribosomal subunit as declared by Leclercq et al. [17]. Kishk et al. demonstrated that ribosomal methylation has impaired binding of erythromycin to its target [18].

In current study, an increased resistance to erythromycin and clindamycin, drugs of choice in
case of serious penicillin allergy, had been observed as the highest level of resistance was reported against clindamycin where 76% were resistant followed by cefotaxime and erythromycin with resistance levels of 68.5% and 61.1% respectively.

However, another study by El Masry et al., showed similar results regarding clindamycin resistance (51.7%) and cefotaxime resistance (69%) while their isolates showed lower resistance to erythromycin (37.9%) and higher resistance to penicillin (37.9%) and to levofloxacin (27.5%) [8].

Other studies as a study by Jalalifar et al. showed high clindamycin resistance (47%) and erythromycin resistance (52%) [19]. Also, other studies showed similar results regarding high penicillin and vancomycin sensitivity levels with rates of 100% for both [13].

The emergence of higher resistance for different antibiotics used has ethnic and geographic variations. The rates of resistance to macrolides and lincosamides differ between countries and even within the same country regionally, which could be explained by the different regimen of antimicrobials used and different strains in different regions.

Clindamycin is considered an alternative for treatment of GBS infections [20]. MLSB antibiotics resistant strains have emerged due to the misuse of these antibiotics as reported by Gadepalli et al. [21].

Erythromycin resistance and clindamycin resistance are related. It is difficult to identify the inducible MLSB phenotype in routinely performed tests as isolates appear to be clindamycin-susceptible and erythromycin resistant in vitro. This false clindamycin result in iMLSB isolates may lead to therapeutic failure if patients are treated with lincosamides [19]. So, performing D test for revealing iMLSB was mandatory.

On performing D test, out of the 33 erythromycin-resistant isolates, only five isolates (15.1%) showed phenotype (iMLSB), 18 isolates (54.5%) showed constitutive phenotype (cMLSB), while 10 isolates (30.3%) showed M phenotype, so induced clindamycin resistance was the least demonstrated phenotype.

Another study by Compain et al. showed higher constitutive resistance than ours as the distributions of the cMLSB, iMLSB, and M phenotypes were 64%, 17%, and 18% respectively [22].

Unlike present study, higher rate of inducible resistance phenotype was seen in a study in Brazil by Nakamura et al. as inducible MLSB, constitutive MLSB and M phenotype had 46.67%, 33.3% and 20% rates respectively [23].

It was our aim in the study to identify inducible clindamycin resistant isolates, so it was important to evaluate the prevalence of resistant phenotypes to clindamycin and erythromycin in all GBS isolates isolated from every colonized pregnant woman allergic to penicillin to prevent emergence of clindamycin resistance shortly after start of therapy. Also, resistance phenotypes detection will enable proper choice of the antimicrobial to be used in IAP, as one of the safest methods, pending availability of vaccines to reduce neonatal morbidity and mortality [14].

In spite of the geographic variation of resistance patterns, the prevalence of erm genes appears to be quietly the same in different countries as reported [18].

In present study, all the 33 erythromycin resistant isolates using primary selective enrichment broth cultures underwent conventional PCR using a primer for the ermA gene, four specimens (12.1%) were positive for ermA gene using the ermA PCR assay. Positive specimens showed specific bands with amplicon size of 190 bp in size.

A total of 5 specimens (15.1%) of the 33 erythromycin resistant ones were found to be positive for iMLSB by phenotypic disk diffusion D-test method, and out of the 5 D-test positive specimens, ermA PCR assay detected 4 as positive (80%), So ermA gene was predominant in iMLSB isolates.

Similarly, other studies by Nabavinia et al. demonstrated that most of their iMLSB-GBS isolates harbored the ermA gene [24], same results by Motallebirad et al. in Iran [25], and in Boston by Heelan et al [26].

Also, in France, a study performed in 2014 by Compain et al. stated that 84.5% of iMLSB isolates had ermA gene which indicates its predominance as well [22], another one by Nakamura et al. had concordant results as ermA gene was expressed in both iMLSB and cMLSB isolates which suggests that the ermA gene has mutated such that it is constitutively expressed in some strains of GBS [23].

With respect to different resistance mechanisms to macrolides, lincosamides, and
streptogramin groups. Different classes of erm genes coding for ribosomal methylation resistance mechanism are also expressed as ermB, ermC, ermTR...etc, up to 40 erm genes have been reported till now, these genes may be solely responsible for the MLSB resistance phenotypes or there may be more than one gene present on the same genome and they share in phenotypic resistance appearance together [17].

In current study we focused on ermA gene being the most predominant one expressed in Streptococcus agalactiae isolates as stated in different studies.

Given high positive predictive value, D-test is recommended to detect sensitivity to clindamycin, which can help in guiding clinicians towards the wise use of clindamycin.

Although more expensive compared to standard phenotypic methods, our study proved that the PCR technique, using ermA gene as target was not only more rapid but also has a higher sensitivity in detecting inducible clindamycin resistant GBS isolates during pregnancy. This allows for more accurate and rapid diagnosis of GBS which will be reflected into more effective treatment of colonized females.

**Conclusion**

In this study, the prevalence of *S. agalactiae* was remarkable and antibiogram is mandatory to detect the inducible resistance phenotype. Further studies are needed to provide a comprehensive data about the GBS antibiogram in other health care facilities to reduce neonatal morbidity and mortality.

**Conflicts of interest**

The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

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None.

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