Felemban *et al. J Pure Appl Microbiol,* **13**(**1**), 257-263 | March 2019
Article 5461 | https://dx.doi.org/10.22207/JPAM.13.1.27

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE

Correlation Between Strain Distribution and Antibiotic Resistance Genes Pattern of *Streptococcus agalactiae* Group B from Patients in Taif, Saudi Arabia

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Abstract

The Group B *Streptococcus* (*Streptococcus agalactiae*) (GBS) is one of the natural flora bacteria in the female reproductive system. In the recent years, GBS has become the major bacterial infections throw the perinatal period causing many troubles. This study was undertaken to determine the prevalence and antibiotic genes of GBS colonization in pregnant women of obstetrics hospital in Taif, Saudi Arabia. Fourteen *Streptococcus agalactiae* isolates obtained after screening about 134 swabs samples from genitourinary tract specimens of women patients from obstetrics hospital in Taif governorate, Saudi Arabia. These isolates were studied for antibiotic resistance and virulence genes. All obtained isolates were identified as *Streptococcus agalactiae* by the 16S rDNA gene sequence. These strains were found by Disc diffusion method sensitive against Meropenem, Cefotaxime, Cefepime, Amoxicillin, Penicillin G, Daptomycin, Chloramphenicol, Linezolid and Levofloxacin. The highest resistance was for Tetracyclin (85.7%) whereas the lowest resistance was found for Vancomycin (21.4%). Resistance against Erythromycin and Clindamycin was 71.5%, and 28.5% respectively. PCR based detection revealed 50% of isolates were carrying the *tetT* genes, while 92.8% of isolates were carrying *tetO* and *tetM* genes associated with Tetracycline resistance. All isolates were harboring genes that associated with Erythromycin resistance like *Erm*B1, *Erm*B2, and *Erm*(A|TR) genes, but only 28.5% of isolates were carrying *ErmTR* gene. Molecular detection of virulence associated genes revealed that out of fourteen isolates of *S. agalactiae*, only one isolate was carrying the *LinB* gene, while all isolates were positive for *Mre*A and *Van*A genes respectively. We can conclude that the GBS isolates were found sensitive to many antibiotic while most isolates were resistance to Tetracycline due to the existence of *tetO* and *tetM* genes. Resistance against Erythromycin and Clindamycin was 71.5%, and 28.5% respectively. The two erythromycin resistance genes (*Erm*B and *Erm*TR) were found in all isolates, while, the third erythromycin resistance gene *Erm*(A|TR) was found only in 25.8% of the isolates.

Keywords: *Streptococcus agalactiae* group B, antibiotic resistance, virulence genes, Saudi Arabia.

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(Received: 03 October 2018; accepted: 20 November 2018)

Citation: Ebaa M. Felemban, Daifellah A.M. Al Juaid, Walaa F. Alsanie, Mohamed M. Hassan and Ahmed Gaber, Correlation Between Strain Distribution and Antibiotic Resistance Genes Pattern of *Streptococcus agalactiae* Group B from Patients in Taif, Saudi Arabia, *J Pure Appl Microbiol*., 2019; **13**(**1**):257-263 doi: 10.22207/JPAM.13.1.27

INTRODUCTION

Group B streptococci (GBS)are a component of intestinal bacteria and human genitourinary microflora. These types are often associated with the disease found in new borns, affecting life-threatening diseases such as meningitis and sepsis. Also, these strains are linked to complications during pregnancy and postpartum[1]. GBS colonization of the Umayyad reproductive tract was considered the most important risk factor for the development of neonatal disease[2,3]. Moreover, GBS is a reason for infection in older infants and non-pregnant adults, particularly older people or those with any primary medical conditions. Clinical manifestations include urinary tract infections, skin infections or soft tissue, osteomyelitis, and pericardial infarction[3,4]. Territories of concern incorporate an expanded recurrence of perinatal disease and a higher death rate in grown-ups than in the neonatal group[5]. GBS was reliably defenseless against penicillin and other β-lactams. But, antimicrobial resistance utilized as an elective treatment, particularly macrolides, lincosamides and fluoroquinolones, has been recorded in various countries[6-8].The most widely recognized macrolide resistance mechanisms in streptococcus is the change of the ribosome by methylase, which is encoded by Erm genes. These enzymes also give resistance to the formation of resistance to lycosamides and streptopogen B, which characterizes macrolide-linuxamidestry-ptoline B (MLSB) phenotypes. Another common mechanism is the flow of drugs via membrane-specific protein encoded by the MEF gene, which is linked with M phenotype[9]. Protection from fluoroquinolones in GBS was first depicted in 2003 and is related with point mutations in gyrA and parC[10].In spite of the clinical impact of GBS disease and expanded protection from some antimicrobial, there is a limited number of reports indicating antibiotic resistance among the GBS strains in Saudi Arabia[7,11], even from non-symptomatic colonization or clinical contamination[12-15]. In present study, fourteen streptococcus *agalactiae* of 134 strains, isolated from genitourinary tract specimens of women patients from obstetrics hospital in Taif, Saudi Arabia from December 2017 to August 2018, were tested for antimicrobial susceptibility and macrolide resistance genes.

MATERIALS AND METHODS

Streptococcus strains

Fourteen *S. agalactiae* isolates obtained after screening about 134 swabs samples from genitourinary tract specimens of women patients from obstetrics hospital in Taif, Saudi Arabia. The bacterial species were identified using the fully automated VITEK-2 COMPACT microbiology system (Bio M'rieux, Inc., Durham, NC, USA). Purchased from strain control *S. agalactiae* ATCC® BAA1138™) from ATCC, USA.

Antibiotic Susceptibility Testing

Using the recommended clinical standard (CLSI) standard, World Health Organization method, the susceptibility of *S. agalactiae* against 16 antibiotics was determined[11,16].

The 16S rDNA gene sequencing

The genomic DNA was isolated using DNA extraction kit (Gena Bioscience, Germany) from all *S. agalactiae* isolates accordance the manufacturer's instructions. For each isolate and as per previously described methods, one fragment of the DNA (about 1,400 barrels) was amplified from the 16S rDNA gene[11]. The pieces were punctuated using the QIA quick PCR purification kit (QIAGEN, Valencia, CA, USA) and sequenced in DNA Analyzer 3146 Applied Bioscience (Applied Biosystems, USA). The sequencing texts were edited and compiled using DNASTAR software (Laser gene, Madison, WI, USA). Using the NCBI server, BLAST searches were performed (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Detection of Antimicrobial Resistance Genes

The antimicrobial resistance genes (The*kpsII,ermB1ermB*2,*ermBA/TR, mreA, ,ermTR, linB, mefA, vanAtetT*, *tetO* and *tetT*) were detected in the *S. agalactiae* strains[17,18]. Antibiotic resistance and virulence genes were detected in multiple-drug resistant enterococcal isolates by PCR with primers synthesized by the macrogen Co., Ltd. (Seoul, Korea) (Table 1).

Ethics Statement

This study was accepted by the ethics council of obstetrics hospital in King Faisal complex, Taif, Saudi Arabia, (1-439-6050). All subjects gave written informed consent before their consideration in the study.

Table 1. PCR primers for detection of some antibiotic resistance and virulence genes in *Streptococcus agalactiae* group Bisolates

Primers name	Primer sequence (52'→32')	Size (bp)
Erm(B1)	GAA AAG GTA CTC AAC CAA / AGT GGT ACT TAA ATT GTT TAC	640
Erm(B2)	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	420
Erm(TR)	GAA GTT TAG CTT TCC TAAGCT TCA GCA CCT GTC TTA ATT GAT	495
Erm(A/TR)	TCA GGA AAA GGA CAT TTT ACCATA CTT TTT GTA GTC CTT CTT	425
MreA	AGA CAC CTC GTC TAA CCT TCTCT GCA GGT AAG TAA GTG CG	498
LinB	CCT ACC TAT TGT TTG TGG AAATA ACG TTA CTC TCC TAT TC	925
MefA	AGT ATC ATT AAT CAC TAG TGCTTC TTC TGG TAC TAA AAG TGG	328
Van-A	GTAGGCTGCGATATTCAAAGC CGATTCAATTGCGTAGTCCAA	231
KpsII	GCGCAT TTGCTGATA CTGTTG CAT CCA GAC GAT AAG CAT GA	272
Tet(M)	GTMGTTGCGCGCTATATTCC GTGAAMGRWAGCCACCTAA	696
Tet(O)	GCGGAACATTGCATTTGAGGGCTCTATGGACAACCCGACAGAAG	538
Tet(T)	CAGTGGGAATATAAGGACACGTCCAAGCCTTCTCTACAGCATC	644

RESULTS

Enterococcus isolates identification

Out of 134 isolates, fourteen strains (10.4%) were identified as *Streptococcus agalactiae* by the using a fully automated VITEK-2 COMPACT microbiology system (BioMɪrieux, Inc., Durham, NC, USA). These results were confirmed by 16S rDNA sequencing (data not shown). All isolates were compared to the partial sequences of the 16S rRNA genes of *S. agalactiae* that deposited in the Gen Bank database and were found to be similar to the obtained sequence. Ribosomal operons are greatly relevant for the study of bacterial evolution and phylogeny. When re-constructing phylogenetic relationships of microorganisms, sequencing of 16S rDNA has been widely used. Phylogenetic analysis of the partial 16S rDNA sequences from the studied *S. agalactiae* strains, together with related sequences deposited in GenBank were positioned *Streptococcus* isolates into *Streptococcus agalactiae* group B (Fig. 1).

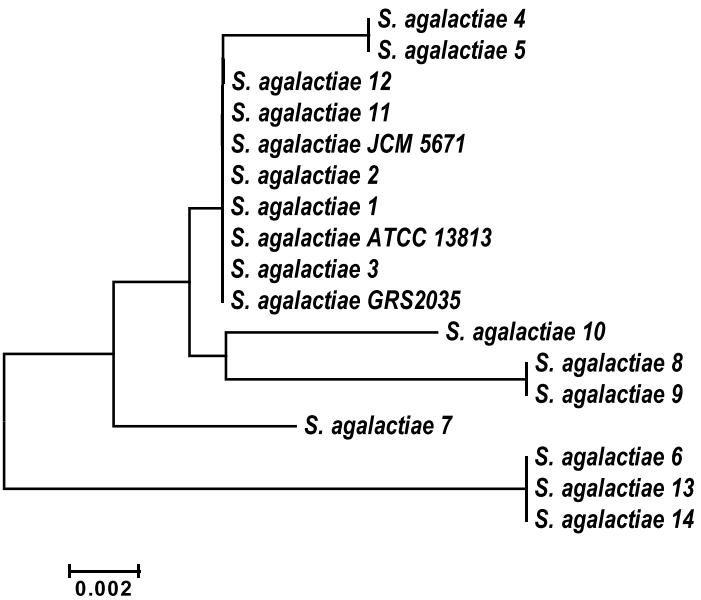

Fig. 1. Phylogenetic relationship of *Streptococcus agalactiae* group B isolates and related genera based on full size16SrDNA sequences. The tree was constructed using neighbor joining algorithm with Kimura 2 parameter distances in MEGA 7.1 software. The bar indicates the Juke-Cantor evolutionary distance.

Streptococcus specie sensitivity to antibiotics

All the fourteen *Streptococcus* strains were sensitive to Meropenem, Cefotaxime, Cefepime, Amoxicillin, Pencillin G, Daptomycin, Chloramphenicol, Lienezolid and Levofloxacin. The highest resistance was for Tetracyclin (85.7%) whereas the lowest resistance was found for Vancomycin (21.4%). Resistance against Erthromycin and Clindamycin was 71.5%, and 28.5% respectively (Fig. 2).

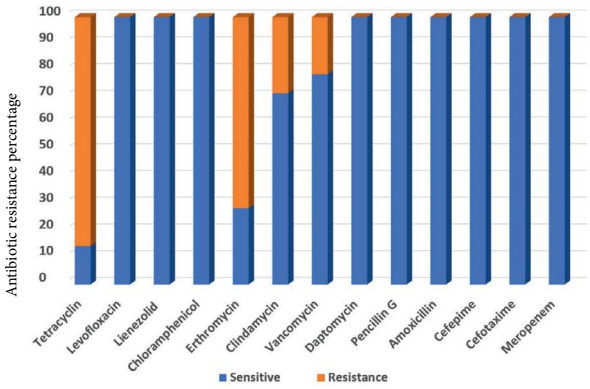

Fig. 2. Antimicrobial resistance profiles of nineteen multi-Drug resistance *Streptococcus agalactiae* group B against fourteen antibiotics.

Occurrence of antimicrobial resistance genes

The *kpsII, ermB1ermB*2, *ermBA/TR, mreA,* and *vanA* genes were detected in all *Streptococcus* isolates, while, *ermTR, linB, mefA,* and *tetT* genes were detected in 28.5, 7.1, 64.2, 50, and 50% of *Streptococcus* isolates, respectively. While, both *tetM* and *tetO* that associated with Tetracycline resistance were detected in 92.8 % of *Streptococcus* isolates. Table 2 shows the detection of these antimicrobial resistance genes in all isolates. The *emeB1, ermB2* and *ermA/TR* genes were found in all isolates. In addition to drug efflux, such *ermTR* gene that were present in 28.5% of the erythromycin-sensitive enterococci and 7.1% of the linezolid-sensitive *Streptococcus*, indicating no expression of the *emeB*gene in some *Streptococcus* isolates. Further, the *emeB* gene occurred at a greater significance in the

Table 2. Molecular characterization of virulence and Antibiotic genes in *S. agalactiae* group B

Strain	Antibiotic resistance genes										
	Erm B1	*Erm B2*	*Erm TR*	*ErmA/ TR*	*MreA*	*LinB*	*MefA*	*Van-A*	*TetM*	*TetO*	*TetT*
S-1	+	+	-	+	+	-	+	+	+	+	+
S -2	+	+	-	+	+	-	+	+	+	-	-
S -3	+	+	+	+	+	-	+	+	+	+	+
S -4	+	+	-	+	+	-	-	+	+	+	+
S -5	+	+	-	+	+	-	-	+	-	+	-
S -6	+	+	-	+	+	-	-	+	+	+	-
S -7	+	+	-	+	+	+	+	+	+	+	+
S -8	+	+	+	+	+	-	+	+	+	+	+
S -9	+	+	-	+	+	-	+	+	+	+	-
S -10	+	+	+	+	+	-	+	+	+	+	+
S -11	+	+	-	+	+	-	+	+	+	+	-
S -12	+	+	-	+	+	-	+	+	+	+	-
S -13	+	+	+	+	+	-	-	+	+	+	-
S -14	+	+	-	+	+	-	-	+	+	+	+

fluoroquinolone-resistant enterococci than that in the fluoroquinolone-sensitive *Streptococcus*. This indicates that the distribution of the *emeB*gene was related to the resistance to the three fluoroquinolones in the *Streptococcus* species (Fig. 3).

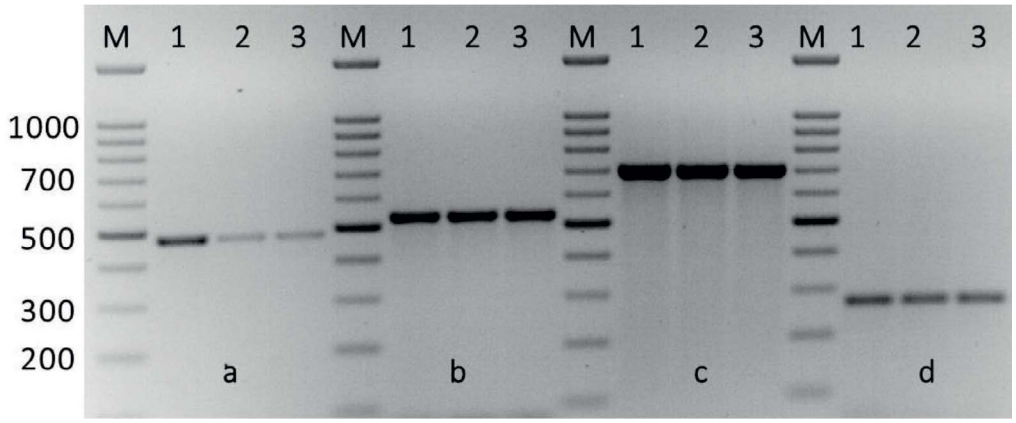

Fig. 3. Amplification of some specific genes producing in some *Streptococcus agalactiae* group B isolates by single PCR. (A) *MreA* gene with size about of 498 bp. (B)*Tet(O)*gene specific for tetracycline with size about of 538 bp. (C) *Tet(M)*gene specific also for tetracycline resistance with size about of 696 bp. (D) *KpsII* gene specific for capsule formationgene with size about of 272 bp. First lane on each panel is 100 bp molecular weight markers

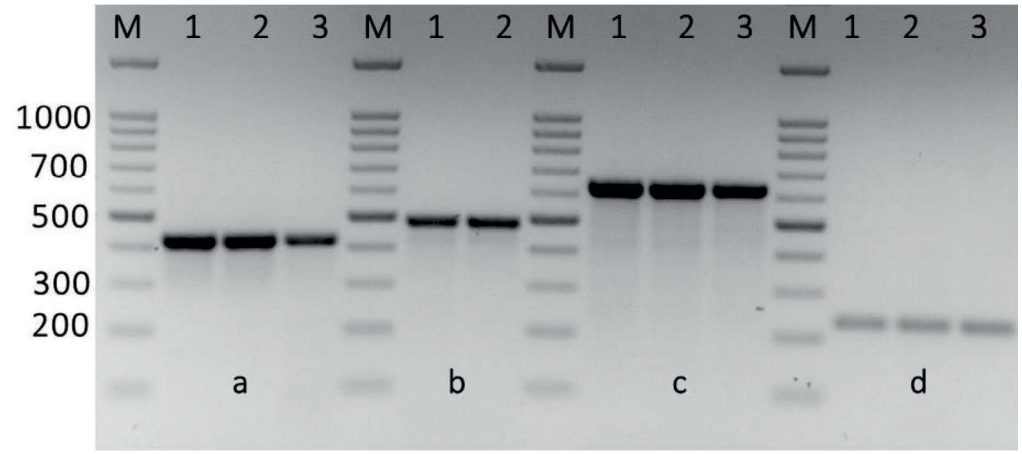

Fig. 4. Amplification of some specific genes producing in some *Streptococcus agalactiae* group B isolates by single PCR. (A) *Erm(A/TR)* gene specific for erythromycin resistance with size about of 425 bp. (B)*Erm(TR)*gene specific for erythromycin with size about of 495 bp. (C) *Erm(B1)* gene specific also for erythromycin resistance with size about of 640 bp. (D) Van-A gene specific for vancomycin resistance with size about of 231 bp. First lane on each panel is 100 bp molecular weight markers.

DISCUSSION

Protection from erythromycin, clindamycin and tetracycline antibiotics was seen among the considered Streptococcus isolates. The resistance frequency of *S. agalactiae* group B to such antibiotics have wide-ranging depending on the region and time assessed, as reported in experiments accompanied in various countries[8,9]. In Saudi Arabia, available recorded data about *S. agalactiae* group Bantimicrobial susceptibility were obtained from studies performed in different governorates[12,13,15,19,20]. In this investigation, the general occurrence of GBS colonization amongst pregnant ladies was observed to be 10.4%. Comparative discoveries have been accounted for in other African countries, for example, in Malawi,

Egypt, Zimbabwe, Gambia, and Tanzania; the prevalence of GBS in these countries ranges from 16.5 to 23%[21-23]. Nevertheless, low colonization rate was stated in a report directed in Ethiopia (9%) and some Latin American countries, for example, Peru (6%)[24,25]. Diverse examinations directed in Saudi Arabia indicated results higher than present investigation, 27.6% at Riyadh governorate[12]. On other hand, other reports showed results lower than present investigation as documented in 3-5% carriage rate in term of pregnant women at Aseer governorate[13] and 4.76% at Abha Maternity Hospital[14]. As shown in our results, Tetracycline and clindamycin resistance rates were fundamentally the same as those saw in past investigations directed in differentarea[26,27]. A point of worry is the discovery of an Erthromycin-resistant isolate (Figure 2). GBS extremely resistant to Erthromycin was first depicted in Japan[10],and from that point forward, it found in different countries[7,11]. Here, we report for the first time, the existence of Erthromycin resistance among *S. agalactiae* group B in Saudi patients at Taif governorate which it be recognized by molecular techniques. With respect to resistance against erythromycin, the elective treatment to penicillin unfavorably susceptible patients, results got in this investigation far surpassed the rates recently detected in the equivalent geological area. Despite the fact that there is no accessible information on macrolide utilization in other area in Saudi Arabia, it is well recognized that, for related species, protection from this antibiotic is specifically connected to its utilization[25]. Generally, it well known that the phenotypes of macrolide resistance isolates are well associated with genotypes, excluding for that one isolate displayed a cMLSB phenotype and did not have any of the related genes. Similar results for recognizin g*erm* and *mef* genes in *S. agalactiae* group B phenotype have been reported somewhere else[8]. Also, several strains that have *ermA*+ *ermB* genes and displayed an iMLSB phenotype has been reported in different areas, including Saudi Arabia[15,19]. But, combination of *ermA*+ *mefA/*E genes in an iMLSB phenotype had never been detected in erythromycin-resistant isolates that found in Saudi Arabia.

CONCLUSION

In the present study, it was found that the GBS isolates are resistance to Tetracycline due to the existence of *tet O*and *tetM* genes. Also most of the isolates were resistance to the antibiotic Erthromycine, that is because all isolates are carrying *ErmB1*, *ErmB2*, and *Erm*(*A*|*TR*)genes. Therefore, according to the results found in this study, the antibiotics Meropenem, Cefotaxime, Cefepime, Amoxicillin, Pencillin G, Daptomycin, Chloramphenicol, Lienezolid and Levofloxacin can be reliable replacements of erythromycin in curing or avoiding GBS infection in women who suffer from GBS colonization.

ACKNOWLEDGEMENT

This study was funded by the Deanship of Scientific Research, Taif University, KSA (research project number 1-349-6050).

CONFLICT OF INTEREST

The author declares that there are no conflict of interest.

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