MAEDICA – a Journal of Clinical Medicine
2014; 9(2): 157-161

MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

High Incidence of Macrolide and Tetracycline Resistance among *Streptococcus Agalactiae* Strains Isolated from Clinical Samples in Tehran, Iran

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ABSTRACT

Background: Streptococcus agalactiae *or Group B Streptococci (GBS) is an important bacterial pathogen that causes a wide range of infections including neonatal sepsis, meningitis, pneumonia and soft tissue or urinary tract infections.*

Material and methods: *One hundred and fifteen isolates of* Streptococcus agalactiae *collected from urine specimens of patients attending a hospital in Tehran. All isolates were screened for their capsular types and genes encoding resistance to the macrolide and tetracycline antibiotics by PCR and multiplex PCR–based methods.*

Results: *Most of isolates belonged to capsular types III (49%), V (19%), II (16%), and Ib (6%). Twelve isolates (10%) were nontypable. All isolates were susceptible to penicillin and Quinupristin-dalfopristin, but were resistant to clindamycin (35%), chloramphenicol (45%), erythromycin (35%), linezolid (1%) and tetracycline (96%). The most prevalent antimicrobial resistance gene was* tetM *found in 93% of the isolates followed by* ermTR, ermB, *and* tetK, *found in 23%, 16%, and 16% of isolates, respectively. The genes,* tetL, tetO, ermA, ermC *and* mefA *were not detected in any of the S. agalactiae isolates. Of the 110 tetracycline resistant* S. agalactiae, *89 isolates harbored the* tetM *gene alone and eighteen isolates carried the* tetM *gene with the* tetK *gene. All erythromycin-resistant isolates exhibited*

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Article received on the 14th of October 2013. Article accepted on the 19th of December 2013.

cMLSB resistance phenotype, 22 isolates harbored the ermTR *gene alone and five isolates carried the* ermTR *gene with the ermB gene. The rate of coexistence of genes encoding the erythromycin and tetracycline resistance determinants was 34%.*

Conclusion: *The present study demonstrated that* S. agalactiae *isolates obtained from urine samples showed a high rate of resistance to tetracycline, chloramphenicol and macrolide antibiotics and were commonly associated with the resistance genes* temM, ermTR *or* ermB.

Keywords: *Streptococcus agalactiae,* capsular type, *ermTR, tetM*

INTRODUCTION

Streptococcus agalactiae or Group B Streptococci (GBS) is both a human commensal and an important bacterial pathogen that cause a wide range of infections including neonatal sepsis, meningitis, pneumonia and soft tissue or urinary tract infections (1). Penicillin is currently the drug of choice for the treatment of S. agalactiae infections. Macrolide, lincosamide and group B streptogramins (MLS_B) antibiotics are often used as an alternative in the penicillin-allergic patient (2,3). However, the emergence of MLS_B resistance among *S. agalactiae* is an increasing problem in many parts of the world (4-7).

Resistance against MLS_B antibiotics among *S. agalactiae* may be occurring through two mechanisms: through target site modification and through an active efflux pump (8). Target site modification is encoded by the erythromycin ribosome methylase (*erm*) genes [*ermB* and ermTR], conferring resistance to MLS_B antibiotics. Phenotypic expression of MLS_B resistance can be inducible ($iMLS_B$) or constitutive ($cMLS_B$). On the other hand, an active drug efflux system that functions via a transmembrane pump encoded by the *mefA* or *mefE* gene is responsible for macrolides and group B streptogramins resistance only (9-11).

In streptococci, resistance to tetracycline is encoded by ribosome protection genes including *tetM* and *tetO* or by efflux pumps is encoded by the *tetK* or tetL genes (12). Tetracycline resistance genes are often found on the same mobile element as erythromycin resistance genes (13).

Despite the clinical impact of *S. agalactiae* infections and increasing resistance rates to some antibiotics, there is currently little information on the antibiotic resistance patterns and capsular types of *S. agalactiae* in Iran. The aim of the present study is to provide information regarding the prevalence of erythromycin, clindamycin, and tetracycline resistance determinants and capsular types among *S. agalactiae* isolates in Tehran, Iran. ❑

MATERIALS AND METHODS

2.1. Bacterial isolates

One hundred and fifteen *S. agalactiae* isolates were recovered from urine specimens of patients attending a hospital in Tehran (Pars Hospital) from May 2010 to October 2010. Only one isolate per patient was included. Isolates were identified to species level using standard biochemical methods (14). The study was approved by the Ethics Committee of Tehran University of Medical Sciences.

2.2. Antimicrobial susceptibility testing

Testing for susceptibility to the following antibiotics was performed using a disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (15): clindamycin (2μg), chloramphenicol (30μg), erythromycin (15μg), linezolid (30μg), penicillin (10 units), quinupristin-dalfopristin (15μg) and tetracycline (30μg).

Streptococcus pneumoniae ATCC 49619 and Staphylococcus aureus ATCC 25923 were used as control strains. The macrolide resistance phenotypes were determined by the double disc diffusion method with erythromycin and clindamycin discs on Mueller-Hinton agar supplemented with 5% sheep blood.

All figures including frequency and antimicrobial susceptibility results were rounded down if they were <0.5, and were presented as whole numbers if they were ≥ 0.5.

2.3. DNA extraction and gene detection

Total DNA was extracted from *S. agalactiae* isolates as previously described (16). Genes encoding resistance to the MLS_B (*ermA, ermB,*

ermC, ermTR, and *mefA*) and tetracycline (*tetM, tetL, tetK,* and *tetO*) antibiotic were investigated by PCR and multiplex PCR–based methods, using specific primers (4,9,17).

The primers used to detect Ia, Ib, II, III, and V capsular types; conditions of PCR; and size of the amplified products were as described by Poyart et al. (18). ❑

RESULTS

Of 115 *S. agalactiae* isolates, 56 (49%) were capsular type III, 22 (19%) were type V, 18 (16%) were type II, 7 (6%) were type Ib and 12 (10%) were non typeable. No strains of type I was found.

The antimicrobial resistance patterns of the strains are shown in Table 1. The results show that 110 (96%) isolates were resistant to tetracycline. Resistance to clindamycin (35%), chloramphenicol (45%), and erythromycin (35%) were prevalent but only 1 (1%) isolate was resistant to linezolid. All *S. agalactiae* were susceptible to penicillin and quinupristin-dalfopristin.

The antimicrobial resistance genes distribution among *S. agalactiae* isolates are shown in Table 2. The most prevalent gene was *tetM* found in 93% (107/115) of the isolates followed by *ermTR, ermB,* and *tetK* found in 23% (27/115), 16% (18/115), and 16% (18/115) of isolates, respectively. The genes, *tetL, tetO, ermA, ermC* and *mefA* were not detected in any of the *S. agalactiae* isolates in this study.

All erythromycin-resistant isolates exhibited $cMLS_B$ resistance phenotype, 22 isolates harbored the *ermTR* gene alone and five isolates carried the *ermTR* gene with the *ermB* gene.

Of the 110 tetracycline resistant *S. agalactiae,* 89 isolates harbored the *tetM* gene alone and eighteen isolates carried the *tetM* gene with the *tetK* gene. Three isolates did not have any of tested the tetracycline resistance genes. The rate of coexistence of genes encoding the erythromycin and tetracycline resistance determinants was 34% (39/115).

No significant differences in the distribution of the erythromycin and tetracycline resistance genes were found between strains of the various capsular types. ❑

DISCUSSION

The capsular polysaccharide is a major *S. agalactiae* virulence factor and it is commonly used for strain typing. Ten distinct capsular types have been recognized: Ia, Ib and II to IX (19). In this study, of 115 S. agalactiae isolates tested, more than 90% were capsular types III, V, II, and Ib. These results are similar to those from Germany, Portugal, Angola, Poland, Romania, and Brazil (20-25). However the predominance of particular capsular type differs noticeably between countries (26-29). This may reflect the emergence of particular clones in different geographical area or source of bacterial isolation.

Macrolide antibiotics, especially erythromycin, are important therapeutic agents for penicillin-allergic patients suffering from *S. agalactiae* infections. In the present study, the erythromycin and clindamycin resistance was 35%, this rate of resistance can be compared to similar reports from Romania (31%) and Tunisia (40%) but it is higher than rates reported from Portugal (19%) and Japan (12.8%) (7,21,24, 29). Expression of macrolide resistance in *S. agalactiae* isolates may be constitutive or inducible. When expression is constitutive, the isolates are resistant to erythromycin and clindamycin antibiotics. However, when the expression is inducible the isolates are only resistant to erythromycin. In our study, macrolide -resistant phenotype correlated well with the major antimicrobial resistance genes, all macrolide-resistant isolates exhibited constitutive (cMLSB) resistance phenotype and all of them contained the *ermTR* or *ermB* genes and negative for the *mefA* gene. In the current study, as in many reports the coexistence of the *ermTR* and *ermB* genes among macrolide-resistant isolates was common (21,28).

	N(%) of isolates		
Antibiotic	**Resistant**	**Intermediate**	**Susceptible**
Clindamycin	40 (35)	5 (4)	70 (61)
Chloramphenicol	51 (45)	5 (4)	59 (51)
Erythromycin	40 (35)	4 (3)	71 (62)
Linezolid	1 (1)	-	114 (99)
Penicillin	-	-	115 (100)
Quinupristin-dalfopristin	-	-	115 (100)
Tetracycline	110 (95)	1 (1)	4 (4)

TABLE 1. The antibiotic resistance patterns of Streptococcus agalactiae strains isolated from urine culture.

SD, standard deviation; EAT, epicardial adipose tissue; HDL, high density lipoprotein; TG, triglyceride; BSA, body surface area; FPG, fasting plasma glucose.

Gene	Resistance pattern	Capsular types	Number of isolates
tetM	TET*	Ib, II, III, V	40
tetM	TET, C	Ib, II, III, V	15
ermTR	ERY, CD	NT#	1
tetM + *tetK*	TET	II, III,V	9
tetM + *tetK*	TET,C	II, III, NT	4
tetM + *ermTR*	TET, ERY, CD, C	II, III,V	17
tetM + *ermTR*	TET, ERY, CD	III, NT	2
tetM + *ermB*	TET, ERY,CD, C	II, III, NT	9
tetM + *ermB*	TET, ERY, CD	III, V	2
tetM + *tetK* + *ermTR*	TET, ERY,CD, LZD	III	1
tetM + *tetK* + *ermTR*	TET, ERY, CD	V	1
tetM + *tetK* + *ermB*	TET, ERY,CD,C	Ib, V	2
tetM + *ermB* + *ermTR*	TET, ERY,CD,C	Ib, II, V, NT	4
tetM + *tetK* + *ermB* + *ermTR*	TET, ERY,CD	II	1

TABLE 2. Distribution of the antimicrobial resistance genes and capsular types among *Streptococcus agalactiae* isolates.
* TET, tetracycline; C, chloramphenicol; CD, clindamycin; ERY, erythromycin; LZD, linezolid
# NT, non typeable

In the present study, the tetracycline-resistance rate among *S. agalactiae* isolates was 96% which is slightly lower than similar reports from Turkey (100%) and Tunisia (97.3%) and more than Kuwait (89.5%), Italy (68.1%) and Japan (46.5%) (5,7,28-30). In our study, as in many reports tetM was the most prevalent tet gene, encountered in more than 93% of tetracycline-resistant isolates (7,28,30). The second most detected tet gene in this study is tetK, the protein encoded by which confers an efflux-mediated resistance to tetracycline. These results indicated that ribosome protection was the most important mechanism of tetracycline resistance in our isolates and it is mainly mediated by the tetM gene.

We found the co-occurrence of genes encoding the erythromycin and tetracycline resistance determinants among our *S. agalactiae* isolates, as did Acikgoz et al and Gherardi et al. (5,30). However, the mechanisms of this coexistence are yet not clear.

In the current study, resistance to chloramphenicol was high (45%), which is difficult to explain since chloramphenicol is rarely used in Iran. However, high prevalence of chloramphenicol resistance in *S. agalactiae* was also detected in Turkey (44. 2%) and Kuwait (30%) (5, 28). We also found one linezolid-resistant isolate, which could indicate an emerging public health problem. Our results are also consistent with those of others who found that *S. agalactiae* isolates are almost always susceptible to penicillin and quinupristin-dalfopristin (5,7,24, 28).

In conclusion, to our knowledge, this study is the first report has demonstrated that *S. agalactiae* isolates obtained from urine samples showed a high rate of resistance to tetracycline, chloramphenicol and macrolide antibiotics and were commonly associated with the resistance genes temM, ermTR or ermB in Tehran, Iran. ❑

Conflict of interests: none declared.
Financial support: none declared.

Acknowledgment
This research has been supported by Tehran University of Medical Sciences & Health Services grant 15952/90-04-30 and Ilam University of Medical Sciences grant 9190161116.

The authors of this manuscript wish to thank the participants in this study. A special thanks to Dr. Shahram Bromandi in Pars Hospital for his assistance in specimen collection.

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