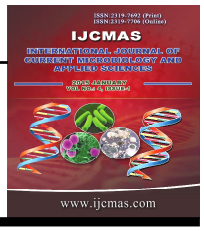
Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 419-436

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 1 (2015) pp. 419-436**
http://www.ijcmas.com



Original Research Article

Prevalence of macrolide resistance genes among Group B *Streptococci* in pregnant women

Zainab Oday Hamid*, Neihaya H. Zaki and Munim R. Ali

Department of biology, College of science, the University of Mustansiriya, Baghdad, Iraq
**Corresponding author*

Keywords

Group B Streptococcus (GBS), Septicemia, Macrolides, *erm*B, *erm*A/TR, *erm*C

A B S T R A C T

Group B streptococcal septicemia is caused by the bacterium *Streptococcus agalactiae***,** which is commonly called or Group B *Streptococcus* (GBS), this opportunistic bacterium can destroy suboptimal host defenses to cause invasive disease and tissue damage. Transplacental infection or an ascending infection from the cervix may be caused by this bacteria that colonize the mother's genitourinary tract the neonate acquires the microorganisms as it passes through the colonized birth canal at delivery. Septicemia is an infection in the bloodstream that may travel to different body organs and causes an invasive infection in newborn infants, in women around the time of childbirth, and in older individuals with underlying chronic illnesses. Macrolides are an important class of antibiotics effective against a number of pathogenic bacteria. These mostly bacteriostatic drugs, and continue to be viewed as excellent antibiotics with high potency and low toxicity. Macrolides belong to one of the most commonly used families of clinically important antibiotics used to treat infections caused by Grampositive bacteria. One hundred and eighty nine vaginal swabs were collected from Ibn alBalady Hospital, Central Public Health Laboratory and Kamal alSamarrai Hospital. Fortysix *Streptococcus agalactiae* isolates were obtained during October 2013 to February 2014. The most prevalent age among GBS infected pregnant women was among (3537) years old with 76.08% rate of infection. The isolates were initially identified phenotypically by blood agar culture media and chromogenic culture media and further identification was performed by VITEK 2 system and by molecular methods by 16s rRNA based PCR. A comparative analysis were performed between the three phenotypic detection methods and molecular method were best tool for *S. agalactiae* detection, where in which 46 isolates were identified while 37, 36, 30 isolates were detected by VITEK 2 system, chromogenic agar and blood agar respectively. VITEK Antibiotic Susceptibility Test for different antibiotics were performed to all detected isolates, the highest prevalence of resistance was 65.2% toward tetracycline and followed by 58.6% and 45.6% for erythromycin and clindamycin respectively. Macrolide resistance genes were screened and the resistant rate in total isolates was 69.5%. Ribosome methylation genes (*erm*B, *erm*A/TR, *erm*C) were screened in all examined isolates; the most prevalence gene was *erm*B with 63.04%, whereas 23.9% was the prevalence of *erm*A\TR gene and despite of changing of different PCR conditions, this study failed to detect *erm*C gene in any of examined isolates. The results of the study displayed that the prevalence rate of efflux pumps encoding genes (*mef*A and *mreA*) were 8.69% and 69.5% respectively. The current study showed that 14 (30.4%) of total isolates have not recognized as a carrier of any erythromycin resistance genes. One isolate of all screened isolates was harboring four erythromycin resistance genes except *erm C* gene.

Introduction

Group B Streptococci (GBS) are Grampositive bacteria that are common asymptomatic colonizers of healthy adults. However, this opportunistic organism can also subvert suboptimal host defenses to cause severe invasive disease and tissue

damage (Rajagopal, 2009). GBS is an important cause of invasive infection in newborn infants, in women around the time of childbirth, and in older individuals with underlying chronic illnesses (Schuchat, 1998). Although GBS has the capacity to produce lifethreatening infection in susceptible hosts (Yamamoto *et al*., 2005).

This bacterium possessing an array of immune resistance phenotypes and secreted toxins that render it capable of producing serious disease in susceptible hosts, in particular the human neonate (Nizet *et al*., 2000). Antibiotic resistance in the group B *Streptococcus* (GBS) is not widely appreciated and many clinical laboratories do not undertake full antibiotic susceptibility tests of clinical isolates (Liddy and Holliman, 2002). Recently, increasing antimicrobial resistance consider implications for GBS disease treatment and Intrapartum prophylaxis (Borchardt *et al*., 2006).

GBS has been continuously susceptible to penicillin and other βlactams. However, resistance to antimicrobials used as alternative therapy, especially macrolides, lincosamides and fluoroquinolones has been documented in different countries (Nakamura *et al*., 2011). Macrolides are an important class of antibiotics effective against a number of pathogenic bacteria. These mostly bacteriostatic drugs, it were introduced into medical practice almost 60 years ago and continue to be viewed as excellent antibiotics with high potency and low toxicity Kannan and Mankin, 2011). Macrolides belong to one of the most commonly used families of clinically important antibiotics used to treat infections caused by Gram positive bacteria (Zhang *et al*., 2010).

Erythromycin A was the first macrolide introduced into clinical use in the early 1950s (Kannan, K. and Mankin, 2011). Erythromycin is an organic compound produced by the actinomycete *Streptomyces erythraeus*, currently known as *Saccharopolyspora erythraea* (Rantala, 2009).

Macrolides, which are primarily antibiotics, belong to the polyketide group of natural products. They derive their name from their characteristic structural features, a macrocyclic lactone ring to which various deoxy sugars (Steel *et al*., 2012).Many natural and synthetic antibiotics inhibit bacterial growth by interfering with protein synthesis. Most of these drugs act upon the ribosome (Tenson and Mankin, 2001). Erythromycin binds to domain V of the 23S rRNA within a tunnel of the peptidyltransferase center (Rantala, 2009). Erythromycin blocks the polypeptide exit tunnel and thus prevents the extension of the growing peptide and provokes the premature release of the unmature peptide chain (Rantala, 2009).

Macrolide were shown to compete with the peptidyl tRNA for binding to the ribosome and therefore can cause spontaneous dissociation of the peptidyltRNA before the nascent peptide enters the exit channel (Tenson and Mankin, 2001). Some macrolides with extended side chains reach close to the catalytic center and interfere with peptide bond formation (Poulsen *et al*., 2000).

Macrolide resistance mechanisms in GBS represented by two mechanisms: Target Site Modification by Erythromycin ribosomal methylase, mediated by *ermB*, *Erma*, *ermTR*, or *ermC* genes which confers cross resistance to macrolides, lincosamides, and streptogramin B (MLSB phenotype) (Cai *et al*., 2007). *Erm* genes encode methylase 23S

rRNA, which is responsible for methylation of erythromycin and clindamycin receptor sites in ribosomes (BrzychczyWłoch *et al*., 2010). This resistance can be constitutive macrolide–lincosamide–streptogramin B (cMLSB) resistance, as well as inductive macrolide–lincosamide– streptogramin B (iMLSB) resistance (Gherardi *et al*., 2007).

The second resistance mechanism that found in GBS are Macrolide efflux pump, mediated by *mef* genes, which confers resistance to 14 and 15 membered macrolides only In addition, a novel efflux system distinct from the Mef pump and encoded by *mreA* (for macrolide resistance efflux) was recently reported in a unique strain of *S.agalactiae* COH31 γ/δ (Clancy *et al*., 1997). However, susceptible GBS strains have also been shown to possess the *mreA* gene, and it might function as a housekeeping gene (Allam and Bahgat, 2006).

Materials and Methods

Samples collection and bacterial isolation

One Hundred eighty nine swap were taken from the mucus of the vaginal tissues of pregnant women at 3640 weeks of gestation during 20 October 2013 to 22 February 2014 from IbnAlbalady hospital, Central health laboratory, KamalAlsamaraie hospital. Then, swabs were transported to the laboratory to be cultured using Amies transport media. Each swab was cultured firstly on Columbia blood agar by gently streaking on sterile prepared plates and incubated at 37°C for 2448 hours. Then, a significant growth was obtained. After that, colonies were subcultured on CHROM strep B agar, a selective media for GBS isolation. Pink and blue colonies were obtained. The pink to moave colonies were sub cultured in the same previous condition to get a pure single colony while blue colonies, which belonged to other bacteria, were neglected. Morphological examination, latex agglutination test, catalase test, camp test, microscope examination and 16srRNA genetic identification were done to confirm GBS identification.

Determination of the Minimum Inhibitory Concentration (MIC)

Susceptibility to antibiotics and Minimum inhibitory concentration was determinated for all isolates using Vitek 2 system as described in Vitek idenification with some differences listed in Kit brochures depending on the manufacturing company.

Amplification of 16srRNA gene and Macrolide Resistance Genes

Preparation Template DNA

Wizard genomic DNA purification kit was used for DNA extraction and the instruction was followed. All DNAextracted isolates were checked to ensure that the products of extraction procedure were sufficient to be amplified. From each sample 10µl of extracted DNA were placed in the agarosegel well and colored by 2µl of preprepared loading dye after well mixing. TBE buffer (1X) was poured in to gel tank and the tray were placed horizontally placed in the electrophoresis tank. Electrophoresis were done with 7V/cm power supply for 1.5 to 2 hours.

Then five microliters of DNA ladder (100bp) was loaded in single lane. Finally all extraction product were visualized using UV transilluminator .the positive samples were used to further investigation while negative ones were repeated to obtain DNA for certain genomic detection.

PCR Amplification Procedure

All isolate were detected as *S. agalactiae* by 16srRNA gene. All GBS isolate screened for three macrolide resistance genes ermC, ermB and ermA/ TR and two efflux pump genes mreA and mefA.

PCR condition were optimized by repeated changing temperatures ranged (55 to 60) and number of cycle (30 to 35) as listed in table. usually process started with initial denaturation step (96°C for 5 minutes) followed by repeated cycles which consist from denaturation step ranged from 94°C to 96°C, annealing step depend on primer then extension step (mostly at 72°C) followed by final extension step (usually at 72°C) (Joshi and Deshpande, 2011). The amplified PCR product were resolved by horizontal agarose gel electrophoresis.

Results and Discussion

Group B Streptococci Isolation

One Hundred and eighty nine vaginal swab was collected from pregnant women during gestation period (3840) week and from different periods of age ranging (1846) years old as listed in table 9. The collected samples were from (IbnAlbalady, kammalAlsamaraie hospitals and central health center). All swabs was transported by Amies transport media to the laboratory for bacterial isolation and further identification by culture methods, Vitek system and PCR amplification.

The prevalence of *S. agalactiae* among Iraqi pregnant women was 24.3%. Forty six group B *Streptococcus* was collected out of all swabs and identified and confirmed with many tests before be used. The study showed that the highest percentage of GBSinfected women was 35 strain (76.08%), which were occurred between (3537) years old while, the lowest incidence of infection was one specimen (2.17%) between (1824) years old pregnant women as shown in table (9).

As well as, the pregnant women with (2535) and (3746) years old displayed a moderate rate of infection reached three (6.52%) and seven (15.2%) strains respectively as shown in table (3).

Genotypic Identification

Molecular GBS identification performed by *16srRNA* gene based PCR. The entire one hundred and eighty nine collected specimen were tested by PCR to ensure accuracy and to find the most suitable method for GBS detection. In vaginal specimens, 45 (23.8%) GBS were detected by *16srRNA* gene based PCR as agreed with (Mian, 2009). Sensitivity of PCR in diagnosis of *S.agalactiae* can be improved by choice of a suitable extraction method (Kim *et al*., 2001; AlSoud *et al*., 2000). The advantage of this method is that results can be obtained within 1 day as compared to traditional microbiological testing (Nilsson *et al*., 2003). In addition, previous work on other bacteria has indicated that *16srRNA* gene is accurate rapid tool for identification (Nilsson *et al*., 2003; Sacchi *et al*., 2002)

Prevalence of Antibiotic Susceptibility and Minimum Inhibition Concentration (MIC) of GBS Isolates

The entire isolate were examined by Vitek 2 system to determine its antibiotic susceptibility and minimum inhibition concentration for each tested antibiotic. Certain antibiotics were used in the study which is include erythromycin since the macrolide consider are the recommended secondline agents for GBS prophylaxis in

the case of betalactam allergy as mentioned in (Fluegge *et al*., 2004).

Erythromycin susceptibility testing showed that 27 (58.6%) of all GBS isolates were resistant to erythromycin. As compared to reports from other countries with erythromycin resistance rates up to 50.7% (Back *et al*., 2012) while, (Hsueh *et al*., 2001) found the resistance ratio reached Four (26.7%) of the penicillinno susceptible isolates were resistant to erythromycin. (Jain *et al*., 2012) reported in his study (33%) erythromycin resistance and (von Both *et al*., 2003*)* found 10% resistance among all tested isolates.

In Tunisian hospital, 40% of GBS were resistant to erythromycin (Hraoui *et al*., 2013) also, (Lin *et al*., 2000*)* found (22%) was the resistant proportion in his study. The study showed a close result from Taiwan (4346%) and the USA (54%) (Hsueh *et al*., 2001; DiPersio *et al*., 2006), the high rate of macrolide resistance appeared to be related to the high usage of these antibiotics. In contrast to the previous, another Arabian recent studies reported low resistance, which is decreased to 0.7% in Kuwait and 13.15% in Egypt (AlSweih *et al*., 2005), in many European countries [Germany (12%), Belgium (16.7%), Spain (818%) and France (1821.4%) (Ruess *et al*., 2000; Decoster *et al*., 2005; Fitoussi *et al*., 2001), in Japan (3.0%) and in Canada (18%) (Matsubara *et al*., 2001; de Azavedo *et al*., 2001).

In our study, 21 (45.6%) of GBS isolate were not susceptible to clindamycin which did not come in line with (Panda *et al*., 2009; Lambiase *et al*., 2012) who showed decreased rate of resistance reached to (3%) to (21%) for clindamycin. As well as (Decoster *et al*., 2005) and (Zeng *et al*., 2006) found similar result to the previous one up to (18%) resistance and (17%) resistance isolates, respectively. Whereas (Back *et al*., 2012) showed approximately elevated rate of clindamycin resistance equal to 38.4%.

The result showed 15 (32.6%) of erythromycin resistant isolate displayed crossresistance to clindamycin. Resistance to erythromycin and clindamycin emerged during the last decade in many countries. Clindamycin. Other report revealed resistance to both erythromycin and clindamycin, and the prevalence was considerably higher, ranging from 16.4% to 70% in the study period, showing a statistically significant increment (Leclercq *et al*., 2002); this finding is very far from our study which indicating significant country variations.

The increased resistance to macrolides, particularly to erythromycin observed all over the world, can be ascribable to the treatment of Chlamydia infections of the lower reproductive tract however we have no explanation for the higher rate of resistance in our population. A hypothesis is that the variation may be due to differences in techniques as well as characteristics of the population investigated (Lambiase *et al*., 2012). Penicillin G was used in the study since it is consider the antibiotic of choice for prophylaxis (Rausch *et al*., 2009; Lin *et al*., 2001). The data demonstrate that all fortysix GBS isolates was susceptible to benzyl penicillin, ceftriaxone, linezolid, trimethoprim/sulfamethoxazole and vancomycin.

Despite of that Worldwide, there have been only a few reports penicillin resistance. Our study concurred with (Heelan *et al*., 2012) and (Lin *et al*., 2000) and all GBS was susceptible to penicillin and vancomycin. Susceptibility to Ampicillin and levofloxacin was examined and displayed

three (6.5%), one (2.1%) rate of resistance respectively which agreed with (Barros *et al*., 2012) which indicate low levels of resistance.

High rate of resistance recorded in the result of the study reached 30 (65.2%). Tetracycline resistance in GBS is due to acquisition of two mechanisms: an effluxmediated mechanism encoded by *tetK* or *tetL* genes; and ribosomal protection proteins, which protect the ribosome from the action of tetracycline, mediated by *tetM*, *tetO*, *tetS*, *tetP*, *tetQ* and *tetU* resistant determinant (Hraoui *et al*., 2012). Tetracycline is a broad spectrum antibiotic, not widely used as treatment for streptococcal infections; however, resistance to these drugs has become widespread among GBS. A high rate of tetracycline resistance was noted as was described in other studies performed in different countries; USA (96%), Germany (74.5%) and France (88.1%) (Florindo *et al*., 2010; Portillo *et al*., 2001; Aracil *et al*., 2001).

Out of (30) tetracycline resistance isolates 15 (50%) express cross resistance to erythromycin in current research. Tetracycline resistance genes are often found on mobile genetic elements that Carry macrolide resistance genes, so cooccurrence of resistance to both classes of drugs can be observed as (Clancy *et al*., 1997) reported. As well (Hraoui *et al*., 2012) showed that the Resistance to tetracyclines in *S. agalactiae* is assumed to be mainly due to the presence of conjugative transposons and *tetM* can be transferred via Tn916 related transposons, which may account for its spread.

Incidence of Ribosome Methylation Genes

Three different ribosome methylation genes were used to screen all GBS isolates in the study. They are *ermB*, *ermC* and *ermA* (subclass *TR*). Figure (2) illustrate an ethidium bromide stained agarose gel showing DNA fragments produced by PCR amplification of the *ermB* gene from *S. agalactiae*. As it is clear, the positive results in lanes 1, 2, 3, 4, 5 that belong to the V2, V17, V31, V35, V43 isolates with amplified size 454 bp.

This result was relatively closed with the result of (Marimón *et al*., 2005) who showed 31.8% of all screened isolates was harbored *ermA/TR* gene while (Zeng *et al*., 2006) report approximately low proportion of resistance encoded by *ermA/TR* reach to 9%. Another study demonstrate 8.12% of all screened GBS was owned this gene. In Turkish hospital, the prevalence of resistance by previous gene was 2.9% (Acikgoz *et al*., 2004). Another low rate of resistance through *ermA/TR* gene was illustrated by (Marimón *et al*., 2005) reached to (0.8%).

In china, approximate elevated level of macrolide resistance isolates by *ermA/TR* were detected as 17.9% among all isolate (Zeng *et al*., 2006). Whereas, study in Iran showed 23% of examined isolates were resistance to erythromycin drug (Emaeini *et al*., 2014). Moreover, Canadian report demonstrate on its result 20% of all screened GBS was harbored this gene (de Azavedo *et al*., 2001). As expected in other studies, the *ermA/TR* and the *ermB* genes were the most prevalent among erythromycin resistant GBS strains (Zeng et al., 2006).

As the third ribosomal methylation gene (*ermC*) screened, no detectable isolate was noticed. Previous result are concurred with the result of (Arana *et al*.,2014) which indicate the absence of *erm C* gene among all examined isolates as shown in figure (4).

Investigation of Efflux Pumps Genes among GBS Isolates

All resistant isolates were screened to examine the prevalence of efflux pumps mechanisms, which encoded by *mreA* and *mefA* genes. Figure (5) displayed a number of erythromycin resistant isolate which gave a positive result for harboring *mreA* gene occupied 1, 2, 3, 4, 5, 6 lanes with 498 bp amplification size of V1, V5, V11, V14, V23 and V41 isolates.

The result showed that the rate of *mreA* existence reached to 100% among resistant isolates, since its consider a housekeeping gene and high conserved among resistant strains as reported in study carried out by (Clarebout *et al*.,2001) and the previously mentioned gene display 100% of presence. Moreover, (Clancy *et al*., 1997) showed that *mreA* gene found in all investigated resistant isolates and 10 of susceptible ones which concurred with the result of thestudy.

The putative tertiary structure of *mreA* gene is probably not consistent with that of a protein, which spans a biological membrane, but it has short recurrent hydrophobic regions of approximately 10 amino acids that may associate transiently with the cell membrane or perhaps with specific membrane proteins. Further studies are needed to determine whether additional membrane associated functionalities are required to effect macrolide efflux. If this were, so, functionally equivalent structures would appear to be present in grampositive and gramnegative bacteria, since experiments suggest that *mreA* functions in both *E. coli* and *S. agalactiae*. The distant but extensive homologies to secreted proteins of some prokaryotic and eukaryotic organisms suggest that *mreA* plays a role in conveying the macrolide to the inner face of themembrane (Clancy *et al*., 1997).

The second efflux gene *mefA*, which confers efflux pump resistance only to 14 and 15 membered macrolides (de Azavedo *et al*., 2001), has been examined among the entire isolates. *Mef* gene is believed to encode a hydrophobic membrane protein, which uses the energy of the proton motive force to pump macrolides from the interior of the cell (Clancy *et al*., 1997). The result showed that the mentioned gene was detected in 12.5% of resistant isolates.

Figure (6) displayed agarose gel electrophoresis of PCR product for efflux pump *mef* gene. As it is obvious lanes size 328 bp while lane 5 showed 100 bp DNA marker 1, 2, 3 and 4 showed a positive result for V4, V12, V32 and V41 isolates.

A study have been done in Tunis reported that *mefA* gene prevalence was 75% among resistant isolates, which consider high rate as compared with another study (Aracil *et al*., 2002) that, recorded 25% of *mefA* existence among examined isolates. Another low percentage was displayed as 9.3% of total isolates (Marimón *et al*., 2005). Furthermore, many studies in France, Spain and Canada showed descending rank of *mef A* incidence (Fitoussi *et al*., 2001; Betriu *et al*., 2001; de Azavedo *et al*., 2001). Whereas, a study carried out in Iran reported that *mefA* not detected by.

One isolate (V12) showed coexistence with genes combination which they are (*ermB*, *ermA*/*TR*, *mefA* and *mreA*). In current study (10/11) of (*ermA*/*TR*harboring) isolates (V1, V3 V8, V14, V20, V24, V26, V40, V44 and V46) were possessed a combination of genes include (*ermB* and *mreA* only) which consider high rate as compared to (Marimón *et al*., 2005) which indicate in their study 2.3% of gene combination. Although (Acikgoz *et al*., 2004) revealed 8.6% among resistance isolates were harbored of

previously mentioned genes. This coexistence of the *ermA/TR* and *ermB* genes found in many reports as (Emaeini *et al*., 2014; Udo *et al*., 2014; Florindo *et al*., 2010).

Only one phenotypically and genetically susceptible isolate (V9) was harbored *mreA* gene alone. On other hand, all *mefA*harboring isolates were containing *mreA* gene whereas, thirtyone *ermB* containing isolates were possess *mreA* gene. Current study also showed that three susceptible isolates to erythromycin by Vitek examination were detected as erythromycin resistant in genetic screening by using ribosomal methylation genes and efflux pumps gene, which could explained as lack of gene expression (Krishnaveni *et al*., 2014).

Table.1 PCR primers sequences for target genes used in experiments. Manufactured by Promega, USA.

Target Gene	Primers (5' to 3')	Product	Amplicon (bp)	Reference
16S rRNA	**F:** GCCTCATAGCGGGGATAAC	16S Ribosomal RNA	328	Mian *et al*., 2009
	R: ACGTTCTTCTCTAACAACA			
ermB	**F:** GGTAAAGGGCATTTAACGC	Erythromycin resistant methylase	454	Poyart *et al*., 2003
	R: CGATATTCTCGATTGACCA			
ermA/TR	**F:** TCAGGAAAAGGACATTTTC	Erythromycin resistant methylase	423	Poyart *et al*., 2003
	R: ATACTTTTTGTAGTCCTTCT			
ermC	**F:** TCAAAACATAATATAGATA	Erythromycin resistant methylase	649	Poyart *et al*., 2003
	R: GCTAATATTGTTTAAATCGTCAAT			
mefA	**F:** AGTATCATTAATCACTAGG C	Macrolide efflux protein	328	Poyart *et al*., 2003
	R: TTCTTCTGGTACTAAAAGTGG			
mreA	**F:** AGACACCTCGTCTAACCTTC	Macrolide resistance efflux protein	498	Poyart *et al*., 2003
	R: TCTGCAGGTAAGTAAGTGG			

Table.2 Solutions mixture used for genes amplification procedure. The final volume was 25µl for each PCR tube

Target gene	Template DNA (µl)	F+R Primers (10 pmol/ml) (µl)	GOTaq®Green Master Mix (1x) (µl)	Nuclease free water (µl)
16S rRNA	5	1.5	12.5	4.5
ermB	5	1.5	12.5	4.5
ermC	5	2	12.5	3.5
ermA/TR	5	2	12.5	3.5
mefA	6	1.5	12.5	3.5
mreA	5	1.5	12.5	4.5

Table.3 Reaction conditions of PCR used for genes amplification. Elongation conditions for all genes were 72°C for 1min and final extension 72°C for 7min.

Genes	Initial denaturation	Number of cycle	Denaturation	Primer annealing
16S rRNA	96°C/5min	30°C	96°C/30sec	55°C
ermB	95°C/5min	30°C	95°C/1min	58°C
ermC	95°C/5min	35°C	96°C/1min	55°C
ermA/TR	95°C/5min	35°C	95°C/30sec	53°C
mefA	95°C/5min	35°C	95°C/1min	61°C
mreA	95°C/5min	30°C	95°C/30sec	49°C

Table.4 Age of infected women and percentage of infection among them.

Age	(18-24)	(25-45)	(35-37)	(38-46)
Number of infected women	1/46	3/46	35/46	7/46
Percentage	2.17%	6.52%	76.08%	15.20%

Figure.1 An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for 16S rRNA gene. Showing 328bp of DNA amplification fragments produced by PCR for 16S ribosomal RNA gene from S. agalactiae. Lanes 1, 2, 3, 5, 7, 8, 9, 10, 11, 12 and 13: 16S rRNA gene amplification fragments; Lane 6: 100 bp ladder

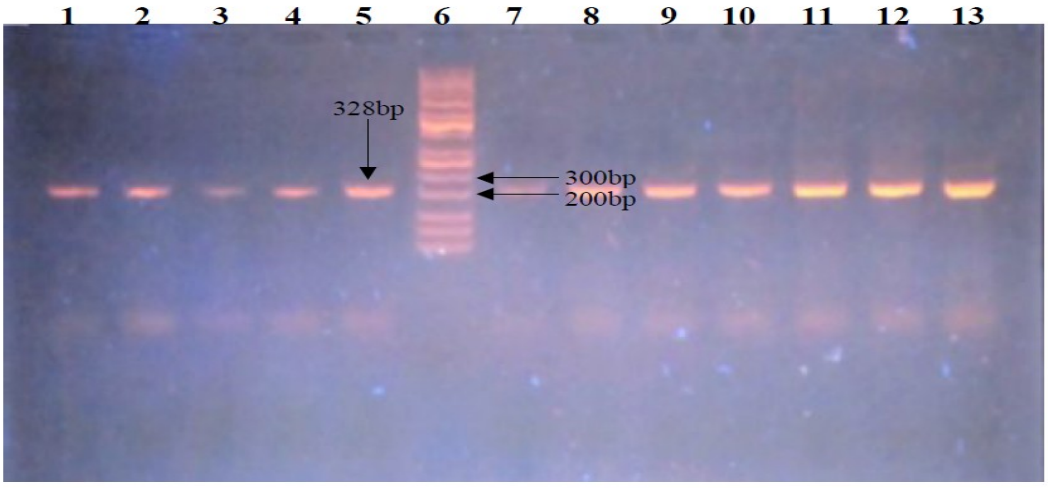


Table.5 Frequency of erythromycin resistance genes among bacterial isolates

	*erm*B	*erm*C	*erm*A/TR	*mef*A	*mre*A
No. of resistant isolates	29/46	0/46	11/46	4/46	32/46
Prevalence in total Isolates	63. 04%	0. 00%	23. 91%	8. 69%	69. 56%
Prevalence in resistant Isolate	90. 62%	0. 00%	34. 37%	12. 50%	100. 00%
Strain	***erm*B**	***erm*C**	***erm*A/TR**	***mef*A**	***mre*A**
V1	+	-	+	-	+
V2	+	-	-	-	+
V3	+	-	+	-	+
V4	-	-	-	+	+
V5	+	-	-	-	+
V6	-	-	-	-	-
V7	-	-	-	-	-
V8	+	-	+	-	+
V9	-	-	-	-	+
V10	+	-	-	-	+
V11	+	-	-	-	+
V12	+	-	+	+	+
V13	-	-	-	-	-
V14	+	-	+	-	+
V15	-	-	-	-	-
V16	+	-	-	-	+
V17	+	-	-	-	+
V18	-	-	-	-	-
V19	+	-	-	-	+
V20	+	-	+	-	+
V21	+	-	-	-	+
V22	+	-	-	-	+
V23	-	-	-	-	-
V24	+	-	+	-	+
V25	-	-	-	-	-
V26	+	-	+	-	+
V27	-	-	-	-	-
V28	+	-	-	-	+
V29	-	-	-	-	-
V30	-	-	-	-	-
V31	+	-	-	-	+
V32	-	-	-	+	+
V33	+	-	-	-	+
V34	+	-	-	-	+
V35	+	-	-	-	+
V36	-	-	-	-	-
V37	+	-	-	-	+
V38	-	-	-	-	-
V39	+	-	-	-	+
V40	+	-	+	-	+
V41	+	-	-	+	+
V42	-	-	-	-	-
V43	+	-	-	-	+
V44	+	-	+	-	+
V45	-	-	-	-	-
V46	+	-	+	-	+

Where: (erm) Erythromycin resistant methylase gene, (+) Positive result, (-) Negative result.

Figure.2 The prevalence of antibiotics susceptibility among GBS isolates. Where: R (Resistance), (S) Sensitive, (EM) Erythromycin, (CN) Clindamycin, (LZ) Linezolid, (AMP) Ampicillin, (CTX) Cefotaxime, (CRO) Ceftriaxone, (SXT) Trimethoprim/Sulfamethoxazole, (TET) Tetracycline, (VAN) Vancomycin, (BPC) Benzyl penicillin, (LEV) levofloxacin

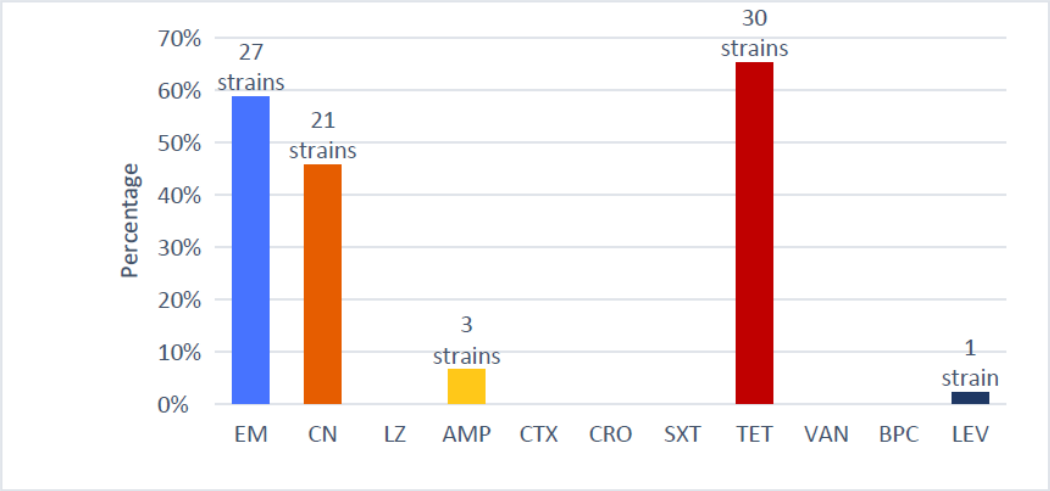


Figure 3. An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for ermB gene. Showing 454bp of DNA amplification fragments produced by PCR for erythromycin resistant methylase gene (ermB) from S. agalactiae. Lane 2, 3, 4, 5 and 6: ermB gene amplification fragments; Lane 1: 100 bp ladder

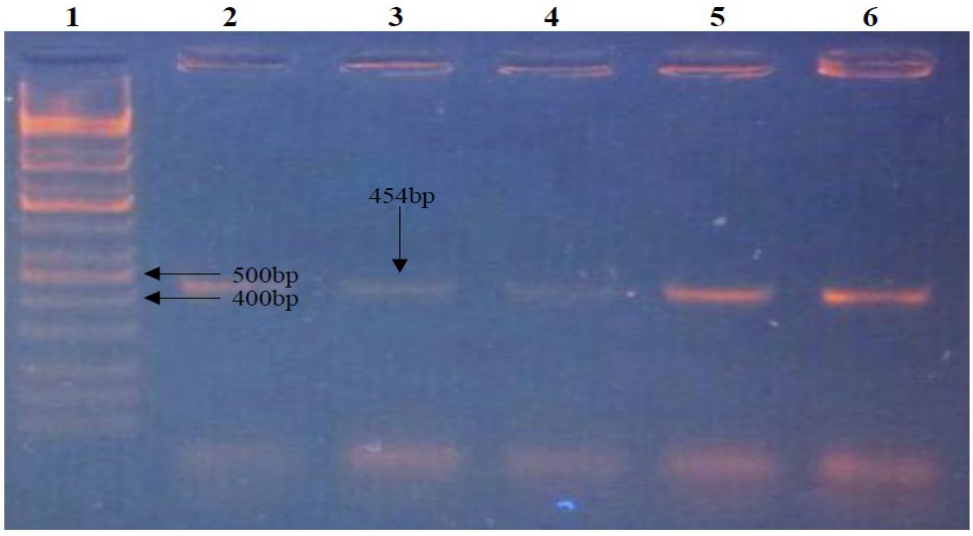


Figure 4. An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for ermA/TR gene. Showing 423bp of DNA amplification fragments produced by PCR for erythromycin resistant methylase gene (ermA/TR) from S. agalactiae. Lanes 2, 3, 4, 5, 6, 7 and 8: ermA/TR gene amplification fragments; Lane 1: 100 bp ladder.

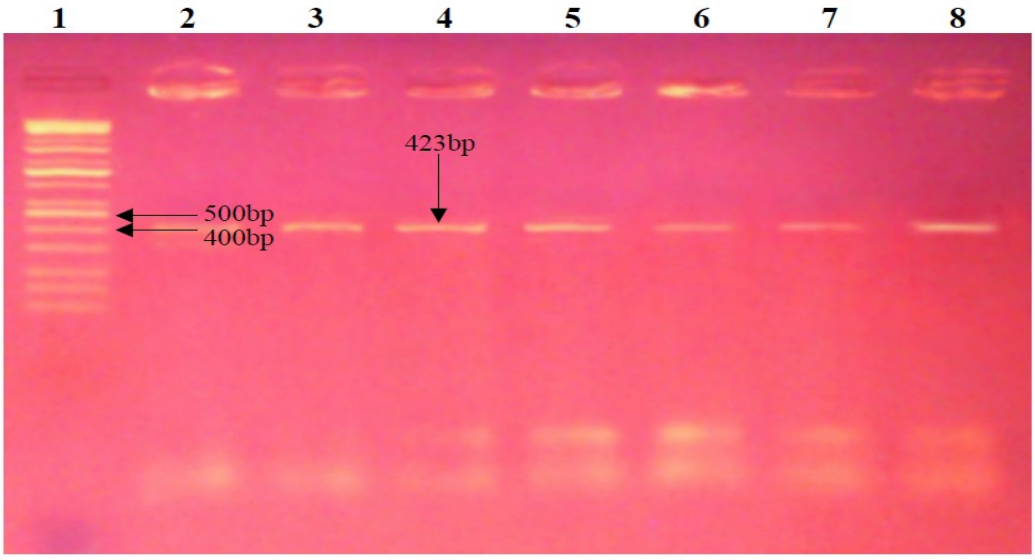


Figure 5. An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for ermC gene. Showing negative results for erythromycin resistant methylase gene (ermC) from S. agalactiae. 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12 and 13: ermC gene negative results; Lane 6: 100 bp ladder

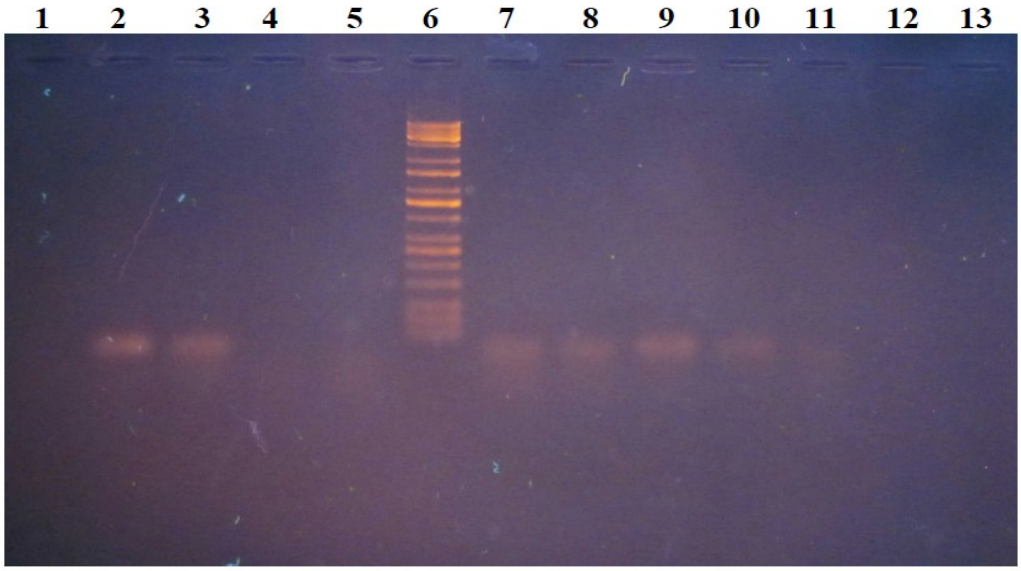


Figure 6. An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for mreA gene. Showing 498bp of DNA amplification fragments produced by PCR for macrolide resistant efflux pump gene (mreA) from S. agalactiae. Lanes 1, 2, 3, 5, 6 and 7: mreA gene amplification fragments; Lane 8: negative result; Lane 4: 100 bp ladder.

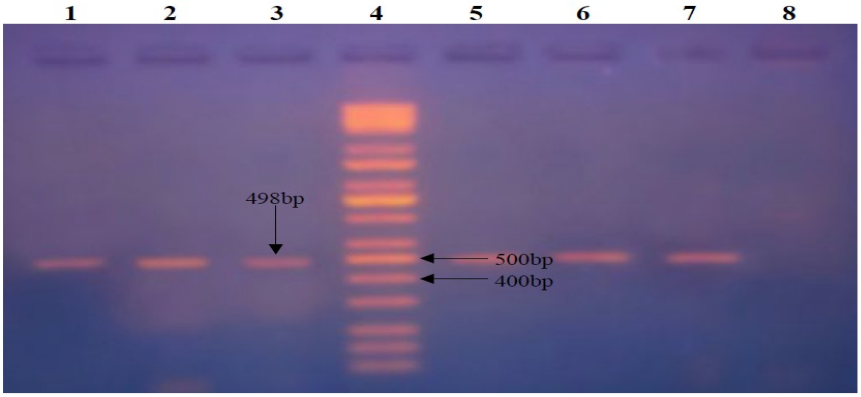
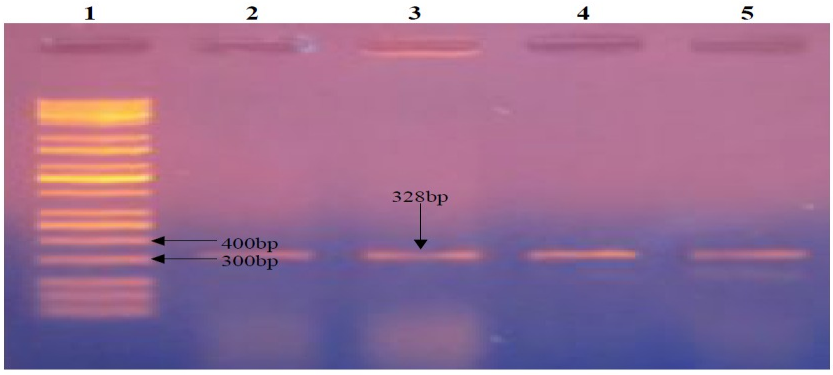


Figure 7. An ethidium bromide stained agarose gel electrophoresis with 1% agarose and electric field strength 7V/cm for 90min for *mef*A gene. Showing 328bp of DNA amplification fragments produced by PCR for macrolide resistant efflux pump gene (*mef*A) from *S. agalactiae*. Lanes 2, 3, 4 and 5: *mef*A gene amplification fragments; Lane 1: 100 bp ladder.



References

Acikgoz, Z. C.; Almayanlar, E.; Gamberzade, S. and Gocer, S. (2004). Macrolide resistance determinants of invasive and noninvasive group B streptococci in a Turkish hospital. *Antimicrob. Agents Chemother*. 48(4): 14101412.

Allam, A. A. and Bahgat, M. A. (2006). Phenotype and Genotype of Some Clinical Group B Streptococcus Isolates Resistant to Erythromycin in Egypt. *Egypt. J. Med. Microbiol*. 15(1): 7177.

AlSoud, W. A.; Jönsson, L. J. and Rådström, P. (2000). Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *J.*

Clin. Microbiol. 38(1): 345350.

AlSweih, N.; Jamal, M.; Kurdia, M.; Abduljabar, R. and Rotimi, V. (2005). Antibiotic susceptibility profile of group B streptococcus (*Streptococcus agalactiae*) at the Maternity Hospital, Kuwait. *Med. Princ. Pract.* 14(4): 260263.

Aracil, B.; Miñambres, M.; Oteo, J.; De La Rosa, M. and Gómez-Garcés, J. L. (2002). Susceptibility of strains of *Streptococcus agalactiae* to macrolides and lincosamides, phenotype patterns and resistance genes. *Clin. Microbiol. Infect.* 8 (11): 745748.

Arana, D. M.; Bezares, B. R.; Manrique, C. T. and Alós, J. I. (2014). First clinical isolate in Europe of clindamycinresistant group B Streptococcus mediated by the lnu (B) gene. *Rev. Esp. Quimioter*. 27(2): 106109.

Back, E. E.; O'grady, E. J. and Back, J. D. (2012). High rates of perinatal group B Streptococcus clindamycin and erythromycin resistance in an upstate New York hospital. *Antimicrob. Agents Chemother.* 56(2): 739742.

Barros, R. R.; Kegele, F. C. O.; Paula, G. R. D.; Brito, M. A. D. and Duarte, R. S. (2012). Molecular characterization of the first fluoroquinolone resistant strains of Streptococcus agalactiae isolated in Brazil. *Braz. J. Infect. Dis.* 16(5): 476478.

Betriu, C.; Culebras, E.; Gómez, M.; RodríguezAvial, I.; Sánchez, B. A.; Agreda, M. C. and Picazo, J. J. (2003). Erythromycin and clindamycin resistance and telithromycin susceptibility in *Streptococcus agalactiae*. *Antimicrob. Agents Chemother*. 47(3): 11121114.

Borchardt, S. M.; DeBusscher, J. H.; Tallman, P. A.; Manning, S. D.; Marrs, C. F.; Kurzynski, T. A. and Foxman, B. (2006). Frequency of antimicrobial resistance among invasive and colonizing Group B streptococcal isolates. *BMC Infect. Dis.* 6(1): 57.

BrzychczyWłoch, M.; Gosiewski, T.; Bodaszewska, M.; Pabian, W.; Bulanda, M.; Kochan, P. and Heczko, P. B. (2010). Genetic characterization and diversity of Streptococcus agalactiae isolates with macrolide resistance. *J. Med. Microbiol.* 59(7): 780786.

Cai, Y.; Kong, F. and Gilbert, G. L. (2007). Three new macrolide efflux (mef) gene variants in *Streptococcus agalactiae*. *J. Clin. Microbiol.* 45(8): 27542755.

Clancy, J.; DibHajj, F.; Petitpas, J. W. and Yuan, W. (1997). Cloning and characterization of a novel macrolide efflux gene, mreA, from *Streptococcus agalactiae*. *Antimicrob. Agents Chemother*. 41(12): 27192723.

Clarebout, G.; Villers, C. and Leclercq, R. (2001). Macrolide Resistance Gene mreA of *Streptococcus agalactiae* Encodes a Flavokinase. *Antimicrob. Agents Chemother.* 45(8): 22802286.

de Azavedo, J. C.; McGavin, M.; Duncan, C.; Low, D. E. and McGeer, A. (2001). Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. *Antimicrob. Agents Chemother*. 45: 35043508

Decoster, L.; Frans, J.; Blanckaert, H.; Lagrou, K. and Verhaegen, J.

(2005). Antimicrobial susceptibility of group B streptococci collected in two Belgian hospitals. *ACTA Clin. Belg*. 60(4): 180184.

Decoster, L.; Frans, J.; Blanckaert, H.; Lagrou, K. and Verhaegen, J. (2005). Antimicrobial susceptibility of group B streptococci collected in two Belgian hospitals. *ACTA Clin. Belg*. 60(4): 180184.

Del Grosso, M.; d'Abusco, A. S.; Iannelli, F.; Pozzi, G.and Pantosti, A. (2004). Tn2009, a Tn916like element containing mef (E) in Streptococcus pneumoniae. *Antimicrob. Agents Chemother*. 48(6): 20372020

Del Grosso, M.; d'Abusco, A. S.; Iannelli, F.; Pozzi, G.and Pantosti, A. (2004). Tn2009, a Tn916like element containing mef (E) in Streptococcus pneumoniae. *Antimicrob. Agents Chemother*. 48(6): 20372020

DiPersio, L. P. and DiPersio, J. R. (2006). High rates of erythromycin and clindamycin resistance among OBGYN isolates of group B Streptococcus. *Diagn. microbial. Infect. Dis*. 54: 7982

Emaeini, M.; misrsaehian, A.; Beigvierdi, R.; Fooladi, A. A. I.; Asadi, F.; Jabalameli, F., and Ilam, I. (2014). High Incidence of Macrolide and Tetracycline Resistance among Streptococcus Agalactiae Strains Isolated from Clinical Samples in Tehran, Iran. *MAEDICA–a Journal of Clinical Medicine*. *9*(2), 157161.

FigueiraCoelho, J.; Ramirez, M.; Salgado, M. J.and MeloCristino, J. (2004). Streptococcus agalactiae in a large Portuguese teaching hospital: antimicrobial susceptibility, serotype distribution, and clonal analysis of macrolideresistant isolates. *Microbial Drug Resistance*. *10*(1), 3136.

Fitoussi, F.; Loukil, C.; Gros, I.; Clermont, O.; Mariani, P.; Bonacorsi, S.and Bingen, E. (2001). Mechanisms of macrolide resistance in clinical group B streptococci isolated in France. Antimicrobial agents and chemotherapy, 45(6), 18891891.

Florindo, C.; Viegas, S.; Paulino, A.; Rodrigues, E.; Gomes, J. P.and Borrego, M. J. (2010). Molecular characterization and antimicrobial susceptibility profiles in Streptococcus agalactiae colonizing strains: association of erythromycin resistance with subtype III-1 genetic clone family. *Clinical Microbiology and Infection*. *16*(9). 14581463.

Fluegge, K.; Supper, S.; Siedler, A. and Berner, R. (2004). Antibiotic susceptibility in neonatal invasive isolates of Streptococcus agalactiae in a 2year nationwide surveillance study in Germany. *Antimicrobial agents and chemotherapy*. *48*(11), 44444446.

Garland, S. M.; Cottrill, E.; Markowski, L.; Pearce, C.; Clifford, V.; Ndisang, D. and Daley, A. J. (2011). Antimicrobial resistance in group B streptococcus: the Australian experience. *Journal of medical microbiology*.*60*(2), 230235.

Gherardi, G.; Imperi, M.; Baldassarri, L.; Pataracchia, M.; Alfarone, G.; Recchia, S. and Creti, R. (2007). Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy. *Journal of clinical microbiology*. *45*(9), 29092916.

Gherardi, G.; Imperi, M.; Baldassarri, L.; Pataracchia, M.; Alfarone, G.;

Recchia, S. and Creti, R. (2007). Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy. *Journal of clinical microbiology*. *45*(9),29092916.

Heelan, J. S.; Hasenbein, M. E. and McAdam, A. J. (2004). Resistance of group B streptococcus to selected antibiotics, including erythromycin and clindamycin.*Journal of clinical microbiology*. *42*(3), 12631264.

Hraoui, M.; Boubaker, I. B. B.; Rachdi, M.; Slim, A. and Redjeb, S. B. (2012). Macrolide and tetracycline resistance in clinical strains of Streptococcus agalactiae isolated in Tunisia. *Journal of medical microbiology*. *61(8*), 11091113.

Hraoui, M.; Boubaker, I. B. B.; Rachdi, M.; Slim, A. and Redjeb, S. B. (2012). Macrolide and tetracycline resistance in clinical strains of Streptococcus agalactiae isolated in Tunisia. *Journal of medical microbiology*. *61(8*), 11091113.

Hsueh ;P. R.; Teng, L. J.; Lee, L. N.; Ho, S. W.; Yang, P. C. and Luh, K. T. (2001). High incidence of erythromycin resistance among clinical isolates of Streptococcus agalactiae in Taiwan. *Antimicrobial agents and chemotherapy*, *45*(11), 32053208.

Hsueh ;P. R.; Teng, L. J.; Lee, L. N.; Ho, S. W.; Yang, P. C. and Luh, K. T. (2001). High incidence of erythromycin resistance among clinical isolates of Streptococcus agalactiae in Taiwan. *Antimicrobial agents and chemotherapy*, *45*(11), 32053208.

Joshi, M. and Deshpande, J. D. (2011). Polymerase Chain Reaction: Methods, Principles and Application. *International Journal of Biomedical Research*, 2(1), 8197.

Kim, C. H.; Khan, M.; Morin, D. E.; Hurley, W. L.; Tripathy, D. N.; Kehrli Jr, M. and Kakoma, I. (2001). Optimization of the PCR for detection of Staphylococcus aureus nuc gene in bovine milk. *Journal of dairy science*, *84*(1), 7483.

Krishnaveni, N.; Isloor, S. K.; Hegde, R.; Suryanarayanan, V. V. S.; Rathnma, D.; Veeregowda, B. M.; and Sundareshan, S. (2014). Rapid detection of virulence associated genes in Streptococcal isolates from bovine mastitis.*African Journal of Microbiology Research*, *8*(22), 22452254.

Lambiase, A.; Agangi, A.; Del Pezzo, M.; Quaglia, F.; Testa, A.; Rossano, F. and Catania, M. R. (2012). In vitro resistance to macrolides and clindamycin by group B Streptococcus isolated from pregnant and nonpregnant women. *Infectious diseases in obstetrics and gynecology*, *2012.*

Leclercq, R. (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases*, 34(4), 482492.

Liddy, H. and Holliman, R. (2002). Group B Streptococcus highly resistant to gentamicin. *Journal of Antimicrobial Chemotherapy*.*50* (1), 142143.

Lin, F. Y. C.; Brenner, R. A.; Johnson, Y. R.;Azimi, P. H.; Philips III, J. B., Regan; J. A. and Clemens; J. D. (2001). The effectiveness of riskbased intrapartum chemoprophylaxis for the prevention of earlyonset neonatal group B streptococcal disease. *American journal of obstetrics and*

gynecology.*184*(6), 12041210.

Lin, F. Y.; Azimi, P. H.; Weisman, L. E.; Philips, J. B.; Regan, J.; Clark, P., Rhoads, G. G.; Clemens, J.; Troendle, J. and other authors (2000). Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 19951998. Clin Infect Dis 31, 76–79.

Lin, F. Y.; Azimi, P. H.; Weisman, L. E.; Philips, J. B.; Regan, J.; Clark, P., Rhoads, G. G.; Clemens, J.; Troendle, J. and other authors (2000). Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 19951998. Clin Infect Dis 31, 76–79.

Marimón, J. M.; Valiente, A.; Ercibengoa, M.; GarcíaArenzana, J. M. and PérezTrallero, E. (2005). Erythromycin resistance and genetic elements carrying macrolide efflux genes in Streptococcus agalactiae. *Antimicrobial agents and chemotherapy*. *49*(12), 50695074. 44. Matsubara, K.; Nishiyama, Y.; Katayama, K.; Yamamoto, G.; Sugiyama, M.; Murai, T. and Baba, K. (2001). Change of antimicrobial susceptibility of group B streptococci over 15 years in Japan. *Journal of Antimicrobial Chemotherapy*, *48*(4), 579582.

Nakamura, P. A.; Schuab, R. B. B.; Neves, F. P.; Pereira, C. F.; de Paula, G. R.and Barros, R. R. (2011). Antimicrobial resistance profiles and genetic characterisation of macrolide resistant isolates of Streptococcus agalactiae.*Memórias do Instituto Oswaldo Cruz*. *106*(2), 119122.

Nilsson, W. B.; Paranjype, R. N.; DePaola, A., and Strom, M. S. (2003). Sequence polymorphism of the 16S rRNA gene of Vibrio vulnificus is a possible indicator of strain virulence. *Journal of clinical microbiology*, *41*(1), 442446.

Nizet, V. I. C. T. O. R.; Ferrieri, P. A. T. R. I. C. I. A. and Rubens, C. E. (2000). Molecular pathogenesis of group B streptococcal disease in newborns.*Streptococcal infections: clinical aspects, microbiology and molecular pathogenesis. Oxford University Press, New York, NY.* 180221.

Panda, B.; Iruretagoyena, I.; Stiller, R. and Panda, A. (2009). Antibiotic resistance and penicillin tolerance in anovaginal group B streptococci. *Journal of MaternalFetal and Neonatal Medicin*e, 22(2), 111114.

Portillo, A.; Lantero, M.; Olarte, I.; RuizLarrea, F. and Torres, C. (2001). MLS resistance phenotypes and mechanisms in βhaemolytic group B, C and G Streptococcusisolates in La Rioja, Spain. *Journal of antimicrobial chemotherapy*. *47*(1). 115116.

Poulsen, S. M.; Kofoed, C. and Vester, B. (2000). Inhibition of the ribosomal peptidyl transferase reaction by the mycarose moiety of the antibiotics carbomycin, spiramycin and tylosin. *Journal of molecular biology*. *304*(3), 471481.

Rachdi, M.; Boutibaben Boubaker, I.; Hraoui, M. and Ben, R. S. (2009). High rates of macrolide resistance among clinical isolates of Streptococcus agalactiae in Tunisia. *Archives de l'Institut Pasteur de Tunis*. *87* (12), 3542.

Rajagopal, L. (2009). Understanding the regulation of Group B Streptococcal virulence factors. *Future Microbial*. 4(2): 201–221.

Rantala, M. (2009). Antimicrobial resistance in Streptococcus pneumoniae in Finland with special

reference to macrolides and telithromycin.

Rausch, A. V.; Gross, A.; Droz, S.; Bodmer, T.and Surbek, D. V. (2009). Group B Streptococcus colonization in pregnancy: prevalence and prevention strategies of neonatal sepsis. *Journal of perinatal medicine*. *37*(2), 124129.

Ruess, M.; Mu¨ ller, U.; Sander, A. and Berner, R. (2000). Antimicrobial susceptibility patterns of Streptococcus agalactiae in a German university hospital. *Scand J Infect Dis* 32, 623–626.

Sacchi, C. T.; Whitney, A. M.; Reeves, M. W.; Mayer, L. W.; and Popovic, T. (2002). Sequence diversity of Neisseria meningitidis 16S rRNA genes and use of 16S rRNA gene sequencing as a molecular subtyping tool. *Journal of clinical microbiology*, *40*(12), 45204527**.**

Schuchat, A. (1998). Epidemiology of group B streptococcal disease in the United States: shifting paradigms. *Clinical Microbiology Reviews*.*11* (3), 497513.

Steel, H. C.; Theron, A. J.; Cockeran, R.; Anderson; R. and Feldman, C. (2012). Pathogenand hostdirected anti-inflammatory activities of macrolide antibiotics.*Mediators of inflammation.*

Tenson, T. and Mankin, A. S. (2001). Short peptides conferring resistance to macrolide antibiotics. *Peptides*, *22*(10), 16611668.

Udo, E. E.; Boswihi, S. S. and AlSweih, N. (2013). Genotypes and virulence genes in group B streptococcus isolated in the Maternity Hospital, Kuwait.*Medical Principles and Practice*. *22*(5), 453457.

Uh, Y.; Hwang, G. Y.; Jang, I. H.; Cho, H. M.; Noh, S. M.; Kim, H. Y. and Yoon, K. J. (2007). Macrolide resistance trends in βhemolytic streptococci in a tertiary Korean hospital. *Yonsei medical journal*.*48* (5), 773778.

Von Both, U.; Ruess, M.; Mueller, U.; Fluegge, K.; Sander, A. and Berner, R. (2003). A serotype V clone is predominant among erythromycinresistant Streptococcus agalactiae isolates in a southwestern region of Germany.*Journal of clinical microbiology*, *41*(5), 21662169.

Yamamoto, Y.; Poyart, C.; Trieu-Cuot; P., Lamberet; G., Gruss, A. and Gaudu, P. (2005). Respiration metabolism of Group B Streptococcus is activated by environmental haem and quinone and contributes to virulence. *Molecular microbiology*. *56*(2), 525534.

Zeng, X.; Kong, F.; Wang, H.; Darbar, A. and Gilbert, G. L. (2006). Simultaneous detection of nine antibiotic resistancerelated genes in Streptococcus agalactiae using multiplex PCR and reverse line blot hybridization assay. *Antimicrobial agents and chemotherapy*, *50*(1), 204209.

Zhang, L.; Song, L.; Liu, Z.; Li, H.; Lu, Y.; Li, Z. and Ma, S. (2010). Synthesis and antibacterial activity of novel 3*O*carbamoyl derivatives of clarithromycin and 11, 12cyclic carbonate azithromycin. *European journal of medicinal chemistry*.*45* (3), 915922.