



Susceptibility of *Streptococcus agalactiae* Isolated from Pregnant Women to Antibiotic

Tarek Elbashir Nakuaa¹

¹Medical Microbiology Department. Faculty of Science. Zintan University, Libya

Abstract

Background: *Streptococcus agalactiae* often called as Lancefield group B streptococcus (GBS) which is a gram-positive coccus and capsulated bacterium that colonizes the genitourinary tract and is associated with a developed risk of preterm labor.

Aims of study: The aim of study was to evaluate the susceptibility of *Streptococcus agalactiae* isolates from pregnant women to antibiotics.

Methods: A total two hundred and seven swabs were collected from the vagina of pregnant women at 35-40 of gestational weeks from Yafrin and Zintan general hospital. Then, the swabs were inoculated on 5% Columbia blood agar, the plates were then examined for showing beta hemolytic and identification of *Streptococcus agalactiae* colonies were done by cultural morphology, catalase test, Gram stain, CAMP test, and confirmed by latex agglutination test.

Results: During the study. From 207 of pregnant women included, 18 (8.7%) of them has positive of *Streptococcus agalactiae*. All strains isolated of *Streptococcus agalactiae* were susceptible to penicillin G, ampicillin, and amoxicillin - clavulanic acid (100%), clindamycin, Erythromycin (94.4%, 88.8%) respectively, and vancomycin (94.4%). Nevertheless, the antimicrobial agent that *S. agalactiae* isolates were most resistant was to tetracycline (83.3%).

Conclusion: Based on susceptibility profiles, i findings suggest the use of penicillin or ampicillin for intrapartum chemoprophylaxis to prevent newborn *Streptococcus agalactiae* infections.



Keywords Microbial Sensitivity, Penicillin's, Pregnant women, *Streptococcus agalactiae*,

دراسة حساسية بكتيريا *Streptococcus agalactiae* المعزولة من النساء الحوامل
للمضادات الحيوية

المخلص:

المقدمة: *Streptococcus agalactiae* غالبًا ما يُطلق عليها Lancefield Group B Streptococcus (GBS)، عبارة عن بكتريا موجبة لصبغة جرام، تكون كبسولة وتستعمر الجهاز البولي التناسلي وترتبط بخطر تطور الولادة المبكرة.

أهداف الدراسة: تهدف الدراسة الحالية الى دراسة مقاومة بكتيريا *Streptococcus agalactiae* المعزولة من النساء الحوامل لبعض المضادات الحيوية.

المواد والطرق: تم تجميع مئتان وسبعة مسحة مهبلية من النساء الحوامل، كما تراوحت مدة الحمل ما بين 35-40 اسبوع ، في مستشفى يفرن والزنتان العام . بعد ذلك، تم تلقيح المسحات على 5% Columbia blood agar، فحصت الاطباق لوجود انحلال الدم (بيتا)، تم التعرف على مستعمرات *Streptococcus agalactiae* عن طريق Culture characters الخصائص الزرعية، واختبار Catalase (إنزيم الكتاليز)، Gram stain (صبغة جرام)، اختبار CAMB (الكامب) واستخدم اختبار Latex agglutination (تراص اللاتكس) للتأكد من البكتيريا.

النتائج: من بين 207 من النساء الحوامل ، 18 (8.7%) منهن مصابات بالمكورات العقدية *Streptococcus agalactiae*. جميع السلالات المعزولة من *S. agalactiae* كانت حساسة للبنسلين G ، الأمبيسيلين ، الأموكسيسيلين - حمض الكلافولانتيك (100%) ، الكلينداميسين ، الاريثروميسين (94.4% ، 88.8%) على التوالي ، والفانكوميسين (94.4%). ومع ذلك ، فإن العامل المضاد للميكروبات الذي كانت عزلات *S. agalactiae* الأكثر مقاومة هو التتراسيكلين (83.3%).



الخلاصة: بناءً على ملفات تعريف الحساسية ، تشير النتائج التي توصلت إليها إلى استخدام البنسلين أو الأمبيسيلين للوقاية الكيميائية أثناء الولادة للوقاية من عدوى *Streptococcus agalactiae*.

الكلمات المفتاحية: الحساسية الميكروبية، البنسلين، النساء الحوامل، *Streptococcus agalactiae*

Introduction

Streptococcus agalactiae is also named as Lancefield Group B streptococcus (GBS), is a gram-positive coccus and capsulated bacterium that colonizes the genitourinary tract [Fatima, et al 2017]. Although approximately 10-40% of pregnant women are Asymptomatic rectovaginal colonized with *S. agalactiae* widely worldwide [Farzana, and Khadijah, 2015]. Hence, pregnant woman can transmit the bacterium to her fetus through the birth canal [Fatima, et al 2017]. However, *S. agalactiae* has become the major cause of preterm birth, stillbirth, and meningitis [Anita and Patricia, 2004]. The most common kind of neonatal *S. agalactiae* illness is early onset infection (occurs in neonates less than 7 days of age), and late onset infection (occurs in greater than 7 days and up to 3 months of age) [Hughes et al, 2017].

Vaccination is an attractive preventative strategy, but currently there is not a vaccine available for *S. agalactiae*, the strategy for early onset *S. agalactiae* disease prevention is maternal antibiotic treatment [Farzana et al 2015. Hughes et al, 2017].

Intrapartum antibiotic prophylaxis became the recommended treatment in the many countries of the world, which lead to a dramatic decreased in the incidence of mortality of *S. agalactiae* disease at a faster rate from 3% per year previously to 5% per year currently [Susan et al 2004].



Penicillin or ampicillin is drug of choice for treatment of *Streptococcus agalactiae* disease. The standard suggestion for the prevention of *GBS* transmission from colonized women to their infants during delivery is to administer intravenous each four hours for the period of delivery [Max Roberto et al, 2018]. However, other studies have suggested that *S. agalactiae* susceptibility to penicillin has reduced [Soodabeh et al, 2021]. Whereas erythromycin, clindamycin, and vancomycin would to be the second line of choice for pregnant women who demonstrated an anaphylactic or severe allergy to penicillin [Max Roberto et al, 2018. Soodabeh et al, 2021]. However, several emerging erythromycin and clindamycin resistance strains have been reported across the world [Fatima, et al 2017 . Soodabeh et al, 2021].

There was limited research in Libya on the prevalence of *S. agalactiae* in pregnant women, and the sensitivity of the bacteria to antibiotics, as the maternal colonization rate was 11.8% in Tripoli Medical Center [Haifa et al, 2010]. And 19% in Al-Galaa Hospital [Tarek, 2009]. Both are located in the city of Tripoli, Libya.

According to the CDC's guidelines [Verani et al, 2010]. The current study assessed the susceptibility of *S. agalactiae* isolated from pregnant women to antibiotic who attended to a Yafrin and Zintan general hospitals.

Materials and methods

In the present study, two hundred and seven swabs were collected from the vaginal of pregnant women at 35-40 gestational weeks age from Yafrin and Zintan general hospital. Pregnant women who had been using antimicrobials in the last seven days or who used vaginal ointment at the time of collection were excluded from the study.

The swabs were inoculated on 5% Columbia blood agar (Oxoid, UK), by streaking on sterile prepared plates. Then, inoculated culture plates were kept aerobically in the incubator overnight at 37°C, the plates



were then examined for showing beta hemolytic activity. Negative plates were reexamined for hemolytic activity by reincubation. Positive colonies were subcultured on 5% Columbia blood agar to get a pure single colony accordingly using standard bacteriological procedures. [Mucheye et al, 2019. Marianna et al, 2019].

Initial identification of *S. agalactiae* colonies were done by a combination of standard laboratory procedures. Cultural morphology, catalase test activity, microscope examination, CAMP test, (Christie, Atkins and Munch-Petersen) and finely latex agglutination test were done in accordance with the manufacturer's instructions to confirm *S. agalactiae* identification. These were done according to the standard procedures [- Mohammad et al, 2020].

Assessment of antimicrobial susceptibility of isolates was carried out via disc diffusion technique. Fresh subcultures of *S. agalactiae* growing on Columbia blood agar were resuspended in sterile saline to a 0.5 McFarland turbidity standards, then was inoculated on Mueller-Hinton medium (Liofilchem, Italy), supplemented with 5% sheep blood agar and incubated aerobically for 24 hours at 37°C. These were done according to the [Clinical and Laboratory Standards Institute, 2013].

In the present study, we tested the following antibiotics, penicillin G (P) (10 IU), ampicillin (AMP) (10µg), tetracycline (TE) (30µg), clindamycin (2µg), erythromycin (E) (15µg), amoxicillin and clavulanic acid (15µg), and vancomycin (VA) (30µg) (Bioanalyses, Turkey).

Results

A total of two hundred and seven pregnant women at 35-40 weeks of gestation from antenatal of Yafrin and Zintan general hospital were enrolled in this study.

In the current study, from 207 pregnant women included in the study, 18 sample (8.7%) of them Has positive *S. agalactiae*. Figure



(1), the results obtained in this study for susceptibility patterns of 18, *S. agalactiae* isolates from pregnant women participating against 7 antimicrobial agents are summarized in table (1). The results found *S. agalactiae* were susceptible to penicillin G, ampicillin, and amoxicillin - clavulanic acid (100%), clindamycin, Erythromycin (94.4%, 88.8%) respectively, vancomycin (94.4%)., And 5.5% of the isolates were sensitive to tetracycline. The intermediate susceptibilities of *S. agalactiae* isolates to tetracycline, and vancomycin were found to be 11.1 % and 5.6 %, respectively. On the other hand, the antimicrobial agent that *S. agalactiae* isolates were most resistant was to tetracycline 15 (83.3%), Erythromycin 2 (11.2%), and Clindamycin 1 (5.4%).

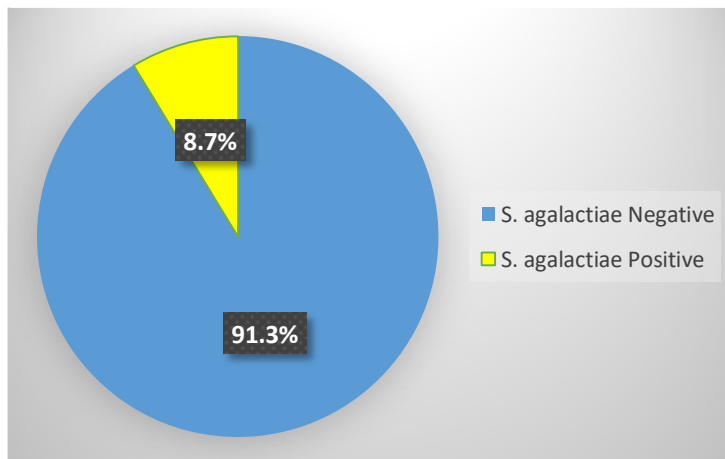
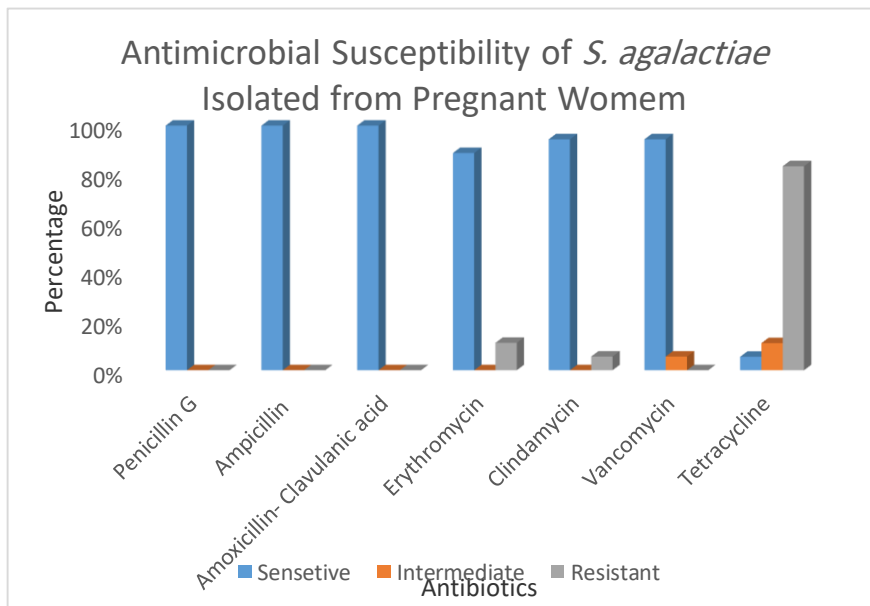


Figure (1). Percentage of *Streptococcus agalactiae* positive and negative in Pregnant Women

Table (1). Antimicrobial susceptibility profile of *Streptococcus agalactiae* isolated from pregnant women



Drugs	Susceptibility %	Intermediate %	Resistant %
Penicillin G	18 (100%)	0 (0%)	0 (0%)
Ampicillin	18(100%)	0 (0%)	0 (0%)
Amoxicillin-Clavulanic acid	18 (100%)	0 (0%)	0 (0%)
Erythromycin	16 (88.8%)	0 (0%)	2 (11.2%)
Clindamycin	17 (94.4%)	0 (0%)	1 (5.6%)
Vancomycin	17 (94.4%)	1 (5.6%)	0 (0%)
Tetracycline	1 (5.5%)	2 (11.1%)	15 (83.4%)



Discussion

Maternal *Streptococcus agalactiae* colonization remains the most important risk factor for illness development in the neonate. However, there are still few data available from Libya.



In present study, the prevalence of *Streptococcus agalactiae* among pregnant women was 8.7% (18 out of 207). Similar results have been found in Tuzla, Bosnia and Herzegovina 8.5% [Fatima, et al 2017], and in Misiones, Argentina 7.6% [Quiroga, 2008].

Reports of high colonization rates of *Streptococcus agalactiae* in pregnant women were 28.4% in Parana State, Brazil [Mohammad et al, 2020]. Aljala Hospital, Libya 19% [Tarek, 2009]. The bacterium has been isolated from the vagina of pregnant women in Gaza strip, Palestine 21% [Nabil, 2017].

Studies of low colonization rates of *Streptococcus agalactiae* in pregnant women were 1.8% in Maputo, Mozambique [Sibone, 2008]. The prevalence of *Streptococcus agalactiae* maternal colonization is affected by culture techniques, such as the number and type of sites cultivated and the medium used [Quiroga, 2008, Fatima, et al 2017. Nabil et al, 2017. Soodabeh et al, 2021].

Penicillins are the first-line antibiotics recommended for the prevention and treatment of *Streptococcus agalactiae* infections [Farzana, and Khadijah, 2015. Hughes et al, 2017]. The results indicated that there was a uniform susceptibility to penicillin G, ampicillin and amoxicillin -clavulanic acid, similar to some previous studies [Tarek, 2009. Nkembe, 2018].

The Centers for Disease Control and prevention recommends intravenous penicillin as the treatment of choice for antimicrobial prophylaxis in pregnant women infected with *S. agalactiae* at the onset of delivery [Verani et al, 2010]. Unfortunately, there is a probability for pregnant women could be sensitive to this treatment. According to a Study conducted by Kimberly and Harold, and Mark et al 8% to 10% of pregnant women have an allergy to this treatment [Mark, 2009. Kimberly and Harold, 2013].

The sensitivity to clindamycin and erythromycin is highly varied from region to region and from study to study. In the current study



demonstrated that the overall of the clindamycin sensitivity isolates were 94.4%, this result is higher than that Nabil et al. who detected 31% sensitivity to clindamycin [Nabil et al, 2017]. Clindamycin sensitivity rates were 96 % in a study conducted in Missions, Argentina [Quiroga, 2008]. Another study in Tripoli, Libya was 86.8% of isolates sensitive to Clindamycin [Tarek, 2009]. Moreover, sensitivity to erythromycin was 88.8%, similar findings were reported in study conducted In Tripoli [Tarek, 2009]. But it differed from the study conducted in Nigeria who detected a sensitivity of 100% to erythromycin [Dahal, et al, 2019]. Another study detected 27.7 % sensitivity to erythromycin [Tahereh, et al, 2021].

There are two mechanisms of erythromycin resistance in *Streptococcus agalactiae*, the first consist of target site modification which conveyed by the action of a family of methyltransferase enzymes encoded by the *erm* genes (erythromycin ribosome methylase) [Quiroga, 2008]. The second, include active drug efflux which mediated by the *mef* gene (macrolide efflux) which lead to resistance to many member of macrolide compounds [Clancy, 1996].

On the other hand, susceptibility of *S. agalactiae* to tetracycline, as identified by this study and found to be a highest level of resistance (83.3%). Similar results were registered in other studies, including study in Tripoli with 81.5% % [Tarek, 2009]. Tunisia with 97.3% [Hraoui, 2012]. Highly resistance to tetracycline can be explained by the fact that these antibiotics have a narrow action on *Streptococcus agalactiae* or related to the widely used drug previously [Hraoui, 2012].

Pregnant women who are allergic to penicillin and clindamycin-resistant isolates, vancomycin is another alternative [Mark, 2009]. However, vancomycin sensitivity was studied, and the study showed (94.44 %) that the rates are comparable to those recorded globally [Max Roberto et al, 2018. Addisu et al, 2022. Kalyani et al. 2022].



Conclusion

As a result of carried out work, all isolated strains of *Streptococcus agalactiae* showed uniform susceptibility to penicillin. Hence, this make up the first line for intrapartum prophylaxis to prevent neonatal *Streptococcus agalactiae* infections. Furthermore, we recommend that large scale epidemiological studies should be conducted in other parts of the country to assess the sufficiency of the antimicrobial susceptibility of *Streptococcus agalactiae* during the pregnancy.

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