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Resistance Rates of *Streptococcus agalactiae* Strains Isolated from Urine Samples to Various Antibiotics

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ABSTRACT

Aim: Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, are Gram-positive, encapsulated bacteria found in the normal human gastrointestinal and urogenital flora. A wide variety of infectious diseases can cause urogenital system infections in newborns, elderly people, immunocompromised people, pregnant women, and adults. In this study, we aimed to inform clinicians about the agents that can be used in intrapartum antibiotic prophylaxis by examining the antibiotic susceptibility of GBS strains isolated from urine samples from the Gynecology and Obstetrics Clinic in our laboratory.

Material and Methods: Isolates of the *S. agalactiae* isolated from a total of 222 urine samples from the Gynecology and Obstetrics Clinic that came to our Medical Microbiology laboratory between January 2022 and December 2022 were included and the data of these isolates were evaluated retrospectively. The clinical samples that came to our laboratory were inoculated on Sheep blood agar medium and incubated at 37°C for 18-24 hours. Quantitatively seeded urine culture ≥104 CFU/ml grown bacteria was considered a uropathogenic. Urogenital skin flora element or three and more different types of bacteria growing cultures as contamination evaluated.

Results: Due to the group selected as the sample, 100% of the patients were female. The age range of the patients ranged from 12 to 72, and the mean age was calculated as 30.16. All GBS isolates were susceptible to penicillin, linezolid, trimethoprim-sulfamethoxazole, tigecycline, teicoplanin, and vancomycin.

Conclusion: Penicillin resistance was not found in our study. However, resistant strains were detected in erythromycin, clindamycin, and levofloxacin, which stand out as other options in cases where penicillin cannot be used.

Keywords: Group B streptococcus; penicillin; antimicrobial susceptibility.

İdrar Örneklerinden İzole Edilen *Streptococcus agalactiae* suşlarının Çeşitli Antibiyotiklere Direnç Oranları

ÖZ

Amaç: *Streptococcus agalactiae* olarak da bilinen Grup B Streptokoklar (GBS) insan normal gastrointestinal ve ürogenital florasında bulunan Gram pozitif, kapsüllü bakterilerdir. Yenidoğanlarda, yaşlılarda, bağışıklığı baskılanmış kişilerde çok çeşitli bulaşıcı hastalıklar kişilerde, gebelerde ve erişkinlerde ürogenital sistem enfeksiyonlarına neden olabilirler. Bu çalışmada Kadın Hastalıkları ve Doğum Kliniği'nden laboratuvarımıza gelen idrar örneklerinden izole edilen GBS suşlarının antibiyotik duyarlılıklarını inceleyerek intrapartum antibiyotik profilaksisinde kullanılabilecek ajanlar hakkında klinisyenleri bilgilendirmek amaçlandı.

Gereç ve Yöntemler: Ocak 2022- Aralık 2022 yılları arasında Kadın Hastalıkları ve Doğum Kliniği'nden Tıbbi Mikrobiyoloji laboratuvarımıza gönderilen toplam 222 klinik idrar örneğinden izole edilen *S. agalactiae* izolatları dahil edildi ve bu izolatlara ait veriler retrospektif olarak değerlendirildi. Laboratuvarımıza gelen klinik örnekler Koyun kanlı agar besiyerine ekildi ve 37°C'de 18-24 saat inkübe edildi. Kantitatif olarak ekim yapılan idrar kültüründe ≥104 CFU/ml üremesi olan bakteriler üropatojen olarak kabul edilirken ürogenital cilt flora elemanı veya üç ve daha fazla farklı tipte bakteri üreyen kültürler kontaminasyon olarak değerlendirildi.

Bulgular: Seçilen hasta grubu nedeniyle hastaların %100'ü kadındı. Hastaların yaşları 12 ile 72 arasında değişirken yaş ortalaması ise 30,16'ydı. GBS izolatlarının tümü penisilin, linezolid, trimetoprim sulfametoksazol, tigesiklin, teikoplanin ve vankomisine duyarlıydı.

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Sonuç: Çalışmamızda penisilin direnci saptanmamıştır. Ancak penisilinin kullanılamadığı durumlarda diğer seçenekler olarak öne çıkan eritromisin, klindamisin ve levofloksasine dirençli suşlar tespit edilmiştir.
Anahtar Kelimeler: Grup B streptokok; penisilin; antimikrobiyal duyarlılık.

INTRODUCTION

Group B Streptococci (GBS), also known as *Streptococcus agalactiae*, are gram-positive, encapsulated bacteria found in the normal human gastrointestinal and urogenital flora (1). *Streptococcus agalactiae* can cause meningitis, sepsis, skin and soft tissue infections, pneumonia, urinary tract infections in neonates and adults with comorbidities, and postpartum endometritis in pregnant women (2-4). Also, this bacterium is a critical reason for mortality or morbidity in non-pregnant adults, mostly in the elderly and those with underlying diseases (5). While approximately 10% to 30% of women are carriers of this bacterium during pregnancy, 60% of them transmit the bacterium to their children during pregnancy or delivery (6,7). Due to this vertical transmission, the mortality rate from GBS infections in infants is estimated to be between 2% and 4%, but this rate is even higher in premature infants (8). The widespread use of intrapartum antibiotic prophylaxis to prevent the early onset of this infection has led to concerns about resistance that may occur in GBS isolates (9). To prevent multi-drug resistance that may develop, to prevent human colonization and related early-onset diseases in newborns, 35-37th gestational days of pregnancy. Vaginal and rectal colonization screening is performed in the first weeks of gestation and selective intrapartum antibiotic prophylaxis is recommended for all women with positive screening (9). Despite these precautions, GBS has been shown to gain resistance to antibiotics in many studies.

Penicillins are the first-choice agents for the treatment of GBS-related infections due to their sensitivity to penicillin (10,11). Ampicillin, vancomycin, teicoplanin, first, second and third-generation cephalosporins, imipenem, and meropenem are other antibiotics to which it is sensitive (12,13). Clindamycin and erythromycin are preferred as alternative options in people with penicillin allergy (12,14). According to the statistics published by the Center for Disease Control (CDC) in the United States (USA) in 2006 and 2009, the resistance of GBS to erythromycin and clindamycin has increased by 25-32% and 13-20% (15). In a study by Mucheye et al. in 2019, it was reported that GBS in Africa is resistant to several antibiotic families (16). Determining the prevalence and antimicrobial susceptibility profile of GBS in different regions is very important for therapeutic strategies. In another study conducted in Cameroon in 2018, it was shown that 14% of GBS were sensitive to β-lactams but resistant to erythromycin (17). These studies have shown that the antibiotic susceptibility and resistance profiles of GBS should be regularly examined and monitored both locally and globally.

In this study, we aimed to inform clinicians about the agents that can be used in intrapartum antibiotic prophylaxis by examining the antibiotic susceptibility of GBS strains isolated from urine samples from the Gynecology and Obstetrics Clinic in our laboratory.

MATERIAL AND METHODS

Between January 2022 and December 2022, *S. agalactiae* isolates isolated from a total of 222 clinical urine samples from the Gynecology and Obstetrics Clinic were included in our Medical Microbiology laboratory, and the data of these isolates were evaluated retrospectively.

The clinical samples that came to our laboratory were inoculated on Sheep blood agar medium and incubated at 37°C for 18-24 hours. Quantitatively seeded urine culture ≥104 CFU/ml grown bacteria were considered a uropathogenic. Urogenital skin flora element or three and more different types of bacteria growing cultures as contamination evaluated. Bacteria with Gram-positive cocci in Gram staining and negative catalase test were identified by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker, Germany). Antibiotic resistance status was determined with the VITEK®-2 (BioMérieux, France) device. Resistance rates were determined according to CLSI (Clinical and Laboratory Standards Institute) standards.

The study was approved by the Health Sciences University Gulhane Scientific Research Ethics Committee with the decision dated 14.02.2023 and numbered 2023-66. The research process was carried out by the Declaration of Helsinki.

Statistical Analysis

Descriptive data; is presented as numbers, percentages, and min-max values.

RESULTS

A total of 6624 samples came to our laboratory from the clinic, pathogenic bacteria growth was detected in 949 (14.32%) of them, no growth was found in 4046 (61.08%) samples, and contamination was detected in 1511 (24.6%) samples. The number of GBS-isolated urine samples was 222. Due to the group selected as the sample, 100% of the patients were female. Only four of the samples (1.8%) were from inpatients, while the remaining samples were all from outpatients. Of these, 108 (48.6%) came to our laboratory from the pregnancy follow-up outpatient clinic, 16 (7.2%) from the urogynecology outpatient clinic, and 94 (42.3%) from the obstetrics and gynecology outpatient clinic. The age range of the patients ranged from 12 to 72, and the mean age was calculated as 30.16. While 166 (74.77%) of these patients were followed up due to pregnancy, 47 (21.17%) of them were patients of reproductive age but admitted for non-pregnancy reasons, and 9 (4.06%) of them were followed up due to menopause. There was no significant difference in resistance profiles between these patient groups ($p>0.05$). All GBS isolates were susceptible to penicillin, linezolid, trimethoprim-sulfamethoxazole, tigecycline, teicoplanin, and vancomycin. Tetracycline, erythromycin, clindamycin, and moxifloxacin resistance rates of the isolates were 91%, 33.4%, 17.2%, and 28.8%, respectively. The antibiotics studied and their susceptibility profiles are summarized in Table 1.

Table 1. Distribution of *Streptococcus agalactiae* antibiotic susceptibility results [n (%)]

Antibiotic	Susceptible	Resistant	Total
Penicillin	222 (100)	0	222 (100)
Moxifloxacin	158 (71.2)	64 (28.8)	222 (100)
Clindamycin	184 (82.8)	38 (17.2)	222 (100)
Erythromycin	148 (66.6)	74 (33.4)	222 (100)
Linezolid	222 (100)	0	222 (100)
Trimethoprim-sulfamethoxazole	222 (100)	0	222 (100)
Tetracycline	20 (9)	202 (91)	222 (100)
Tigecycline	222 (100)	0	222 (100)
Teicoplanin	222 (100)	0	222 (100)
Vancomycin	222 (100)	0	222 (100)
Chloramphenicol	194 (87.3)	28 (12.7)	222 (100)
Daptomycin	216 (97.3)	6 (2.7)	222 (100)
Levofloxacin	160 (72.4)	62 (27.6)	222 (100)
Nitrofrontain	195 (87.8)	27 (12.2)	222 (100)
Rifampin	89 (40.1)	133 (59.9)	222 (100)

DISCUSSION

GBS, which is found as normal vaginal and gastrointestinal tract flora in approximately 10-30% of the female population, can cause serious disease patterns ranging from nonsymptomatic bacteriuria to sepsis (10,18). Penicillins are preferred as the first choice both in the treatment and prophylaxis of infections of these bacteria. Although penicillin resistance has not been encountered so far, it has been reported that there is a decrease in sensitivity, and treatment failures due to penicillin tolerance have been observed for various reasons in recent years (11,19). It is due to the stepwise accumulation of PBP2x mutations (20,21). In a study examining 1974 GBS isolates conducted abroad, penicillin resistance was found to be 33.6% in pregnant women (16). In another study conducted on pregnant women in Iran, penicillin resistance was found in 6.1% of GBS isolates isolated from urine samples (22). Again, in a study conducted in Iran, only one isolate was found to be penicillin-resistant (23). No penicillin-resistant strain was found in GBSs isolated from urine samples made in recent years in our country (24). Similarly, no penicillin-resistant strain was found in our study. This situation suggested that the resistance profiles between centers might be different and that each center should follow its resistance profile.

Although penicillin resistance was not found in GBS in our study and in other studies, a high rate of tetracycline resistance is observed. In our study, this rate was 91%, and similarly high rates of tetracycline resistance were found in other studies (25-28).

Macrolide group in the treatment of GBS in patients with penicillin allergy antibiotics, especially erythromycin, are an important weapon in both treatment and prophylaxis (1,29). However, the decrease in erythromycin sensitivity over the years has led to searches for the choice of secondary drugs, especially in pregnant women with GBS colonization, which may cause serious problems in the future. This situation has emerged not only because of the increase in erythromycin resistance, but also because of the desire to better pass the placental barrier and create a therapeutic effect in the fetus at desired levels, and clindamycin, which provides these, has come to the fore as an alternative to erythromycin (29,30). As mentioned above, erythromycin and clindamycin resistance rates, which are the second choice in GSB treatment and prophylaxis, were found to be 33.4% and 17.2% in our study. In studies conducted in other countries, these rates were 7-45% for erythromycin and 3-25% for clindamycin (31,32). In a study by Atalay et al. with 131 GBS isolates, penicillin and vancomycin-resistant isolates were not detected, while erythromycin and clindamycin resistance rates were found to be 14.5% and 13%, respectively (13). In another study conducted in our country, erythromycin resistance was found to be 24.4%, and clindamycin resistance rate was 19.4% (25). While the erythromycin resistance rate in our study (33.4%) was higher than these two studies, it was found to be lower than the 50% erythromycin resistance rate reported by Savcı et al. in our country (33).

Although low rates of resistance to quinolones have been observed in Europe and the USA, the continued and extensive use of quinolones for both clinical and agricultural purposes and animal nutrition may contribute to increased resistance to quinolones and selection of resistant strains in many microorganisms, including GBS (31,34-37). In studies conducted in South Korea and China, resistance to ciprofloxacin and levofloxacin in *S. agalactiae* isolates isolated from urine samples was reported at a higher rate than colonized strains isolated from pregnant women (36,38). Similarly, in our country, Baba et al. (18) found that quinolone resistance was higher in GBS isolates isolated from the urine of patients with complaints than in the colonized group. This situation has been associated with the frequent use of quinolone antibiotics in the treatment of urinary tract infections (18). In our study, the high resistance to levofloxacin may be due to the isolation of the isolates predominantly from the urinary tract and the use of quinolone group antibiotics as the first choice in urinary tract complaints when over-the-counter antibiotic use was intense.

CONCLUSION

In the light of this information, penicillin resistance was not detected in our study. However, resistant strains have been detected in erythromycin, clindamycin, and levofloxacin, which stand out as other options in cases where penicillin cannot be used.

Authors's Contributions: Idea/Concept: İ.S.A.; Design: İ.S.A.; Data Collection and/or Processing: İ.S.A.; Analysis and/or Interpretation: İ.S.A.; Literature Review: İ.S.A.; Writing the Article: İ.S.A.; Critical Review: İ.S.A.

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