

Identification and antimicrobial susceptibility testing of *Streptococcus agalactiae* associated urinary tract infections using VITEK 2 system

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Received: September 2024, Accepted: November 2024

ABSTRACT

Background and Objectives: As a Gram-positive bacterium, *Streptococcus agalactiae* or Group B Streptococcus (GBS) is normally found as a transient flora of the gastrointestinal and genitourinary tracts of women. The high prevalence of GBS in the urethra warrants investigation of UTIs and antibiotic resistance frequency associated with GBS. Given the paucity of research on antibiotic resistance of GBS in Iran, the present study investigated the UTIs associated with GBS and the antibiotic susceptibility patterns associated with GBS.

Materials and Methods: This study included 65 GBS strains collected from urine samples obtained from the Bouali Laboratory Complex, one of the largest laboratories in western Iran. VITEK 2 GP ID cards were used to identify all GBS isolates. VITEK 2 susceptibility testing for Gram-positive bacteria was performed according to the manufacturer's instructions using the AST-ST card. MIC method was performed after the detection of GBS strains.

Results: We found that 53 (81.5%) of the GBS isolates showed resistance to tetracycline; 47 (72.3%), 40 (61.5%), and 30 (46.15%) of these had a resistance to erythromycin, clindamycin and ampicillin respectively.

Conclusion: In the present study, the VITEK 2 system was validated as a user-friendly system that can serve as a rapid and accurate tool for identification and antimicrobial susceptibility testing of GBS.

Keywords: Identification; *Streptococcus agalactiae*; Urinary tract infections; VITEK 2 System

INTRODUCTION

Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a Gram-positive bacterium typically found as transient flora of the gastrointestinal and genitourinary tracts of women (1, 2). The most critical risk factor for neonatal disease has been reported to be GBS colonization of the maternal

genital tract (3). Vertical transmission of GBS occurs in approximately 30-70% of colonized mothers, and early-onset infections may occur in 1-2% of these mothers (2) in the form of pneumonia and sepsis, while the most common consequence of late-onset disease is meningitis (4). Asymptomatic bacteriuria (ABU), cystitis, pyelonephritis, urethritis, and urosepsis are part of the spectrum of GBS UTI (5). Al-

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though the elderly appear to be most at risk for cystitis due to GBS, GBS ABU is particularly common in pregnant women (6, 7).

In addition, GBS can cause infections in older babies and non-pregnant mothers, and it particularly affects the elderly or those with underlying medical conditions (7, 8). GBS infection in non-pregnant adults, especially older adults, has also become an important issue in this age group (9). An important clinical entity is invasive GBS disease: The most common presentation is primary bacteremia, followed by skin and soft tissue infections, pneumonia, urosepsis, endocarditis, peritonitis, and meningitis (9, 10). The incidence of invasive GBS in adults has increased over time and relapse is relatively common as well. In particular, older age is associated with increased GBS incidence and mortality, and most fatal GBS infections occur in the elderly. Most cases in older adults are associated with underlying medical conditions such as diabetes mellitus, obesity, cirrhosis, stroke, cancer, and cardiovascular disease (6, 9).

Contributing to a variety of infectious diseases in immunocompromised individuals, the elderly, neonates, and pregnant women, GBS account for nearly 2-3% of all urinary tract infections (UTIs) (11). GBS-induced UTIs are also common in diabetics and those with urologic abnormalities, who are at higher risk for bacteremia and/or urosepsis (12, 13). Previous studies have reported sporadic cases of GBS bacteremia in adults, and the most common source of this bacteremia is the urinary tract (14, 15).

The high prevalence of GBS in the urethra warrants investigation of UTIs and antibiotic resistance frequency associated with GBS (16). In general, strains of GBS are susceptible to penicillin. clindamycin is the recommended antibiotic alternative for patients with a history of β -lactam allergy (17, 18). However, the identification of clinical GBS strains resistant to these antibiotics has increased recently (18, 19). Macrolide, lincosamide, and streptogramin B (MLSB) antibiotics are available to reduce the risk of early-onset GBS infection, especially in penicillin-allergic women (20, 21). However, increasing reports of resistance to GBS have become a global health concern (9). There has been an alarming increase in GBS resistance to erythromycin and clindamycin in many countries over the past decade (16, 22). According to the latest Centers for Disease Control and Prevention (CDC) report, clindamycin-resistant GBS is considered as a "threat of concern"

because of the threat it poses to human health (23). This is of concern because it limits antibiotic options for prophylaxis or treatment of infections associated with GBS. Given the paucity of research on the antibiotic resistance of GBS in Iran, the current study examined the UTIs associated with GBS and the antibiotic susceptibility patterns associated with GBS.

MATERIALS AND METHODS

Specimen collection and identification. This was a descriptive analytical cross-sectional study including 65 GBS strains collected from urine samples obtained from Bouali Laboratory Complex, which is one of the largest laboratories in western Iran. Our study was conducted from October 2021 to October 2023. Data including sex and age were recorded. Participants who had taken antibiotics were excluded from the study.

All samples were sent aseptically to the microbiology laboratory for further laboratory processing. Colony morphology, hemolysis, Gram stain, and catalase test were performed for presumptive identification (24). Gram-positive bacteria were detected using the VITEK-2 system and a special cartridge. Isolates were stored at -70°C in trypticase soy broth glycerol. Using 5% sheep blood, we subcultured the isolates twice on Columbia agar and allowed them to grow overnight at 35°C before testing (25).

GBS identification and antimicrobial susceptibility testing (AST). The VITEK 2 system was used for bacterial identification and AST of all isolates. GBS isolates were identified using VITEK 2 GP ID cards. The AST-ST card was used for VITEK 2 susceptibility testing of Gram-positive bacteria according to the manufacturer's instructions.

VITEK 2 card was performed for drug susceptibility testing of Gram-positive bacteria using the following antibiotics: erythromycin, tetracycline, clindamycin, ampicillin, benzylpenicillin, tigecycline, vancomycin, cefoxime, ceftriaxone, sulfamethoxazole/trimethoprim, moxifloxacin, levofloxacin, linezolid, chloramphenicol, and teicoplanin. Standard reference strains were used as control reference strains for identification and AST. Data were automatically analyzed using VITEK, and results were interpreted according to CLSI guidelines. Microbial susceptibility can be categorized into three groups,

namely susceptible (S), intermediate (I) and resistant (R) (26).

Minimum inhibitory concentration (MIC). After detection of GBS strains, the MIC method was performed. The term "minimum inhibitory concentration" (MIC) refers to the in vitro thresholds at which certain bacterial strains become susceptible or resistant to an antibiotic (27). VITEK, an automated system used by many diagnostic laboratories, provides MIC-based susceptibility results (28). After VITEK 2 testing, GBS isolates were stored on slants at room temperature. The MIC was then read by the lead investigator.

Statistical analysis. SPSS version 21 (SPSS Inc. Chicago, IL, USA) was used for data analysis. Descriptive statistics (relative frequencies) were used to describe the data. Chi-square analysis was used for comparisons between antibiotic resistance.

RESULTS

Bacterial isolates. Of the sixty-five isolates, 14 (21.54%) were from male patients and 51 (78.46%) were from female patients. Table 1 shows the age range of the patients.

Antimicrobial susceptibility testing (AST). We found that 53 (81.5%) of the GBS isolates were resistant to tetracycline; 47 (72.3%), 40 (61.5%) and, 30 (46.15%) of them were resistant to erythromycin, clindamycin and ampicillin, respectively. Fig. 1 shows the antimicrobial resistance pattern of GBS-associated urinary tract infections. The rate of tetracycline resistance of GBS isolates in the present study was high (81.53%). In addition, more than three-quarters

of the GBS isolates were susceptible to linezolid 49 (75.38%), teicoplanin 42 (64.6%), and chloramphenicol 40 (61.5%).

Regarding the antimicrobial profile, Table 3 shows the distribution of resistant GBS strains, and Table 2 gives details about the trend of antibiotic resistance during the study.

MIC spectra of GBS strains were obtained for erythromycin 2 and 8 µg/mL, tetracycline 0.5 to 2 and 16, clindamycin 0.25, 1 and 4, ampicillin 0.25 to 0.5, and 2 to 16, benzylpenicillin 1 to 8, tigecycline 0.25 to 1, vancomycin 0.5 to 8, and 32, cefotaxime 0.5 to 1, and 4 to 8, ceftriaxone 0.25 and 4 to 8, sulfamethoxazole/trimethoprim 512, moxifloxacin 0.25 to 0.5 and 2 to 8, levofloxacin 0.25 to 8, linezolid 2 to 8, chloramphenicol 2 to 4, and 16, teicoplanin 0.5 to 1, and 4 µg/ml. Table 3 shows the percentage of resistant strains separately.

DISCUSSION

Due to the recent increase in antibiotic resistance in GBS, the Centers for Disease Control and Prevention (CDC) has recommended that individuals should be tested for antibiotic susceptibility in case therapy is needed. Therefore, due to the dynamic pattern of antimicrobial resistance in different geographical areas, ongoing regional surveillance may be helpful in managing associated infections and optimizing available stewardship strategies. Our results showed updated information about the antimicrobial resistance of *Streptococcus agalactiae* isolates in urinary tract infections in the western Iran.

The CDC published statistics show that in vitro resistance of GBS to erythromycin and clindamycin increased from 25-32% and 13-20%, respectively, during 2006-2009. The WHO has highlighted AMR in GBS as a major public health concern. There is compelling evidence that the increasing resistance of GBS to both erythromycin and clindamycin has become a real concern, both in the general population and in pregnant women (29). Invasive disease caused by GBS infection leads to a variety of clinical diseases. In fact, GBS is considered as a threatening source of morbidity and mortality in high-risk populations such as the elderly, pregnant women, and neonates. Also, non-pregnant adults have been reported to suffer from an increasing incidence of invasive disease caused by GBS infection (30). As shown in our study,

Table 1. Patient’s demographic information

Variable		Frequency (%)
Sex	Female	51 (78.46)
	Male	14 (21.54)
	Total	65 (100)
Age	0-20	5 (7.7)
	21-40	21 (32.3)
	41-60	17 (26.2)
	≥ 61	22 (33.8)
	Total	65 (100)

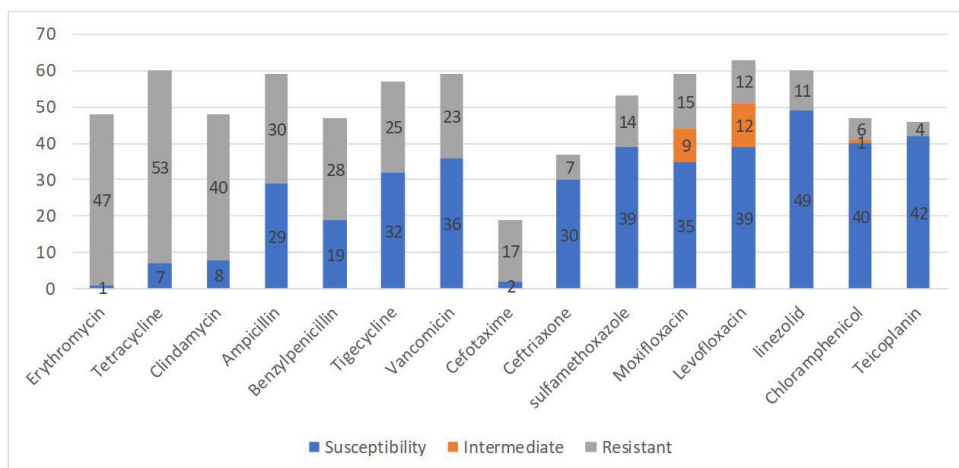


Fig. 1. Antibiotic resistance pattern of GBS associated Urinary Tract Infections

Table 2. Antimicrobial susceptibility profile of GBS obtained from urine samples

Antibiotics	Susceptibility (%)	Intermediate (%)	Resistant (%)	Total (N)
Erythromycin	1 (1.5)	-	47 (72.3)	48
Tetracycline	7 (10.7)	-	53 (81.5)	60
Clindamycin	8 (12.3)	-	40 (61.5)	48
Ampicillin	29 (44.6)	-	30 (46.1)	59
Benzylpenicillin	19 (29.2)	-	28 (43.07)	47
Tigecycline	32 (49.2)	-	25 (38.4)	57
Vancomycin	36 (55.3)	-	23 (35.38)	59
Cefotaxime	29 (44.6)	-	17 (26.15)	46
Ceftriaxone	30 (46.1)	-	17 (26.15)	47
Sulfamethoxazole/trimethoprim	39 (60)	-	14 (21.5)	53
Moxifloxacin	35 (53.8)	9 (15.2)	15 (23.08)	59
Levofloxacin	39 (60)	12 (18.4)	12 (18.46)	63
Linezolid	49 (75.3)	-	11 (16.9)	60
Chloramphenicol	40 (61.53)	1 (1.54)	6 (9.23)	47
Teicoplanin	42 (64.62)	-	4 (6.15)	46

of the 65 isolates studied, 14 (21.54%) and 51 (78.4%) were associated with male and female patients, respectively. In Iran, there have been a few studies on bacterial resistance to major antibiotics, but no study has investigated this resistance in the population of Kermanshah. Therefore, we used phenotypic methods to evaluate antibiotic resistance patterns. According to our results, tetracycline resistance was observed in 53 (81.5%) of the GBS isolates. In addition, 47 (72.3%), 40 (61.5%), and 30 (46.1%) of them were resistant to erythromycin, clindamycin and, ampicillin respectively. In studies conducted in Italy, susceptibility to ceftriaxone and vancomycin was observed

in all 3494 clinical isolates tested. The highest rates of resistance were associated with erythromycin (n=1402, 40.1%) and clindamycin (n=1090, 31.2%) followed by levofloxacin (n=161, 4.6%), penicillin (n=6, 0.2%), and ampicillin (n=5, 0.1%) (17). In another study conducted in Africa in 2019, the greatest resistance of GBS was to tetracycline. Other antibiotics to which GBS was resistant included ciprofloxacin, penicillin, ampicillin, amoxicillin, ceftriaxone, erythromycin, vancomycin, chloramphenicol, and clindamycin (31). A study in Brazil in 2019 found that GBS had the highest resistance to tetracycline, even though this antibiotic is not often used to treat

Table 3. The percentage of GBS strains using MIC (µg/mL)

Minimum Inhibitory Concentration (µg/mL)	0.078125	0.15625	0.3125	0.625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Erythromycin		1							7		40						
Tetracycline							4	2	1		1	52					
Clindamycin						15		32		1							
Ampicillin						29	2		4	2	3	19					
Benzylpenicillin	17	2						2	4	9	13						
Tigecycline	28	1				2	24	2									
Vancomycin							24	11	2	7	8		7				
Cefotaxime		27					2	1	2	2	12						
Ceftriaxone		29				1			1	2	14						
sulfamethoxazole/trimethoprim												10	1	28		8	6
Moxifloxacin		2				20	11	2	9	14	1						
Levofloxacin						1	9	16	19	6	6	6					
Linezolid								2	46	2	10						
Chloramphenicol									2	38	1	6					
Teicoplanin		35				1	2	4		4							

GBS. GBS has also been found to be resistant to clindamycin and erythromycin, which are alternatives to penicillin for GBS infections (32). Bornasi et al. reported that the highest resistance was associated with tetracycline (96.6%), followed by erythromycin (28.3%) and clindamycin (15%), while the lowest resistance was associated with penicillin, ampicillin, and vancomycin (0%), followed by ceftazolin (3.3%) and ceftazidime (5%) (33). The high rates of antibiotic resistance and the prevalence of resistance genes could be explained by the indiscriminate use of antibiotics and their use as a preventive measure. Therefore, further studies are needed to shed more light on this issue. As mentioned earlier, in our study, the greatest resistance of GBS was against tetracycline (81.5%), and a significant susceptibility of GBS was observed against linezolid (75.38%), teicoplanin (64.6%), and chloramphenicol (61.5%). In a study conducted at Babol Islamic Azad University in 2023, tetracycline (94.33%) was again the antibiotic to which GBS had the greatest resistance, and susceptibility to linezolid was observed in all isolates (34). In another study conducted in China in 2023, susceptibility to linezolid, tigecycline, penicillin, vancomycin, ampicillin, and quinupristin–dalfopristin was observed in all isolates tested. Chloramphenicol, levofloxacin, erythromycin, and tetracycline, were other antibiotics to which resistance was observed

(35). In a study conducted in Australia, 100 *S. agalactiae* strains had 32% co-resistance to clindamycin and erythromycin (36). Chinese patients infected with *S. agalactiae* who were allergic to penicillin had fewer antibiotic options due to the rapid rise of *S. agalactiae* resistance. Vancomycin remains active against *S. agalactiae* resistance to second-line antibiotics (37). In a previous study investigating the antibiotic susceptibility of GBS isolates associated with UTIs, antibiotic susceptibility to cefaclor, penicillin, ceftriaxone, and cefuroxime was observed in all isolates, and 80%, 19.5%, and 3.4% of isolates were not susceptible to tetracycline, erythromycin, and levofloxacin, respectively (5). In a study conducted in Tehran on 115 GBS isolates, results showed that 110 isolates (96%) were resistant to tetracycline. Isolates were widely resistant to clindamycin (35%), chloramphenicol (45%) and erythromycin (35%), but only one isolate (1%) was resistant to linezolid. All GBS were susceptible to penicillin and quinpristin–dalfopristin (38). Also, in a study conducted in Mashhad on 66 GBS isolates, the percentage of GBS resistance to clindamycin and erythromycin was 20% and 24.5%, respectively. In this study, 100% of all clinical GBS isolates were resistant to amikacin, gentamicin, nalidixic acid, and kanamycin, and all isolates were fully susceptible to ampicillin, amoxiclav, and ceftriaxone (39).

GBS strains with higher MIC levels can be real problem, and it is important to track how these organisms become susceptible over the time. A number of GBS isolates in our study were resistant to tetracycline, erythromycin and clindamycin, and 75.38%, 64.6%, and 61.5% of the isolates were shown to be susceptible to linezolid, teicoplanin, and chloramphenicol respectively. Although antimicrobial resistance in GBS populations is a worldwide phenomenon, its rate may vary depending on the region and duration of the study conducted. It is therefore important that further research is carried out to shed more light on the susceptibility of GBS strains to antibiotics, as the identification of susceptible strains could help in the selection of an appropriate alternative treatment.

CONCLUSION

According to the results of this study, the VITEK 2 system is a valid and user-friendly system that serves as a rapid and accurate tool for identifying GBS and performing antimicrobial susceptibility testing. The VITEK system can detect a large number of bacteria simultaneously and can also perform antibiotic susceptibility testing on 15 to 20 antibiotics simultaneously. This method is more reliable than manual methods (such as MIC). Our results also confirmed that GBS strains are susceptible to linezolid, teicoplanin, and chloramphenicol and that these antibiotics could be used to treat GBS infections. However, the increase in antibiotic resistance and urinary tract infections associated with GBS necessitates the continued investigation of GBS strains to select the appropriate drug for treatment and prevention of infection.

ACKNOWLEDGEMENTS

The authors would like to express their sincere appreciation to Bouali Laboratory Complex.

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