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Prevalence of *Streptococcus agalactiae* in Urinary Tract Infections of Pregnant Women

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ABSTRACT

Background: Urinary tract infection is the most common bacterial infection in children and adults, especially females. Among urinary tract infection caused by bacteria, *Streptococcus agalactiae* can cause periodic, temporary, or persistent infection in women. The aim of this study was to evaluate the prevalence of *S. agalactiae* isolated from urine samples in pregnant women referred to Qom hospitals, Iran.

Methods: In this descriptive study, 1264 pregnant women suspected for urinary tract infection were assessed. Midstream urine specimens collected from pregnant individuals in Ali-ibn-AbiTaleb hospital medical laboratory were passaged on blood agar media. *Streptococcus agalactiae* was detected using phenotype-based tests. Then, antibiotic susceptibility tests were conducted using the disc diffusion method according to CLSI protocols.

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Keywords:
Antibiotic Resistance, Streptococcus agalactiae, Pregnancy, Urinary Tract Infection.

Results: Out of 1264 pregnant patients, 17.64% were diagnosed with beta-hemolytic *Streptococcus*, and 13.37% were diagnosed with *S. agalactiae*. The highest rates of antibiotic resistance were found for Clindamycin and Erythromycin. In place, the lowest resistance rate was detected for Nitrofurantoin. The prevalence of resistance to Penicillin was about 33%.
Conclusion: Regarding the results of this research, it would be better to perform urine culture tests before week 34 of pregnancy and the last weeks of pregnancy. Also, our results showed that Penicillin can be replaced by alternative antibiotics before week 34 of pregnancy to prevent further *S. agalactiae* antibiotic resistance.

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Introduction

Urinary tract infections (UTIs) include bladder and kidney, are more common in women (1). UTIs can be both symptomatic and asymptomatic due to presence or absence of symptoms (2, 3). Among the aggravating factors of UTI in pregnant women with diabetes are: anemia, increasing gestational age and urinary tract disorders (4). Also, anemia can be an additional outcome of UTI in pregnant women. So, the diagnosis and treatment of bacteriuria during pregnancy is very important (5, 6).

Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS) is a commensal organism in the urogenital tract and rectum in approximately 25% of the healthy adult female (7, 8). The bacterium is the leading cause of bacterial meningitis, pneumonia, and sepsis in human infants (9). In addition, GBS is increasingly associated with invasive infections in not only pregnant women, but also non-pregnant adults. Such an infection is particularly seen in women with underlying conditions such as diabetic mellitus, malignancy, liver disease, and the elderly population (10, 11). GBS has also emerged as a significant cause of acute UTI in adults, especially in the pregnant women (12).

The CAMP test is one of the diagnostic approaches which is routinely used for *S. agalactiae* (13, 14). This test is based on the CAMP factor that enlarges the area of hemolysis formed by the β-hemolysin elaborated from *Staphylococcus aureus* (13, 14). Hippurate hydrolysis test is an alternative test which can be used for detection of *S. agalactiae* (15). This bacterium is also sensitive to bile salts (16). *S. agalactiae* consists of an antiphagocytic polysaccharide capsule which is one of the most important virulence factors of this pathogen (17, 18). Based on antiserums reacting to *S. agalactiae*'s capsule, this bacterium is classified into 9 serotypes, among which Ia, Ib, II, III, and V serotypes are classified as human pathogens (19, 20). Interestingly, this bacterium is a natural member of the intestinal microbiota which can be also detected in the vagina (with a prevalence of about 10% to 30%). It is noteworthy that infants may become infected with *S. agalactiae* during natural birth through the birth canal.

S. agalactiae-associated neonatal infections, including meningitis, pneumonia, and septicemias have been also reported. *S. agalactiae* can cause septicemia or pneumonia in 7-day-old newborns. It can also cause septicemia and meningitis in 7-90-day-old newborns. Newborns' meningitis symptoms are not like adult meningitis and lack of neck stiffness and other non-specific symptoms such as fever or vomiting may lead to delayed diagnosis of the infection (21). However, *S. agalactiae* neonatal infections have been rarely reported (22).

Penicillin G is the most suitable antibiotic during childbirth, and ampicillin could be considered as an alternative. For maximum efficiency, penicillin G should be admitted at least four hours before child birth. Cephazolin is recommended for women who are allergic to penicillin and are in danger of anaphylactic shock. Clindamycin and erythromycin are used for women with high anaphylactic shock risk. Also, in case of penicillin allergy and antibiogram resistant results for clindamycin and erythromycin, vancomycin can be substituted (22).

In previous studies, a prevalence rate of 20.65% to 26% have been reported for *S. agalactiae* in pregnant women. Therefore, the aim of this study was to

assess the prevalence of *S. agalactiae* in UTI-pregnant women referred to Qom hospitals, Iran.

Materials and Methods

Sampling

This cross-sectional study was performed on 223 urine specimens suspicious for *S. agalactiae* infection from total 1264 specimens gathered from patients admitted to Ali-ibn Abi Taleb hospital medical laboratory (March 2019 to January 2020). Information such as age, sex, urine analysis, and antibiotic data retrieved from antibiogram tests are all available in this research. Also, midstream specimens of urine were collected from outpatients suspected of UTI, which had not received any antibiotics and referred to the Pars hospital, a general private hospital in Tehran, Iran. The age range of the patients was from 1 to 96 years.

Bacterial Isolation

Primary isolation of uropathogens was performed by a surface streak plate technique on Tryptic Soy agar (TSA) containing 5% sheep blood (BAP). Agar plates were incubated for 24 hours at 35°C in a 5% CO2 incubator (all media were provided from Merck Co., Germany). Then suspected colonies with beta-hemolytic appearance, whose colony counts were $\geq 10^4$ CFU.mL-1, were examined by Gram staining and catalase test. The definitive identification of GBS isolates carried out using gram staining, catalase test, CAMP reaction, bacitracin and trimethoprim sulfamethoxazole susceptibilities, bile esculin, and 6.5% NaCl tests (8).

Antimicrobial Susceptibility

Antibiotic susceptibility testing was performed for 7 drugs covering all the seven antimicrobial categories comprising Penicillins, Cephalosporins (3rd generation), Cephalosporins (3rd generation (Oral), Macrolides, Folate pathway inhibitors, Lincosamides and Nitrofurantoins, were determined using the disc diffusion susceptibility test according to clinicalal and laboratory standards institute (CLSI) guidelines. Commercial antibiotic discs of Penicillin (10 unit), Ceftriaxone (30 µg), Cefixime (30 µg), Erythromycin (15 µg), Trimethoprim- sulfamethoxazole (30 µg), Clindamycin (2 µg), Nitrofurantoin (300 µg) (MAST Diagnostics, Merseyside UK) were used in disc diffusion test. Then, MDR/XDR/PDR phenotype of these isolates was established according to the results obtained from the disc diffusion test. Briefly, multidrug-resistance (MDR) was defined as resistance to at least three or more different classes, extensive drug-resistance (XDR) was defined as resistance to at least one agent in all but two or fewer antimicrobial categories and pan drug-resistance (PDR) was defined as resistance to all agents in all antimicrobial categories (23, 24).

Results

Among 1264 urine specimens of Ali-ibn Abi Taleb hospital medical lab pregnant clients, 223 cases were suspicious for beta-hemolytic *Streptococcus* (17.64%) and 169 cases were positive for *S. agalactiae*. Other urine culture results are given in table 1. Urine analysis data of 169 pregnant women with *S. agalactiae* infection are available in table 2.

Furthermore, FBS (Fasting Blood Sugar) and OGTT (Oral Glucose Tolerance Test) results for 113 persons (out of 1264 persons) were higher than the normal range, of which 14 people among them suffered from *S. agalactiae* infection. Besides, every positive culture for *S. agalactiae* was determined in terms of colony count. Thus, colony count for 161 cases was less than 40,000 CFU/ml, which 57 of them was less than 10,000 CFU/ml, and colony count for only 8 cases was more than 70,000 CFU/ml. There have been no recorded reports about premature delivery neither any problem during childbirth nor suspicious cases for neonatal septicemia or meningitis in Ali-ibn Abi Taleb hospital. Among all 223 suspicious specimens for *S. agalactiae*, 169 *Streptococcus agalactiae*, 16 *Enterococcus* sp., 6 group D

Streptococcus, and 32 non-ABD *Streptococcus* cases were reported. Results of antibiotic resistance and their susceptibility evaluation are available in table 3. According to figure 1, the highest resistance is related to clindamycin and erythromycin (53.25%), and the lowest resistance belongs to nitrofurantoin (7. 1%). In the studied population, among 169 specimens positive for *S. agalactiae*, 1 PDR case (0.59%), 24 XDR cases (14.2%), 30 MDR cases (17.75%) were reported. Also, 114 cases (67.45%) were not classified in any resistance category and they were considered as negative. (Figure 2).

Discussion

S. agalactiae barely causes any significant infection in pregnant women, but its transmission during childbirth to the newborn is worrying. There is no way to prevent this transmission. Despite medical progression over the past decades, many newborns die every year due to the *S. agalactiae* infection (24). According to previous studies, the prevalence rate of *S. agalactiae* in Iran tend to be very different from 9.1% to 22.7% (12,13). The rate of *S. agalactiae* isolation in other parts of the world included USA (27.2%), India (2.3%), Poland (17.2%), France (16.7%), and Thailand (19.2%) (14). In this study, among 1264 clients of Ali-ibn Abi Taleb hospital medical lab, 223 persons were suspicious for *S. agalactiae*, which 169 of their urine culture results were positive for *S. agalactiae*. In other words, 13.37% of our cases were diagnosed with *S. agalactiae* infection, which is conforming to the global analytical findings (25). In the previous study in Iran, susceptibility to penicillin, clindamycin, vancomycin, and ampicillin was reported 100%, and 61.5% for erythromycin (26). This rate of resistance was much higher than our study.

Excessive use of penicillin, as the first treatment choice or high doses of this antibiotic has led to an increased rate of Penicillin resistance. To reduce resistance to penicillin, the use of other antibiotics such as clindamycin and erythromycin are suitable (27).

Quiroga et al have reported around 7.6% *S. agalactiae* prevalence in 1121 pregnant women. Quiroga et al have also shown that all of the detected *S. agalactiae* isolates have been susceptible to penicillin, ampicillin, and vancomycin. Also, the frequency of sensitivity among the studied strains to nitrofurantoin, trimethoprim, sulfamethoxazole, and tetracycline was 98.3%, 46.8%, and 29%, respectively (28).

Regarding previous study, which respectively reported 95.3% and 100% *S. agalactiae* susceptibility to nitrofurantoin, our findings show 92.9% susceptibility to nitrofurantoin as well. Such findings highlight the fact that nitrofurantoin may be highly effective against symptomatic and non-symptomatic *S. agalactiae* bacteriuria (29).

S. agalactiae UTIs is more common in 21-33 (33% female) and it is less common in 51-60 year-old persons (30). According to our findings, the lowest prevalence rate is for under 20 and over 40-year-old individuals. Among the 21-35 age category, the highest infection rate belongs to 26-30-year-old individuals. There is a meaningful correlation between *S. agalactiae* UTIs rate and sexual activity of different age categories. In this study, due to the number of reported pyurias, epithelial cell detection, and clients' history, 37 of them had urinary tract infection history, among which 17 persons (10.05%) had dysuria and abdominal pains. Besides, in the matter of colony count, only 8 cases have been reported with more than 70,000 CFU/ml. The colony count in the rest cases were less than 40,000 CFU/ml. It shows that the *S. agalactiae* UTIs is mostly non-symptomatic.

In another research, Hamedi et al reported GBS prevalence in 200 pregnant women. All GBS isolated from mothers were involved with premature rupture of membranes (at least 18 hours before delivery). In terms of *S. agalactiae* detection in the vagina, there was a significant correlation between mothers and infants (31-34).

Table 1. Diversity of the micribes detected by urine culture test.

No growth	Beta-hemolytic *streptococcus*	*Streptococcus agalactiae*	*Enterobacteriaceae*	*Pseudomonas aeroginosa*	*Staphylococcus saprophyticus*	*Staphylococcus aureus*	*Candida* sp.
910	223	169	89	8	5	10	19

Table 2. Urine analysis data of 169 pregnant women with *S. agalactiae* infection.

Epithelial cells <10	**WBC* <10**	**Crystal**	**Ketonuria**	**Glycosuria**	**Blood**	**Proteinuria**
19	11	12	2	3	18	29

Table 3. Results of antibiotic resistance and their susceptibility test.

Antibiotics	**Concentration**	**Resistant**	**Sensitive**
Penicillin (19)	10 unit	57	112
Erythromycin (19)	15 µg	90	79
Clindamycin (CC)	2 µg	90	79
Cefixime (CFM) or Ceftriaxone (CRO)	30 µg	12-17	140
Nitrofurantoin (FM)	300 µg	12	157
Cotrimoxazole (SXT)	1.25/ 23.75 µg	75	94

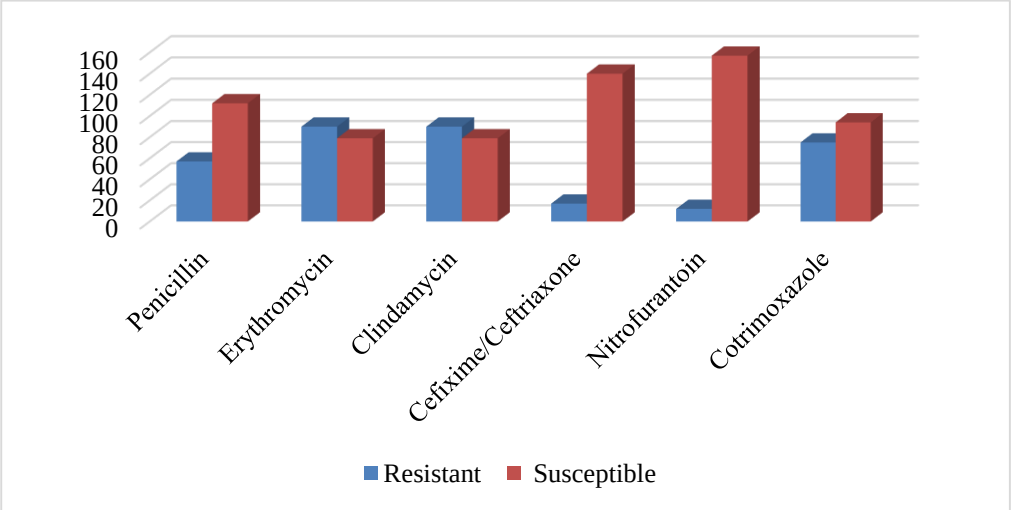

Fig 1. Diagram of antibiotic resistance and susceptibility based on their numbers.

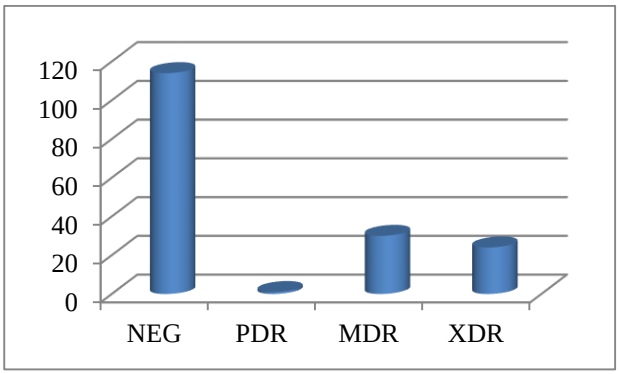

Fig 2. Antibiotic resistance categories.

Conclusion

Considering the detection rate of *S. agalactiae* in pregnant women and high antibiotic resistance, the potential risk of transmission of infection to newborns and the risk of contracting the disease in mothers, it is important to conduct such studies in wider geographical scales.

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Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The informed consent was obtained from all the participants, and informed consent obtained was written.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Timoney JF, Gillespie JH, Scott FW, et al. Hagan and Bruner's microbiology and infectious diseases of domestic animals: *Cornell Univ.* Press; 1988.
2. Abraham S, Chapman TA, Zhang R, et al. Molecular characterization of *Escherichia coli* strains that cause symptomatic and asymptomatic urinary tract infections. *J. Clin. Microbiol* 2012; **50**(3):1027-30.
3. Öztürk R, Murt A. Epidemiology of urological infections: A global burden. *World J. Urol* 2020: **38**:2669-2679
4. Zahedkalaei AT, Kazemi M, Zolfaghari P, et al. Association between urinary tract infection in

the first trimester and risk of preeclampsia: a case–control study. *Int J Womens Health* 2020;**12**:521.
5. Yeh M, Bujaki E, Dolan PT, et al. Engineering the live-attenuated polio vaccine to prevent reversion to virulence. *Cell Host Microbe* 2020; **27**(5):736-51.
6. Szweda H, Jóźwik M. Urinary tract infections during pregnancy-an updated overview. *Dev Period Med*. 2016; **20**(4):263-72.
7. Edwards MS, Baker CJ. *Streptococcus agalactiae* (Group B Streptococci). Bennet J, Dolin R, Blaser M Mandell, Douglas, and Bennett's Principles and practice of infectious diseases 9th ed Philadelphia, PA: Elsevier. 2020:2205-251.
8. Tayebi Z, Saderi H, Gholami M, et al. Evaluation of antimicrobial susceptibility of *Streptococcus agalactiae* isolates from patients with urinary tract infection (UTI) symptoms. *Infect Epidemiol Microbiol* 2016; **2**(4):17-9.
9. Madhi SA, Radebe K, Crewe-Brown H, et al. High burden of invasive *Streptococcus agalactiae* disease in South African infants. *Ann Trop Paediatr* 2003; **23**(1):15-23.
10. Parks T, Barrett L, Jones N. Invasive streptococcal disease: a review for clinicians. *Br Med Bull* 2015; **115**:1.
11. Oyanguren B, Esteban L, Guillán M, et al. Central nervous system involvement in adult patients with invasive infection caused by *Streptococcus agalactiae*. *Neurología* (English Edition). 2015; **30**(3):158-62.
12. Foxman B. The epidemiology of urinary tract infection. *Nat Rev Urol* 2010; **7**(12):653-60.
13. Li Y, Zeng W, Fan W, et al. Structure determination of the CAMP factor of *Streptococcus agalactiae* with the aid of an MBP tag and insights into membrane-surface attachment. *Acta Crystallogr. D: Struct Biol* 2019; **75**(8):772-81.
14. Guo D, Xi Y, Wang S, et al. Is a positive Christie-Atkinson-Munch-Peterson (CAMP) test sensitive enough for the identification of Streptococcus agalactiae? *BMC Infect Dis* 2019; **19**(1):1-5.
15. Nsagha D, Bello C, Kandakai-Olukemi Y. Heppurate hydrolysis and christie, atkins, munch-peterson tests epidemiological diagnostic tools for *Streptococcus agalactiae* carriage in pregnancy. *East Afr Med J* 2000; **77**: 1.
16. Evans J, Klesius P, Gilbert P, et al. Characterization of β-haemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, Liza klunzingeri (Day), in Kuwait. *J Fish Dis* 2002; **25**(9):505-13.
17. Lindahl G, Stålhammar-Carlemalm M, Areschoug T. Surface proteins of *Streptococcus agalactiae* and related proteins in other bacterial pathogens. *Clin Microbiol Rev* 2005; **18**(1):102-27.
18. Cieslewicz MJ, Chaffin D, Glusman G, et al. Structural and genetic diversity of group B *Streptococcus* capsular polysaccharides. *Infect Immun* 2005; **73**(5):3096-103.
19. Smith J, Durfee K, Marymont J. A review of laboratory methods for identification of group B streptococci (*Streptococcus agalactiae*). *AM J Med Technol* 1979; **45**(3):199-204.
20. Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. *Mol Microbiol* 2002; **45**(6):1499-513.
21. Ryan KJ, Ray CG. Medical microbiology. McGraw Hill. 2004;**4**:370.
22. Apgar BS, Greenberg GM, Yen G. Prevention of group B streptococcal disease in the newborn. *Am Fam Physician* 2005; **71**(5):903-10.
23. Magiorakos AP, Srinivasan A, Carey R, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**(3):268-81.
24. Florindo C, Viegas S, Paulino A, et al. Molecular characterization and antimicrobial susceptibility profiles in *Streptococcus agalactiae* colonizing strains: association of

erythromycin resistance with subtype III-1 genetic clone family. *Clin Microbiol Infect* 2010; **16**(9):1458-63.
25. Aarestrup FM, Seyfarth AM, Emborg HD, et al. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* 2001; **45**(7):2054-9.
26. Fazeli Z, Alipour M, Amir Mozafari N, et al. The survey of *Streptococcus agalactiae* carriage in pregnant women and determination of antibiotics susceptibity pattern in Amol city. *Iran J Med Microbiol* 2015; **9**(2):20-6.
27. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Medicinal Chem* 2014; **6**:PMC. S14459.
28. Oviedo P, Pegels E, Laczeski M, et al. Phenotypic and genotypic characterization of *Streptococcus agalactiae* in pregnant women: first study in a province of Argentina. *Braz J Microbiol* 2013; **44**:253-8.
29. Simoes JA, Aroutcheva AA, Heimler I, et al. Antibiotic resistance patterns of group B streptococcal clinical isolates. *Infect Dis Obstet Gynecol* 2004; **12**(1):1-8.
30. Al-Badr A, Al-Shaikh G. Recurrent urinary tract infections management in women: a review. *Sultan Qaboos Univ Med J* 2013; **13**(3):359.
31. Hamid ZO, Zaki NH, Ali MR. Prevalence of macrolide resistance genes among Group B Streptococci in pregnant women. *Int J Curr Microbiol Appl Sci* 2015; **4**(01):419-36.
32. Vansofla AN, Nazarian S, Kordbache E, et al. An IgG/IgY sandwich-ELISA for the detection of heat-labile enterotoxin B subunit of enterotoxigenic *Escherichia coli*. *Gene Reports* 2021; **23**:101099.
33. Hashemi B, Afkhami H, Khaledi M, et al. Frequency of Metalo beta Lactamase genes, *bla IMP1*, *INT1* in *Acinetobacter baumanii* isolated from burn patients North of Iran. *Gene Reports*. 2020; **21**:100800.
34. Theri M, Nazarian S, Ebrahimi F, et al. Immunization evaluation of type III secretion system recombinant antigens and Shiga like toxin binding subunit of *E. coli* O157: H7. *J Babol Univ Med Sci* 2018; **20**(7):47-54.