Prevalence of Group B Streptococci vaginal colonization in pregnant women at Saudi Maternal Hospital, Omdurman, Sudan

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ملخص البحث

الاصابه ببعض الانواع من الباكتيريا تؤثر على النساء الحموامل خاصة فى الاسابيع الاخيرة من الحمل. تعتبر المكورات السبحية من الباكتيريا التى قد تؤدى الى الاجهاض او ربماتنتقل و تصيب الاطفال قبل او اثناء الولادة . اجريت هذه الدراسة على مجموعة من النساء الحموامل فى عمر ٣٥-٣٧ اسبوع من الحمل لعزل وتشخيص الباكتيريا من النوع المكورات السبحية ومدى انتشارها فى المستشفى السعودى للولادة بامدرمان ومدى استجابتها للعلاج بالمضادات الحيوية المعروفة . شملت الدراسة عدد ١٢٥ من المترددات على المستشفى موضوع الدراسة حيث تم اخذ مسحات مهبلية منهن وتم تشخيصها بالاستزراع والتفاعل البيوكيميائى واختبار لانسفيلد ومن ثم تأكيد التشخيص عن طرق التفاعل المتبلمر المتسلسل مع جمع المعلومات عن طريق الاستبان. خلصت الدراسة الى وجود هذا النوع بنسبة تصل الى ٣,٢٪ وسط المترددات على المستشفى. توصى باجراء هذا الفحص كعمل روتينى لجميع النساء الحوامل على نطاق واسع يشمل مستشفيات الولادة لتفادى انتقال العدوى للاطفال حديثى الولادة.

Abstract

Background: Group B Streptococci (GBS) colonization in pregnant women usually has no symptoms, but it is one of the major causes of newborn infection in developing countries.

Objectives: The aim of this study was to determine the prevalence of vaginal GBS colonization in pregnant women at 35 – 37 weeks of gestation at Saudi Maternal Hospital, Omdurman, Sudan and to determine their susceptibility to antibiotics.

Methodology: This is a hospital-based study that surveyed 125 pregnant women who attended Saudi Maternal Hospital at the third trimester (35 – 37 week of gestation) from whom vaginal swabs were collected to investigate GBS streptococcal infection. The data were collected using a questionnaire. The isolated streptococci were identified by biochemical reactions, Lancefield grouping and Polymerase Chain Reaction (PCR).

Result: 3.2 % of the pregnant females attending the Saudi Maternal Hospital had vaginal colonization with GBS.

Conclusion: The prevalence of GBS colonization in pregnant women who attended the Saudi Maternal Hospital during the period of the study was found to be higher than the figure of 1% found on the hospital records. Screening and treatment of pregnant mothers to prevent invasive neonatal GBS disease is recommended.

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Introduction and Literature review

Streptococci are a heterogeneous group of bacteria, including important human pathogens. *S. pneumoniae* is considered a common agent of community-acquired pneumonia, otitis media, and endocarditis. *S. pyogenes* causes a wide array of serious infections, including pharyngitis, soft-

tissue infections, scarlet fever, and toxic shock-like syndromes. *S. agalactiae* is an important cause of serious neonatal infections characterized by sepsis and meningitis. Most other streptococci are members of the normal human floras [(1)].

Streptococci are classified on the basis of colonial morphology, haemolysis, biochemical reactions, and serologic specificity. They are divided into three groups by the type of haemolysis on blood agar: β-haemolytic (clear, complete lyses of red cells), α- haemolytic (incomplete, green haemolysis), and γ-haemolytic (no haemolysis). Serologic grouping is based on antigenic differences in cell wall carbohydrates (groups A to V excluding I and J), in cell wall pili-associated protein, and in the polysaccharide capsule in group B streptococci. Diagnosis is based on cultures from clinical specimens. Group B streptococci typically show hippurate hydrolysis[(1)].

Group B Streptococcus (GBS) is one of the most important bacteria in the majority of maternal and neonatal infections, such as chorioamnionitis, endometritis, bacteremia, sepsis and meningitis. During pregnancy, GBS screening is one of the recommended strategies that are recommended by Center of Disease Control (CDChttps://wwwn.cdc.gov).

In a prospective observational study in Egypt to detect the magnitude of group B streptococcal (GBS) colonization and disease among a sample of pregnant women, 95 pregnant women, 35-37 weeks of gestational age were screened with vagino-rectal swabs by a conventional GBS PCR assay. The study showed that, GBS carriage rate in the study sample was 17.89%. It was concluded that maternal GBS carriage was associated with a significant increase in neonatal infection rate [(2).]

During the last few decades, group B Streptococcus (GBS) has emerged as an important pathogen. The major reservoirs for GBS are the vagina and the peri-anal regions/rectum, and the colonization of these regions is a risk factor for subsequent infection in pregnant women and newborns. A study was performed in India to determine the prevalence of GBS colonization in the vagina and rectum of pregnant women and the antibiotic susceptibility pattern of the isolates. It aimed also to identify risk factors associated with GBS colonization. It showed that GBS colonization rate was 2.3%. It concluded that the GBS colonization rate was low. No resistance to penicillin or clindamycin was seen, while the majority of the isolates were resistant to tetracycline. [(3)]

Group B Streptococci (GBS) are the most frequent cause of severe, life-threatening neonatal infections. Significant infections are also seen in adult women as a complication of pregnancy and in the elderly and immunocompromised hosts. Commonly, 10–40 % of pregnant mothers carry GBS in their genitourinary and gastrointestinal tracts. Maternal colonization with GBS is the predominant risk factor for the development of invasive neonatal GBS disease. GBS carriage screening at weeks 35-37 of gestation and subsequent intrapartum antibiotic prophylaxis (IAP) for culture-positive women is recommended in many countries. [(4)]

A study was conducted in Iran to find out its prevalence among Iranian pregnant women and its vertical transmission to their newborns. It showed that 50% of symptomatic neonates were born from the mothers with positive vaginal culture for group B Streptococcus; whereas para-clinical test was required to detect the infection for the rest of neonates who showed no signs or symptoms[(5)].

The current study is investigating the frequency of Group B streptococci among pregnant women.

Materials and Methods

The study area was the Saudi Maternal Hospital. Lower vaginal swabs were collected in this study. The study population included the pregnant women who attended the study hospital at 35 - 37 week of gestation. All information required from patients were collected by a questionnaire.

Swabs and plastic Petri dishes were purchased readily sterilized. Hydrogen peroxide was prepared

in a concentration of 3% solution and was used for the catalase test.

Defibrinated sheep blood was used in preparing blood agar medium. Blood agar was used in culturing vaginal swabs and it was also used for identification of the type of haemolysis and CAMP reaction.

Bile salt agar was used for detection of growth of the organisms isolated in 40% bile salt agar. Muller Hinton agar was used for sensitivity to antibiotics. Nutrient broth medium (5% serum was added) was used for the catalase test. Hippurate broth was used for the detection of hippurate hydrolysis. All types of media and reagents used were prepared as described by Barrow and Fellthan [6].

Samples collection

Samples were collected from un-treated pregnant women who attended the study hospital at 35 - 37 weeks of gestation.

After collection, all samples of vaginal swabs were kept at refrigerator temperature for laboratory investigation

Culturing and Identification of organism

Swabs were streaked directly on blood agar plates containing 5% sheep blood by the use of bacteriological loop. All plates were then incubated in 10% Co2 overnight at 37º C. When no growth was observed, plates were reincubated for further 48 hrs before they were discarded as negative. Haemolysis was observed but it was not used as a stringent identification criterion.

The Gram stain was done from the overnight growth. Organisms that showed Gram-positive reaction were subjected to further examination and organisms that showed Gram – negative reaction were excluded.

All Gram-positive samples were subjected to catalase test which was done by adding few drops of 3% hydrogen peroxide to the tested organism which was previously cultured for overnight at 37 °C in 5 ml nutrient broth to which 5% serum was added. Positive result was detected by immediate production of air bubbles. Streptococci are considered as catalase-negative. Catalase positive samples were excluded.

Further examinations including different biochemical tests were done as described by Barrow and Fellthan [8].

These tests included growth on 10% and 40% bile, bile solubility, and hippurate hydrolysis in addition to The CAMP reaction. Sensitivity to antimicrobial drugs was then done by the disc diffusion method.

Lancefield grouping was done by using commercial kits according to the manufacturer instruction (Remel Europe Ltd. Clipper Boulevard Kent, D A2 6 PT UK).

DNA extraction was done using saturated bacterial culture which was previously kept in a normal saline solution at freezing temperature. The protocol used was a modified protocol; often referred to as plasmid “mini-prep,” which yielded fairly clean DNA quickly and easily. Samples were centrifuged to pellet the bacterial cells then 0.2 ml of ice-cold solution one (50mM Glucose, 25mM Tris HCl and 10 mM EDTA) was added to each pellet and vortexed. Solution two (0.2 N NaOH and 1%SDS) was then added, mixed by inversion and incubated at room temperature for 5 minutes. Ice-cold solution three (5M acetic acid and 3M potassium acetate) was then added. Sample was vigorously shaken to release DNA and kept at room temperature for 10 min. Centrifugation and transfer of supernatant into fresh micro centrifuge tube using clean disposable transfer pipette was done. This fraction step separates the DNA from the cellular debris. The tube was then filled with Ice-cold Isopropanol which effectively precipitates nucleic acids. The tubes were centrifuged to precipitate the milky pallet of the DNA at bottom of the tube and the supernatant was poured off without dumping out the pallet. Then ice-cold 70% ethanol was added, centrifuged and the supernatant was poured off. Samples were dried then dissolved in double distilled water and kept at – 20 ºC.

PCR amplification was done using a genus- specific primer for streptococci (ST R1 5’- GTA CAG TTG

CTT CAG GAC GTA TC-3' and STR2 5'- ACG TTC GAT TTC ATC ACG TTG-3') (Vivantis Technologies). PCR reaction was performed using a touch-down program profile: 94 °C for 2 min as an initial denaturation followed by 14 cycles during which annealing temperature was 63°C, decreasing per cycle for 14 cycles until the annealing temperature 55 °C was reached. The program was then continued using final temperature as annealing step for additional 35 cycles of: 45 s at 94 °C, 45 s of annealing and 1 min of extension at 72 °C. Finally the reaction was ended with 5 min at 72 °C. The mixes contained 1 µl (10 mM) of each forward and reverse primer, 1 µl of 25 mM MgCl2, 1µl of 10 mM dNTPs, 1 unit of Tag DNA polymerase, 2.5µl of 10X PCR buffer (10 mM tris-HCL PH 8.3, 50 mM KCL), ~150 ng of DNA and the volume was completed to 25 µl by double distilled water. PCR products were subjected to gel electrophoreses using 2% agarose.

All bacterial work was conducted at the Department of Microbiology, Faculty of Veterinary Medicine; University of Khartoum. While all molecular part of the study was conducted at the Center of excellence, Department of Zoology, Faculty of Science, University of Khartoum.

Results

Ninety nine samples out of 125 samples showed Gram-positive cocci, but Gram- negative organisms were also present (8 samples). There was no growth at all in 18 samples. All gram-positive organisms showed catalase-positive reaction except four samples that showed catalase-negative reaction. Gram-negative organisms and catalase-positive reaction organisms were excluded.

The four samples that showed gram-positive staining reaction and catalase-negative reaction were subjected to different types of biochemical reactions including the type of haemolysis which was β haemolysis. Growth on 10% and 40% bile were negative. Hippurate hydrolysis was positive in the four samples

According to these results which were specific criteria of group B streptococci only, the four samples were grouped as group B streptococci.

Using Lancefield grouping kit, the organisms were group B streptococci as shown in Table1.

The sensitivity to antibiotics was slightly different. The isolates were found highly sensitive to Penicillin, sensitive to vancomycin, while they showed resistance to chloramphenicol, tetracycline, ciprofloxacin and gentamicinas shown in Table (2).

Table 1.Biochemical reactions of catalase-negative organisms

Type of reaction	No of organisms	Percentage
Growth on blood agar	107	85.6
No growth	18	14.4
Gram- positive	99	92.5
Gram-negative	8	7.5
Catalase negative	4	4.04
Catalase positive	95	95.96
Haemolysis	4 (β haemolysis)	100
CAMP reaction	4 (positive)	100
Growth on 10% bile	zero	zero
Growth on 40% bile	zero	zero
Hippurate	4 (positive)	100
Biochemical grouping	4 (group B)	100
Lancefield grouping	4 (group B)	100
PCR products	4 (positive)	100

Table 2. Sensitivity to antimicrobial drugs presented in crosses

Antibiotic	Sensitive	Resistant
Penicillin	xxxx	-
Vancomycin	xxx	-
Erythromycin	xx	x
Fusidic acid	x	xx
Cloxacillin	x	xx
Chloramphenicol	-	xxx
Tetracycline	-	xxx
Ciprofloxacin	-	xxxx
Gentamicin	-	xxxx

Discussion

In the last few decades Streptococcus agalactiae or Group B Streptococcus (GBS) has gained importance due to its ability to cause serious neonatal infections. In developed countries GBS is a leading cause of sepsis and meningitis in neonates.[4]

In this study we isolated GBS bacteria from 4 pregnant women out of the 125 studied cases (3.2%). Detection rate reached 4% when gram negative samples were excluded. In agreement with the recommendation of the Center of Disease Control (CDC) and Shirazi[5], our results indicated that a screening test for this group of bacteria during pregnancy is recommended. The results of the current study signify a strong need for further biochemical reactions to be done in addition to antimicrobial sensitivity. Detection rates although slightly less but are in agreement with Shirazi[5] who isolated the same type of bacteria with a ratio of 4.8% and Vijayan [3] who found that the colonization rate was 2.3%.

Maternal colonization by GBS was observed to range from 4% to 40% in several studies conducted worldwide[4]. Although the frequency of GBS colonization was less than the rates in nearby countries like Egypt (17%)[2];we recommend that the screening test for this group of bacteria is better done during pregnancy.

The lower rate of GBS colonization might be due to the fact that only lower vaginal swabs were collected instead of rectovaginal swabs (due to a collection mistake).Recto vaginal swabs could have increased the detection rates. GBS should be screened among pregnant women at 35 - 37 weeks of gestation as stated before by Elbaradie [2] and others.

According to the results of anti biogram obtained Penicillin can be used as a drug of choice for treatment of GBS.

Further studies are recommended to investigate the prevalence of GBS in pregnant women at different maternity hospitals by using recto-vaginal swabs as there was no work in Sudan before

Conclusion

As most samples of this study showed significant bacterial growth, other than GBS, there is a strong need for further screening tests including biochemical reactions to identify the type of bacteria other than Streptococcus agalactiae that colonizes or infects the pregnant women in this hospital. We recommend that further identification methods should be done to reach proper diagnosis of bacteria that colonizes the pregnant women at maternal hospitals. Detection methods should be adopted as routine work at this and other hospitals. Follow-up programs of the neonates of infected women or colonizers might be needed as recommended by the Center of Disease Control

Acknowledgement

The authors express their sincere appreciation to the administration and the staff of Saudi Maternal Hospital. Thanks are also extended to the Matron and sisters of the hospital who assisted in collecting the samples. Prof. A. A. G. is also appreciated for helping in the correction of this manuscript.

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