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Isolation and identification of streptococcus agalactiae colonization in pregnancy woman and resistance to tetracycline in Al_Muthanna proviance

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Abstract--*S.agalactiae* is a Gram-positive, spherical bacteria that causes postpartum sepsis, endometritis, chorioamnionitis, and premature birth in pregnant women.*Streptococcus agalactiae*, often known as group B streptococci (GBS), is a dangerous disease that can infect both humans and animals (GBS) is an important bacterial pathogen that causes a wide range of infections including neonatal sepsis, meningitis, pneumonia and soft tissue or urinary tract infections. The present study demonstrated that *S. agalactiae* isolates obtained from vaginal samples showed a high rate of resistance to tetracycline. Objectives: The study aimed to investigate *streptococcus aqalctae* in pregnancy woman by isolating and diagnosing them from rectovaginal colonization. Result We were collected 206 swab from pregnant female this collected was during gestation period (35-37) week and were collected from different age that including (≤ 25) years, and (≥ 25 years). this sample collected from different hospital included (AL_Rumaitha Hospital, womans and childrens hospital). the collected swab had transfer swabs by Amies transported medium and give to lab. After that diagnosis by microscobic and biochemical test. *S. agalactiae* was spread through pregnant female and infection the infant. This studies appeared that the positive sample 145 sample (70.38%) (Table 1) from total number 206 (29.61 and the negative sample was 61 sample, on other hand the Distribution of *streptococcus agalctiae* by two environment (Rural and city) from total sample (206 sample) was about 130 Sample (63.106%) from Rural environment but the city environment was 76 Sample (36.893%) (Table 2). The distribution of streptococcus agalctiae by % 12.13 sample (

25%) while the negative sample was 49.51 sample(102 ≤25ages was positive (8.7%)(Table 3)18%) and the negative sample was 29.61sample (61 ages the positive sample 25while > Resistance to Tetracycline(30µg) was about 29 (69%) . the ratio is approximately similar to study in Rio de Janeiro The resistance rate was 97% (20) .while (21) the resistance to tetracycline was (87.6%).

Keywords--GBS colonization, *Streptococcus agalactiae*, Tetracycline, pregnant women, chromogenic selective group B.

Introduction

Streptococcus agalactiae (commonly known as Group B *Streptococcus*, or GBS) is a Gram-positive bacteria that colonizes the cervicovaginal tract in 20-30 percent of healthy women and is one of the most common pathogens causing catastrophic newborn infections. GBS can cause various difficulties during pregnancy, yet colonization is asymptomatic. vertically transmitted to newborns peripartum, causing pneumonia, sepsis, or other complications such as chorioamnionitis and urinary tract infections, or vertically transmitted to newborns peripartum, causing pneumonia, sepsis, or other complications such as, chorioamnionitis , urinary tract infections (1).

GBS can infect the amniotic fluid during pregnancy, and vertical transmission during labor can infect the newborn, causing neonatal sepsis and meningitis. Approximately 10-30% of pregnant women are colonized with GBS in the vaginal area, 50-75 percent of their babies are infected from the birth canal(2).

As a commensal organism, *Streptococcus agalactiae* can be found in the maternal vaginal and lower gastrointestinal tracts, but it can also transform into an invasive pathogen that infiltrates a variety of host niches, including the intrauterine compartment, neonatal lung, and several neonatal organs, including the brain, This shows that GBS can efficiently adapt to changing host environments, and that the optimal expression of virulence components in response to the host environment can provide GBS with a survival advantage(3). *Streptococcus agalactiae* is a human pathogen and one of the four hemolytic streptococci (Viridance, agalactiae, pyogens, pneumonia). GBS is extremely damaging to animals and legumes due to its high mortality rates, resulting in massive economic losses in the food industry(4). *Streptococcus agalactiae*, the pathogen, encodes several virulence factors that are essential for the bacteria to cause disease(5).

Biofilms allow bacteria to persist for long periods of time while also protecting them from immune system identification and managing the production of bacterial surface-associated structures like pili and the capsule, which are both important in bacterial biofilm development(6). The organism can penetrate through placental membranes, causing them to weaken. GBS may gain access to the fetus within the amniotic cavity as a result of this process, causing placental membrane rupture and premature birth. The infant lung becomes the principal

focus of GBS infection after aspiration of infected amniotic or vaginal fluid. The bacterium can enter the bloodstream from the lungs, where it subsequently spreads to other organs and tissues (7).

It was discovered that GBS may be transmitted vertically and colonized from mothers to their babies, which occurs in around half of all births (8). This bacteria has the potential to spread disease through secondary transmission pathway into uterus, then taught membrane translocation by rupturing the membrane or translocating the baby into the delivery canal (9). If the mother was high colonized mean female increase danger of colonized in newborn (10)

Materials & Methods

The study included 206 patients from pregnant woman, the samples were collected from different age groups (≤ 25 and > 25) years, these samples taken from Pregnant women in hospitals in Muthanna governorate (Al-Rumaitha General Hospital as well as Women's and Maternity Hospital) for the period from November 2021 to February 2022. The samples were collected from (35_37) weeks by vaginal swap, each placed in the nutrient medium such as brain heart Infusion broth (BHI) for 24 hours at a temperature of 37 ° C in order to activation of bacteria, then transferred to the culture medium that selective chromogenic agar streptococcus group B, this medium specific for *streptococcus agalctiae* for a period of 24 to 48 hours in order to diagnose and isolate the bacteria and assure it with biochemical examinations [11]. The media used are chromogenic agar selective *streptococcus agalctiae*, . Traditional media was also used to confirm the results of the cultures such as blood agar, nutrient agar, Mitis salivarius agar

Sample Collection

206 swabs were taken from the mucus of vaginal tissues of pregnant women at 35-weeks of gestation during 20th October 2013 to 22th February 2014 from Ibn al-Balady Hospital, Central Public Health Laboratory, Kamal al-Sammaraie Hospital. After that, transported swabs to the lab and cultured via used Amies transport media. Bacterial Isolation Every swab had been culturing primary via gently streaking on sterile prepared plates and then incubate at 37°C for 24-48 hours. After that, a typical growth was obtained. After that, colonies that obtained from primary cultured, acted for it sub-cultured on CHROM strep B agar, this media consider a selective media for GBS isolation. We obtained blue and pink colonies. The blue colonies which belong to another bacteria were neglected while the pink to move colonies were sub-cultured into the same condition to get a single colony that was pure.

Detection of Some Virulence Factors of *streptococcus agalctiae*

Detection of Capsule

A drop of India ink stain was applied on the surface of a clean glass slide, then a pure colony was collected and placed on the glass slide, mixed with the stain, and the slide was let to dry before being studied by optical microscopy with an oil lens (100X) (12).

Results and Discussion

In this study, 206 samples were collected from two environment (Rural and city) from all hospitals in Al-Muthanna Governorate for all ages at 35_37 weeks (Table 1). The samples were taken from two environments, the rural and the city. The infection rate in the rural city was very high, in contrast to the city environment for several reasons, including the lack of health centers to care for pregnant women, in addition to the remoteness of the city center that It contains the main hospitals for treatment, in addition to the lack of personal hygiene, as well as the absence of health awareness among patients in the rural environment, as well as the lack of attention to personal hygiene, all of which helped the presence of these bacteria in this period of pregnancy. There is a large health culture in addition to the presence of health organizations that help educate pregnant women and provide advice during this period(Table 2)

Table 1: Distribution of *streptococcus agalctiae* at 35_37 weeks Number of weeks

Weeks	Positive sample	Negative sample	Total
35_37 weeks	145(70.38%)	61(29.611%)	206 sample

Table 2: Distribution of *streptococcus agalctiae* by two environment (Rural and city) from total sample (206)

Place of living	The number of samples
Rural	130 Sample (63.106%)
City	76 Sample (36.893%)

Several antibiotics were used on muller hinton agar medium to confirm the sensitivity and resistance of bacteria. Resistance to tetracycline, chloramphenicol, and other antibiotics was found in *S. agalactiae* isolates isolated from vaginal samples. It was concluded that penicillin is the most useful as it kills bacteria. It can be administered intravenously to pregnant women to prevent infection of newborns with streptococcus agalctiea. The resistance of bacteria to tetracycline was also detected. The causes of resistance are up to and due to the fact that streptococcus bacteria hit the beta-lactam ring, specifically controlling the cell wall area, breaking the peptidoglycan layer and breaking the glycosidic bond, thus canceling the effect of this type of antibiotics on these bacteria. *S. agalactiae* infecting the gastrointestinal tract without causing symptoms, yet it can cause serious infections in some cases. *S. agalactiae*, like other dangerous bacteria, possesses virulence factors (13,14). Other than human infections, *S. agalactiae* can cause mastitis in dairy cattle, resulting in a loss of revenue for the sector. *S. agalactiae* can cause acute or subacute febrile illnesses in cows, resulting in decreased milk output. As a result, a dairy outbreak has an impact. As a result, in recent decades, numerous governments have implemented strategies to decrease *S. agalactiae*'s influence. [15,16), Antibiotics kill pathogenic bacteria and other microorganisms, allowing the body to deal with ailments more effectively. Antibiotics derived from medicinal plants are called natural antibiotics. Antibiotics from nature come in a variety of forms. Clinical studies have shown

that oregano oil, garlic, and other natural antibiotics are the most effective in destroying even the most resistant germs. Recent research [17,18] has also revealed that these natural treatments have positive impacts.

Table 3: Distribution of *streptococcus agalctiae* by ages from total sample(206)

Ages	positive	%	Negative	%
≤25	102	49.51%	25	12.13%
>25	61	29.61%	18	8.7%

Conclusion

The results of this study showed a high incidence of streptococcus bacteria for pregnant women in Al-Muthanna proviance. Several studies were conducted using antibiotics, and the highest rate of resistance to tetracycline was, so we recommend that pregnant women undergo routine examination in clinics and hospitals in Al-Muthanna proviance.

Ethical approval

The study was approved by the administration and officials of Al-Rumaitha Hospital and Women's and childrens Hospitalat Al-Muthanna province/ Iraq. Patient consents were also taken to obtain vaginal samples to be cultured in the laboratory.

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