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ORIGINAL ARTICLE

Carriage of *Streptococcus agalactiae* among Pregnant Women in an Egyptian University Hospital, Serotypes Distribution and Antibiotics Susceptibility

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ABSTRACT

Background*: Streptococcus agalactiae, or Lancefield group B Streptococcus (GBS), is carried by women of childbearing age at variable frequencies. It is responsible for high neonatal infection rates.* ***Objective:*** *Was to detect the frequency of Streptococcus agalactiae carriage among pregnant females attending Benha University Hospital, identify the serotype of the isolated strains and to study their antibiotic susceptibility profile.* ***Methodology****: Two hundred and fifty pregnant females between 35 and 37 weeks gestation were enrolled in this study. Rectovaginal swabs were collected. Identification, serotyping, antimicrobial susceptibility, erythromycin resistance phenotyping by double disc diffusion test were performed.* ***Results****: GBS colonization was detected in 28% of investigated subjects, with serotype V was found in 37.1% of them. All isolates were sensitive to benzylpenicillin, ampicllin, cephotaxim and vancomycin. Resistance to erythromycin and clindamycin was (42.8 and 17.2%) respectively. Inducible and constitutive resistance were 12.9 and 17.1 %. M phenotype was detected in 4.3% of GBS strains.* ***Conclusion****: the results of our study would be useful for implementing prenatal GBS screening and proper choice of antibiotic for intrapartum prophylaxis*

INTRODUCTION

Streptococcus agalactiae, or Lancefield group B Streptococcus (GBS) are beta haemolytic Gram-positive cocci. It is divided into 10 serotypes (Ia, Ib and II-IX) using surface proteins as additional antigenic markers[1].

GBS disease is caused mainly by serotypes I, II and III. Serotype III is the most prevalent serotype in asymptomatic carriers[2].

In healthy adults, the primary reservoir of GBS is the gastrointestinal tract which is the main source of genitourinary colonization. Women of childbearing age carry GBS at variable frequencies of 4.6-31.3% with similar figures in both developing and developed countries[3].

Regardless of the kind of delivery (vaginal or cesarean section), fifty percent of neonates from colonized mothers become also colonized by GBS[4].

It is responsible for 1.8 neonatal infections per 1,000 live births per year[5]. High percentage of neonatal infections due to GBS occurs through vertical transmission from colonized mother to the newborn during labor and birth[6]. Laboratory detection of GBS colonization status in near-term pregnant women is therefore important for the selective prescription of antibiotic prophylaxis at delivery[7].

Since 2002, the Center for Disease Control and Prevention (CDC) recommended GBS screening for pregnant women by culture based method that consists of culturing combined vaginal and anal swab in a selective broth medium[8].

Penicillin and Ampicillin are the choice drugs for GBS. In patients with penicillin allergies or a lack of clinical response, alternatives such as macrolides (e.g. Erythromycin), Lincosamides (e.g. Clindamycin) are often considered. GBS resistance to the macrolides and Lincosamides has increased gradually during the past several years so susceptibility testing are required to guide therapy[9] .

So, the aim of this study was to detect the frequency of *Streptococcus agalactiae* carriage among pregnant females attending Benha University Hospital, identify the serotype of the isolated strains and to study their antibiotic susceptibility profile.

METHODOLOGY

***Patients*:**

After Institutional Research Board approval, the current cross sectional study was conducted in the period from May 2016 to February 2017 .Two hundred and fifty pregnant females between 35 and 37 weeks gestation were enrolled from Benha University Hospital. All women signed an informed written consent after being informed about the study aim and procedures. Use of antimicrobial drugs in the 30 days

prior to study time was the exclusion criteria[10]. Gestational age assessment using last menstrual period and/or early pregnancy ultrasound was used to establish the eligibility for the study.

Sampling procedures:

Sample collection and processing followed the CDC recommendations. A sterile swab was inserted 1-2 cms into the lower entrance of the vagina with swabbing the side walls then the same swab was inserted into the rectum past the external sphincter[5]. Internal examination or visualization of the cervix by speculum examination wasn't used for collection of screening cultures[11]. Collected samples were sent to the laboratory in Amies Transport Media (Oxoid, UK), where the viability of GBS can be maintained for up to 4 days[12].

Microbiological tests for identification of GBS:

All microbiological testing was carried out at Medical Microbiology and Immunology Department Faculty of Medicine, Zagzig University. The swabs were inoculated into Todd Hewitt (Himedia Laboratories, India) enrichment selective medium supplemented with gentamicin (8µg/mL), nalidixic acid (15 µg/mL) and sodium azide 0.02% (Sigma-Aldrich, Inc. MO, USA). The selective medium was incubated at 36°C in 5% CO2 for 18h and then subcultured onto blood agar plates which were incubated at 36°C in 5% CO2 for 24h. After incubation the plates were inspected for β-hemolysis. When no β-hemolytic colonies were observed after 24h, plates were reincubated for another 24h and inspected again. The β-hemolytic colonies with morphology consistent with group B streptococcus were subcultured in broth and submitted to the CAMP (Christie, Atkins, Munch, Petersen) test. The colonies positive for the CAMP test were presumptively considered GBS[5]. Further confirmation was performed by agglutination with a streptococcal grouping kit (Prolex Streptococcal Grouping Latex Kit; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). The procedures were performed according to the manufacturer's instructions[13].

Serotyping by latex agglutination:

A heavy suspension of *S. agalactiae* strains from the blood agar plate was prepared in 250 µl phosphate-buffered saline. Twenty µl aliquot of the bacterial suspension was applied to a disposable reaction card and mixed with 1 µl of latex suspension reagents Ia, Ib, and II to IX (Strep-B-Latex kit; Statens Serum Institut, Copenhagen, Denmark). The reaction card was rotated slowly and observed for agglutination, positive reaction was scored when clear-cut agglutination appeared within 30 seconds[14].

Antibiotic susceptibility testing according to the latest guidelines of CLSI[15]: For benzylpenicillin and ampicillin, E-test (BioMérieux, Sweden) was performed. For other tested antibiotics disc diffusion test was performed with erythromycin (15µg), clindamycin (2 µg), cefotaxim (30µg) and vancomycin (30µg).

Double-disc diffusion test: Double disc diffusion (D-zone test) was used to classify Erythromycin-resistant strains into phenotypes and to detect inducible clindamycin resistance. Erythromycin (15 µg) and clindamycin (2 µg) disks were placed 12 mm apart edge to edge .Blunting (positive DD test) was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disk, indicating inducible Macrolide-Lincosamide-Streptogramin B resistance (iMLSB). Resistance to both erythromycin and clindamycin indicated constitutive MLSB resistance (cMLSB). Resistance to erythromycin but susceptibility to clindamycin without blunting indicated Macrolide-streptogramin B resistance and lincosamide susceptibility (M phenotype) [16].

RESULTS

A total of 250 pregnant women were included in the current study. Their age ranged between 18 and 40 years with the mean was 25.94±5.33. The mean of gestational age of the studied group was 36.22±1.72 ranged between 35- 37weeks.

Rectovaginal GBS colonization was detected in 70/250 (28%) of pregnant women. There were significant associations ($P < 0.05$) between maternal age and the prevalence of rectovaginal GBS colonization; subjects aged ≤ 25 years old were GBS colonized at higher percentage than those aged > 25years old.

Regarding the serotyping for the isolated 70 GBS strains; the highest prevalence was for serotype V (37.1 %); serotypes III, Ia and II were 20, 12 &11.4% respectively, the lowest prevalence rate was for serotype IV (5.7%). Four stains were non-typeable. The distribution of different serotypes is presented by fig 1.

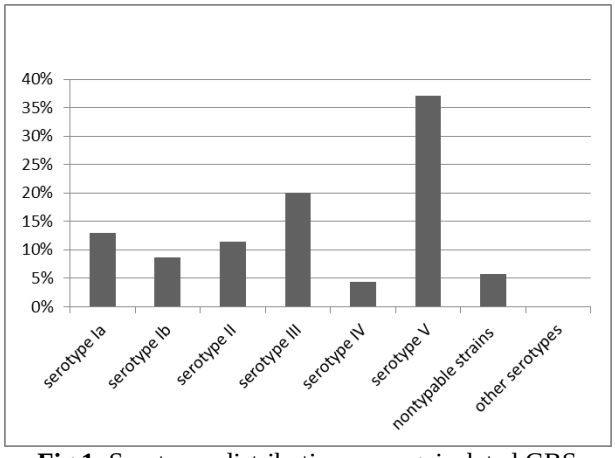

Fig 1: Serotypes distribution among isolated GBS strains

All strains were sensitive to benzylpenicillin, ampicllin, cephotaxim and vancomycin. Sensitivity pattern for clindamycin and erythromycin is shown in fig 2. Resistance to erythromycin and clindamycin was (42.8 & 17.2%) respectively. The Double disk diffusion test detected 12.9% (9/70) strains with inducible macrolide—lincosamide—StreptograminB resistance (iMLSB), 17.1% (12/70) strains with constitutive resistance phenotype (cMLSB), while M phenotype was detected only in 4.3% of the strains (3/70).

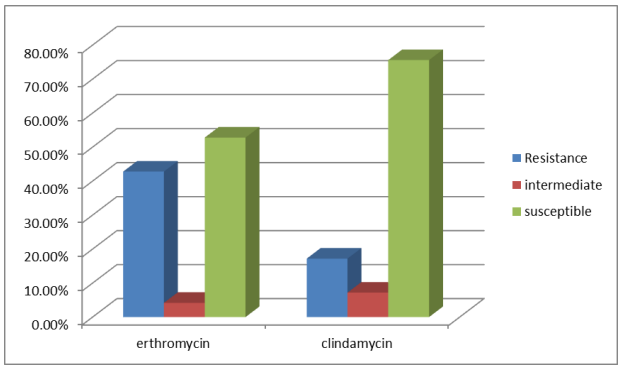

Fig 2: Erythromycin and clindamycin susceptibility profile of the isolated GBS strains

DISCUSSION

S. agalactiae is recognized as a frequent colonizing agent in pregnant women and is an important cause of neonatal sepsis. The rates of GBS-colonized pregnant women range worldwide from 3% to 41%[3]. The aim of this study was to detect the frequency of the *Streptococcus agalactiae* carriage in pregnant females attending Benha university hospital, serotyping of isolated strains and to study their antibiotic susceptibility profile

In the current study rectovaginal GBS colonization was found to be 28%. This prevalence rate is comparable to figures recorded by studies done in other regions from Egypt, in Alexandria (26.5%)[17], from Cairo and Ismalia (29 &25.3%) respectively[18-19].

Studies from other Arabian countries showed different colonization rates Saudi Arabia was (27.6%) [20], United Arab Emirates (10.1%) [21], Kuwait (16.4%)[22], and Tunisia (17%)[23]. In multicenter studies conducted in Netherland, the GBS carriage among African women was 29%, 13% in Asian and 21% in European[24]. Brazilian authors found colonization rates from 5% to 25% in regional studies[10]. The United States and Europe studies reported colonization rates between 6.5% and 36%[25-26].

This worldwide variability is attributed to a well-known fact that maternal GBS colonization is related to different sociocultural, geographic, climatic, biological and methodological determinants and this highlights the importance of individualizing preventive strategies according to the local colonization rates[17].

GBS capsular polysaccharides have chemical and antigenic differences that enable the subdivision of GBS into ten serotypes. The epidemiological distribution of these serotypes varies according to several factors e.g. the geographical region and the population profile being studied. A capsular conjugate vaccines are in clinical trials. Therefore, correct serotyping of clinical isolates is essential to predict vaccine coverage where the common serotypes associated with disease in different populations will be included[27].

Serotype V showed the highest prevalence rate (37.1 %); serotypes III, Ia and II were (20, 12 &11.4%)respectively. This high frequency of serotype V was previously reported by previous studies from Egypt[28], USA, Canada, Zimbabwe, The Gambia, Mynamar, and Australia [29-30].In contrary, for GBS isolates from Saudi Arabia [20] and United Arab Emirates[21] serotype V was the least prevalent serotype.

We reported a frequency of 5.7% for serotype IV which is different from other regional and international reported frequencies this can be explained by the phenomenon of capsular switching and serotype replacement and that GBS seroprevalence is not only dependent on geographical location[31].

Four nontypable strains were reported in the current study. Afshar et al.[14] previously explained nontybable status of GBS by: lack of capsular polysaccharides expression, the expression of undetectable amount of capsular polysaccharides to be detected by the commercially available methods or production of uncharacterized capsular polysaccharide for which typing antibodies are not yet available.

The antibiotic susceptibility profile of the isolated GBS strains is in accordance with previous reports including egyptian studies[32], where all isolated GBS strains were sensitive to benzylpenicillin, ampicllin, cephotaxim and vancomycin

Erythromycin and clindamycin are commonly used in case of allergy to penicillins but the rate of resistance has increased since 1996[32]. Data from the current study showed that 42.8&17.2% of GBS strains are resistant to erythromycin and clindamycin respectively. This coincides within the range of previously reported frequencies (4 to 58.3%) for erythromycin and (2.3% to 57.9%) for clindamycin[33].

In Egypt: Sadaka et al.[17] reported erythromycin and clindamycin to be 22.6 %, and 15% respectively Shabayak et al.[19] found the resistance to erythromycin and clindamycin to be 13.15% and 23.68% respectively

This relatively high resistance rate among GBS isolates in the current study could be explained by the fact that resistance to erythromycin and clindamycin has been associated with serotype V which is the highest isolated serotype. In addition, the increasing resistance to clindamycin points to its increased use for treatment

and prophylaxis of anaerobic infections in dentistry and other clinical settings[34].

Thus when GBS is isolated from pregnant women with penicillin allergy, clindamycin and erythromycin should be tested and reported[15].

Regarding the rate isolation of different resistance phenotypes, Inducible clindamycin resistance (iMLSB) was found in 9/70 (12.9%), and constitutive resistance phenotype (cMLSB) was found in 12/70 (17.1%) of GBS strains. M phenotype was detected only in 3/70 (4.3%) of the strains

The isolation rate of the three phenotypes in previous studies is variable; In Iran Frouhesh-Tehrani et al.[35] reported 9.5% & 10.5% for iMLSB and cMLSB phenotype and 1% for M phenotype. In Egypt 2.6 and 10.5 % [19], 3.8&11.3%[17] were reported for iMLSB and cMLSB phenotype respectively. The prevalence of the M phenotype in other countries is as follows: Canada, 15%; France, 6–7.4%; Spain, 5-9.3%; and Taiwan, 37%[36].

The presence of inducible clindamycin resistance when there is a resistance to erythromycin should be taken in consideration by clinicians[37].

In conclusion, The recorded results about GBS carriage, antimicrobials susceptibility profile would be useful as for implementation of GBS prenatal screening and choice of antibiotics for intrapartum prophylaxsis . The serotyping profile will add to information required to assign protective vaccine and design of a prevention program in Egypt.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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