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Vaginal carriage and antibiotic susceptibility profile of group B *Streptococcus* during late pregnancy in Ismailia, Egypt

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Summary Group B *Streptococcus* (GBS) infection has long been recognized as a frequent cause of morbidity and mortality in newborn infants. The purpose of this study was to determine the colonization rate with GBS and the antibiotic susceptibility profile in pregnant women attending Gynecological clinics in Egypt. One-hundred and fifty vaginal swabs were collected from pregnant women at 35—40 weeks of gestation. In comparison to culture, direct latex agglutination testing revealed 100% sensitivity and 93.75% specificity. Thirty-eight specimens (25.3%) were found to be positive for GBS. Each isolate was tested for susceptibility to penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin and vancomycin. Erythromycin-resistant isolates were further classified by double-disk method. All isolates were susceptible to penicillin G, ampicillin and vancomycin. Resistance to cefotaxime was detected in three isolates (7.89%). Five isolates (13.15%) were resistant to erythromycin and nine isolates (23.68%) were resistant to clindamycin. Four (80%) isolates had constitutive macrolide—lincosamide—Streptogramin$_B$ resistance (cMLSB$_B$) resistance and one (20%) isolate had inducible resistance (iMLS$_B$) resistance. GBS colonization was found to be high in our region. Latex agglutination testing and Islam medium are reliable methods to detect GBS in late pregnancy; however, latex agglutination test is rapid and simpler. Penicillin G remains the first choice antibiotic for treatment of GBS infections.

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Introduction

Streptococcus agalactiae or group B *Streptococcus* (GBS) infection has long been recognized as a frequent cause of morbidity and mortality in newborn infants [1]. Life-threatening complications of GBS bacteremia, such as endocarditis, meningitis and fatal septicemia with multiorgan failure, have been described over the last decades [2].

GBS is found in the vagina and/or rectum of 10—30% of pregnant women as normal flora, and infants born to these women may develop disease due to exposure to bacteria before birth or during the neonatal period [3]. These neonatal GBS infections are divided into early-onset infection and late-onset infection. Early-onset infection, which is the most common type of neonatal GBS disease, occurs within the first week of life, while late-onset infection occurs in infants between 1 week and 3 months of age [4].

Maternal colonization with GBS is the most predominant risk factor for the development of invasive neonatal GBS disease [5], since vertical transmission of GBS before or during delivery can be found in the vast majority of all cases [5]. The revised Centers for Disease Control and Prevention (CDC) guidelines issued in 2002 recommended a culture-based screening for vaginal rectal/colonization with GBS for all pregnant women at 35—37 weeks of gestation for prevention of early-onset GBS disease. If colonization is detected, intrapartum antibiotic prophylaxis is recommended [6]. Implementation of these guidelines has been associated with a falling incidence of neonatal GBS disease in USA [7—9]. No epidemiological data has been published on the colonization rate with GBS of pregnant women in Egypt. The purpose of this study was to determine the current colonization rate with GBS and the antibiotic susceptibility profile in our region.

Materials and methods

Culture and identification procedures

One-hundred and fifty pregnant women (17—43 years) at 35—40 weeks of gestation attending the Gynecological clinic at the Ismailia General Hospital (400 beds) and the Gynecological Department at the University Hospital of Suez Canal University (415 beds) from September 2007 to April 2008 were enrolled in this study. One vaginal swab was collected from each patient with an informed consent, placed in Amies transport medium (Lab M) and kept at 4 °C. Swabs were inoculated in selective enrichment broth medium (Todd-Hewitt broth (oxoid) supplemented with nalidixic acid 15 μg/ml and colistin 10 μg/ml). After 18—24 h incubation at 37 °C in 5% CO_2, broths were subcultured onto Islam medium (Oxoid). Islam media plates were examined for orange pigmented colonies after 24—48 h of anaerobic incubation. In addition, latex agglutination testing (Streptococcal grouping kit, AVIPATH STREP, Omega Diagnostics Ltd.) was performed directly on primary selective broth cultures. Positive selective broth cultures for GBS (either by latex agglutination testing or Islam medium) were further confirmed by the traditional culture method through subculturing onto 5% columbia blood agar (oxoid) supplemented with nalidixic acid 15 μg/ml and colistin 10 μg/ml (CNA medium), incubated for 18—24 h at 37 °C in 5% CO_2. Colonies on CNA plates were identified by Gram stain, hemolysis, catalase, CAMP test (named after Christie, Atkins and Munch-Petersen), hippurate hydrolysis test, and bile esculin hydrolysis test [10,11].

Antibiotic susceptibility testing and macrolide resistance phenotypes

Each isolate was tested for susceptibility to penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin and vancomycin using disk diffusion method according to CLSI (formerly NCCLS) guidelines [12]. Erythromycin-resistant isolates were further classified as having $cMLSB_B$ (constitutive macrolide—lincosamide—$Streptogramin_B$ resistance), $iMLS_B$ (inducible resistance), or M phenotype (macrolide—$Streptogramin_B$ resistance and Lincosamide susceptibility) by double-disk method [12,13].

Results

Colonization rate

Among the 150 patients that were enrolled in the study a total of 38 specimens (25.3%) were found to be positive for GBS by Islam medium. The direct latex agglutination testing on primary selective broth cultures detected 45 (30%) positive specimens.

All specimens found to be positive by culture on Islam medium were also positive by latex agglutination testing. Among the 112 Islam medium negative specimens, 7 were positive by latex agglutination testing. It was noticed that all these seven samples showed positive agglutination testing with

Streptococcus group B and group D. Presumptive identification of *Enterococcus* spp. was achieved using bile esculin test. When these samples were analysed by traditional culturing method, GBS failed to be recovered from primary selective broth cultures and were considered false positive by latex agglutination testing. Thus latex agglutination testing directly on primary selective broth cultures revealed 100% sensitivity and 93.75% specificity.

Antibiotic susceptibility testing and macrolide resistance phenotypes

All the 38 isolates were uniformly susceptible to penicillin G, ampicillin and vancomycin. Resistance to cefotaxime was detected in three isolates (7.89%) while 1 isolate (2.63%) was intermediate. Five isolates (13.15%) were resistant to erythromycin and three (7.89%) were intermediate. Nine isolates (23.68%) were resistant to clindamycin.

Among the erythromycin resistance isolates, four (80%) isolates had constitutive $cMLSB_B$ resistance and one (20%) isolate had inducible $iMLS_B$ resistance.

Discussion

In the present study, the prevalence of GBS in Ismailia, Egypt was found to be 25.3%. Such finding was in accordance with studies from the United States and Europe that reported colonization rates between 6.5% and 36% [14—16,4]. Recent Arabian studies showed similar colonization rates ranging from 10.1% in United Arab Emirates [17], 16.4% in Kuwait [18], 17% in Tunisia [19], and 27.6% in Saudi Arabia [20]. This was consistent with a recent survey by Stoll and Schuchat [21] who demonstrated that despite the geographical variations, GBS colonization rates have similar ranges in developed and developing countries. Different colonization rates during pregnancy may also be attributed to maternal age, parity, ethnicity, marital status, education, smoking and frequent intercourse with multiple partners [22]. According to Badri et al. [23] and Philipson et al. [24] vaginorectal swabs increase the GBS yield by 40—50%, however, vaginal swabs only were involved in the present study and thus the true burden of colonization was underestimated. The GBS colonization rate might have been higher reaching 38% if vaginorectal swabs were used. Considering many obstacles related to cultural believes and ignorance with the importance of GBS screening among pregnant women in Egypt, vaginorectal swabs or rectal swabs were hard to be obtained although vaginal swabs are often taken as a part of routine examination during pregnancy.

Screening for GBS directly on selective enrichment broth cultures by using latex agglutination testing revealed a sensitivity of 100% and specificity of 93.75%. Such sensitivity was higher than those reported by Guerrero et al. [25] and Park et al. [26]. However, the demonstrated specificity was quite similar to that reported by Poilane et al. [27]. The use of latex agglutination test directly on primary broth cultures provided detection of GBS 24—48 h sooner than the culture method using Islam medium. Besides, latex agglutination test is simple, rapid, easy to perform, and does not require special equipments. In contrast to pigment-based culture method using Islam medium which may be cumbersome to use from the standpoint of requiring anaerobic culture conditions.

Concerning the seven GBS latex positive samples with negative subcultures on Islam medium or CNA medium, GBS failed to be recovered from these samples, suggesting the occurrence of a possible antagonistic phenomenon. Park et al. [26] demonstrated that heavy growth of GBS usually lacked concomitant growth or had only very light growth of *Enterococcus fecalis*. Conversely, very light growth of GBS revealed heavy growth of *E. fecalis*. Bourbeau et al. [28] had a similar experience when five of nine GBS specimens failed to multiply in selective enrichment broth. A possible explanation was suggested by Dunne and Holland-Staley [29], who noticed that certain strains of GBS appear to be suppressed by a moderate to heavy growth of *E. fecalis*. Other explanations may include presence of nonviable GBS that could be detected by antigen-detection method but not by culture, as well as inability of culture to detect low bacterial numbers. Antibiotics and feminine hygiene products have also shown to inhibit the detection of GBS by culture but have no detrimental effect on antigenic detection [30]. Inadequate specimen collection and transport from obstetrical clinics to the laboratory may have some effect especially in case of light colonization [31,32].

Susceptibility testing revealed that GBS is uniformly susceptible to penicillin G, ampicillin and vancomycin. Most other studies have found the same [33—36]. Penicillin G is the drug of choice when diagnosis is established. However, an increased resistance to erythromycin and clindamycin, the drugs of choice for women with serious penicillin allergy, has been observed. In this regard, 13.15% of the tested GBS isolates were resistant to erythromycin and 23.68% to clindamycin. Compared to recent Arabian studies,

the present study showed much lower resistance rates to erythromycin and clindamycin than those reported by Dunia et al. in Syria [37]. However, it revealed higher resistance rates to these antibiotics than those reported by Al-Seweih et al. in Kuwait [38]. In regard to recent studies from America and Europe, the present findings were consistent with those reported by Barbaros et al. in Turkey [39], much lower than those reported by DiPersio and DiPersio in USA [40] and higher than those reported by Schoening et al. in Germany [41] and Figueira-coelho et al. in Portugal [42].

The majority of the erythromycin-resistant isolates showed $cMLS_B$ resistance and one had $iMLS_B$ resistance. Quite similar distribution resistance phenotypes were described in studies from Portugal, France and Spain [42—45].

In conclusion, the vaginal GBS colonization rate among Egyptian women was found to be 25.3% and thereby constitutes a group of women whose infants are at great risk of GBS invasive infections. Latex agglutination testing and Islam medium are reliable methods to detect GBS in late pregnancy; however, latex agglutination test is rapid and simpler. Finally, Penicillin G remains the first choice antibiotic for the treatment of GBS infections. The majority of the erythromycin-resistant isolates showed $cMLS_B$ resistance. Continuous monitoring of the occurrence of resistance to antibiotics is essential, as the emergence of resistant strains has become a growing concern on the therapy of GBS. The increasing rates and continued spread of macrolide and lincosamide resistance in GBS have broad implications for both prophylactic and empiric drug treatment strategies.

Conflict of interest statement

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References

[1] Reid TMS. Emergence of Group B Streptococci in obstetric and perinatal infections. Br Med J 1975;2:533—6.
[2] Schrag SJ, Phil D, Zywicki S, Farley MM, Reingold AL, Harrison LH, et al. Group B Streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Eng J Med 2000;342:15—20.
[3] Spellerberg B. Pathogenesis of neonatal *Streptococcus agalactiae* infections. Microbes Infect 2000;2:1733—42.
[4] Schuchat A. Epidemiology of Group B Streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev 1998;11:497—513.
[5] Hickman ME, Rench MA, Ferrieri P, Baker CJ. Changing epidemiology of Group B Streptococcal colonization. Pediatrics 1999;104:203—9.
[6] Centers for Disease Control and Prevention. Prevention of perinatal Group B Streptococcal disease. MMWR 2002;51(RR-11) [inclusive page numbers].
[7] Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, et al. Epidemiology of invasive Group B Streptococcal disease in the United States 1999—2005. JAMA 2008;299:2056—65.
[8] Moore MR, Schrag SJ, Schuchat A. Effects of intrapartum antimicrobial prophylaxis for prevention of Group B Streptococcal disease on the incidence and ecology of early-onset neonatal sepsis. Lancet Infect Dis 2003;3:201—13.
[9] Schrag SJ, Phill D, Zell ER, Stat M, Lynifield R, Roome A, et al. A population-based comparison of strategies to prevent early-onset Group B Streptococcal disease in neonates. N Eng J Med 2002;347:233—9.
[10] Rallu F, Barriga P, Scrivo C, Martel-Laferriere V, Laferriere C. Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for Group B Streptococcus carriage in pregnant women. J Clin Microbiol 2006;44:725—8.
[11] Ross PW. Streptococcus and Enterococcus. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney practical medical microbiology. 4th ed. USA: Churchill Livingstone; 1996.
[12] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; seventh informational supplement. CLSI document, 27(1) (M100-S17). Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2007.
[13] Seppala H, Nissinen A, Yu Q, Huovinen P. Three different phenotypes of erythromycin-resistant *Streptococcus pyogenes* in Finland. J Antimicrob Chemother 1993;32:885—91.
[14] Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal Group B Streptococcal colonisation in European countries. Acta Obstet Gynecol Scand 2008;87:260—71.
[15] Brimil N, Barthell E, Heindrichs U, Kuhn M, Lutticken R, Spellerberg B. Epidemiology of *Streptococcus agalactiae* colonization in Germany. Int J Med Microbiol 2006;296:39—44.
[16] Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Backer CJ. Group B Streptococcal colonization and serotype specific immunity in pregnant women at delivery. Obstet Gynecol 2000;96:498—503.
[17] Amin A, Abdulrazzaq YM, Uduman S. Group B Streptococcal serotype distribution of isolates from colonized pregnant women at the time of delivery in United Arab Emirates. J Infect 2002;45:42—6.
[18] Al-Sweih N, Maiyegun S, Diejomaoh M, Rotimi V, Khodakhast F, Hassan N, et al. *Streptococcus agalactiae* (Group B *Streptococcus*) carriage in late pregnancy in Kuwait. Med Princ Pract 2004;13:10—4.
[19] Ferjani A, Ben Abdallah H, Ben Saida N, Gozzi C, Boukadida J. Vaginal colonization of the *Streptococcus agalactiae* in pregnant woman in Tunisia: risk factors and susceptibility of isolates to antibiotics [abstract]. Bull Soc Pathol Exot 2006;99:99—102.
[20] El-Kersh TA, Al-Nuaim LA, Kharfy TA, Al-Shammary FJ, Al-Saleh SS, Al-Zamel FA. Detection of genital colonization of Group B Streptococci during late pregnancy. Saudi Med J 2002;23:56—61.

[21] Stoll BJ, Schuchat A. Maternal carriage of Group B Streptococci in developing countries [abstract]. Pediatr Infect Dis J 1998;17:499—503.
[22] Benitz WE, Gould JB, Druzin ML. Risk factors for early-onset Group B Streptococcal sepsis: estimation of odds ratios by critical literature review. Pediatrics 1999;103:e77.
[23] Badri MS, Zawaneh S, Cruz AC, Mantilla G, Baer H, Spellacy WN, et al. Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women. J Infect Dis 1977;135:308—12.
[24] Philipson EH, Palermino DA, Robinson A. Enhanced antenatal detection of group B streptococcus colonization. Obstet Gynecol 1995;85:437—9.
[25] Guerrero C, Martinez J, Menasalvas A, Blazquez R, Rodriguez T, Segovia M. Use of direct latex agglutination testing of selective broth in the detection of Group B Streptococcal carriage in pregnant women. Eur J Clin Microbiol Infect Dis 2004;23:61—2.
[26] Park CH, Vandel NM, Ruprai DK, Martin EA, Gates KM, Coker D. Detection of Group B Streptococcal colonization in pregnant women using direct latex agglutination testing of selective broth. J Clin Microbiol 2001;39:408—9.
[27] Poline I, Adam MN, Turlotin JC, Collignon A. Evaluation of a rapid agglutination test for detection of Group B Streptococci in the gastric aspirates of neonates. Eur J Clin Microbiol Infect Dis 1995;14:815—7.
[28] Bourbeau PP, Heiter BJ, Figdore M. Use of gen-probe accuprobe Group B *Streptococcus* test to detect Group B Streptococci in broth cultures of vaginal-anorectal specimens from pregnant women: comparison with traditional culture method. J Clin Microbiol 1997;35:144—7.
[29] Dunne WM, Holland-Staley CA. Comparison of NNA agar culture and selective broth culture for detection of Group B Streptococcal colonization in women. J Clin Microbiol 1998;36:2298—300.
[30] Ostroff RM, Steaffens JW. Effect of specimen storage, antibiotics, and feminine hygiene products on the detection of Group B Streptococcus by culture and the STREP B OIA test [abstract]. Diagn Microbiol Infect Dis 1995;22:253—9.
[31] Rosa-Fraile M, Camacho-Munoz E, Rodriguez-granger J, Liebana-Martos C. Specimen storage in transport medium and detection of Group B Streptococci by culture. J Clin Microbiol 2005;43:928—30.
[32] Stoner KA, Rabe LK, Hillier SL. Effect of transport time, temperature, and concentration on the survival of Group B Streptococci in amies transport medium. J Clin Microbiol 2004;42:5385—7.
[33] Fernandez M, Hickman ME, Baker CJ. Antimicrobial susceptibilities of Group B Streptococci isolated between 1992 and 1996 from patients with bacteremia or meningitis. Antimicrob Agents Chemother 1998;42:1517—9.
[34] Lopardo HA, Vidal P, Jeric P, Centron D, Paganini H, Facklam RR, et al. Six month multicenter study on invasive infections due to Group B Streptococci in Argentina. J Clin Microbiol 2003;41:4688—94.
[35] Mosca A, Russo F, Miragliotta G. In vitro antimicrobial activity of benzalkonium chloride against clinical isolates of *Streptococcus agalactiae*. J Antimicrob Chemother 2006;57:566—8.
[36] Murdoch DR, Reller LB. Antimicrobial susceptibilities of Group B Streptococci isolated from patients with invasive disease: 10 year perspective. Antimicrob Agents Chemother 2001;45:3623—4.
[37] Dunia R, Kowatli K, Abutouk A, Rosa-Fraile M. Epidemiology of Group B *Streptococcus* in pregnant women in Syria. Arab J Pharm Sci 2006;3:81—8.
[38] Al-Sweih N, Jamal M, Kurdia M, Abduljabar R, Rotimi V. Antibiotic susceptibility profile of Group B *Streptococcus* (*Streptopcoccus agalactiae*) at the maternity hospital Kuwait [abstract]. Med Princ Pract 2005;14:260—3.
[39] Barbaros I, Murat C, Mehmet V, Ismet TA, Can K, Sukufe D, et al. The colonization incidence of Group B *Streptococcus* in pregnant women and their newborns in Istanbul. Pediatr Int 2005;47:64—6.
[40] DiPersio LP, DiPersio JR. High rates of erythromycin and clindamycin resistance among OBGYN isolates of Group B *Streptococcus*. Diagn Microbiol Infect Dis 2005;54:79—82.
[41] Schoening TE, Wagner J, Arvand M. Prevalence of erythromycin and clindamycin resistance among *Streptococcus agalactiae* isolates in Germany. Clin Microbiol Infect 2005;11:577—96.
[42] Figueira-Coelho J, Ramirez M, Salgado MJ, Melo-Cristino J. *Streptococcus agalatiae* in large Portuguese teaching hospital: antimicrobial susceptibility serotype distribution and clonal analysis of macrolide-resistant isolates. Microb Drug Resist 2004;10:31—6.
[43] De Mouy D, Cavallo J, Leclercq R, Fabre R. The Aforcopi-Bio Network antibiotic susceptibility and mechanisms of erythromycin resistance in clinical isolates of *Streptococcus agalactiae*: French multicenter study. Antimicrob Agents Chemother 2001;45:2400—2.
[44] Fitoussi F, Doit C, Geslin P, Brahimi N, Bingen E. Mechanisms of macrolide resistance in clinical Pneumococcal isolates in France. Antimicrob Agents Chemother 2000;45:636—8.
[45] Betriu C, Culebras E, Gomez M, Rodriguez-Avial I, Sanchez BA, Agreda MC, et al. Erythromycin and clindamycin resistance and telithromycin susceptibility in *Streptococcus agalactiae*. Antimicrob Agents Chemother 2003;47:1112—4.