Alexandria Journal of Medicine 54 (2018) 69–76

HOSTED BY

Contents lists available at ScienceDirect

Alexandria Journal of Medicine

journal homepage: http://www.elsevier.com/locate/ajme

Group B streptococcal carriage, antimicrobial susceptibility, and virulence related genes among pregnant women in Alexandria, Egypt

Salama Mohamed Sadaka [a], Hala Abdelsalam Aly [a], Marwa Ahmed Meheissen [a,*], Yasser Ibrahim Orief [b], Basma Mohamed Arafa [a]

[a] Medical Microbiology and Immunology Department, Faculty of Medicine, University of Alexandria, Egypt
[b] Obstetrics and Gynecology Department, Faculty of Medicine, University of Alexandria, Egypt

ARTICLE INFO

Article history:
Received 11 November 2016
Accepted 31 January 2017
Available online 21 February 2017

Keywords:
Group B streptococci
Rib gene
Penicillin susceptibility
Vaginal carriage

ABSTRACT

Background and aim: Group B Streptococcus (GBS) has emerged as a leading cause of illness and death among neonates. The study was conducted to estimate the prevalence of recto-vaginal carriage of GBS among pregnant women at 35–37 weeks, gestation, to describe GBS antimicrobial susceptibility profile and to investigate selected virulence genes by PCR.

Subjects and methods: Two-hundred pregnant women at 35–37 weeks of gestation attending antenatal clinic at Al-Shatby University Hospital were enrolled in the study. Both vaginal and rectal swabs were collected from each subject. Swabs were inoculated onto CHROMagar™ StrepB and sheep blood agar plates. All GBS isolates were subjected to antimicrobial susceptibility testing using disc diffusion. Disc approximation test was performed to detect erythromycin resistance phenotype (MLSB). GBS virulence genes *scpB*, *bac*, *bca*, and *rib* were identified by PCR.

Results: Among the 200 pregnant women, 53 (26.5%) were identified as GBS carriers. All carriers had vaginal colonization (100%), four (7.5%) had combined recto-vaginal colonization. None of the carriers had rectal colonization alone. All isolates (100%) were susceptible to penicillin, ampicillin, ceftriaxone, cefotaxime, cefepime, vancomycin, and linezolid. On the other hand, 43.4%, 28.3%, 22.6%, and 15% of isolates were resistant to levofloxacin, azithromycin, erythromycin, and clindamycin respectively. Out of 12 erythromycin resistant isolates, six isolates had constitutive while two had inducible MLSB resistance. *scpB* was identified in 100%, *rib* in 79.2%, and *bac* in 35.8% of GBS isolates. None of the isolates possessed the *bca* gene.

Conclusion: Introduction of GBS screening in Egyptian pregnant women is recommended. Penicillin or ampicillin is still the antibiotic of choice for intrapartum prophylaxis.

1. Introduction

Streptococcus agalactiae (group B streptococcus; GBS) is one of the leading causes of neonatal sepsis and meningitis. GBS neonatal disease is classified as either early-onset disease (<7 days) or late-onset disease (>7–90 days). Early-onset GBS disease (EOD) is mainly caused by vertical transmission of GBS from colonized mothers to their infants during labor or delivery. Approximately 10–40% of pregnant women are colonized with GBS, and the incidence of EOD is 0.3–2 per 1000 live births in different geographical areas.[1]

GBS is also associated with preterm labor or membrane rupture, as well as urinary tract infections, postpartum endometritis, postpartum wound infection, septic pelvic thrombophlebitis and endocarditis in females.[2]

In 1996, the Centers for Disease Control and Prevention (CDC) published consensus guidelines for the prevention of neonatal GBS disease; these guidelines were revised in 2002 then in 2010.[1]The revised guidelines recommended the screening of all pregnant women between 35 and 37 weeks of gestation for vaginal and rectal colonization with GBS. Further, the guidelines recommended intrapartum antibiotic prophylaxis (IAP) for colonized pregnant women.

It has been shown that the screening approach and IAP rather than the identification of maternal clinical risk factors for early-onset neonatal GBS disease are more effective in preventing

Peer review under responsibility of Alexandria University Faculty of Medicine.
* Corresponding author at: Faculty of Medicine, Kartoum Square, Azarita, Alexandria, Egypt.
E-mail address: marwa.meheissen@alexmed.edu.eg (M. Ahmed Meheissen).

http://dx.doi.org/10.1016/j.ajme.2017.01.003
2090-5068/

EOD.[3]In developed countries prenatal screening in pregnant women and IAP have been widely established and successfully reduced the incidence of GBS neonatal disease.[1,4]In low-income settings screening and IAP for prevention of invasive neonatal disease are mostly not implemented due to limitations in resources and infrastructure.[5]

GBS remain fully susceptible to penicillin as well as to most β-lactams.[6–9]However, there are some worrisome reports on reduced penicillin susceptibility in GBS.[10,11]On the other hand, alternative antibiotics are administrated for pregnant women with penicillin allergy, such as clindamycin, erythromycin, or vancomycin.[1] Increased resistance rates to clindamycin and to erythromycin were reported in different parts of the world including reports from Egypt.[9,12–16]

The severity of neonatal disease in GBS infections could be determined mostly by a number of virulence factors encoded. The capsular polysaccharides have been identified as a major virulence factor. Other factors thought to be associated with virulence include the surface-associated C proteins, α and β, encoded by the bca and bac genes, respectively, which participate in adherence, invasion of cervical epithelial cells, as well as resistance to phagocytosis, and the Rib protein encoded by the rib gene which has been found in significant percentage of GBS strains which caused invasive infections in neonates.[17–19]These putative virulence factors have been investigated as possible vaccine candidates because of their ability to elicit protective immunity against GBS infections.[20,21]

In Egypt, there is no national guideline for systematic screening or prophylaxis of GBS in pregnant women. This was the motivation for conducting this study. The objective of the study was to determine the prevalence of GBS carriage among pregnant women at 35–37 weeks of gestation, to evaluate the antimicrobial susceptibility pattern of colonizing GBS isolates, and to investigate selected GBS virulence genes.

2. Subjects and methods

This cross-sectional observational study included 200 pregnant women at 35–37 weeks of gestation attending antenatal clinic at Al-Shatby University Maternity Hospital, Alexandria, Egypt. From all study subjects two swabs, one rectal and one vaginal were collected. For rectal specimens, a swab was carefully inserted approximately 2.5 cm beyond the anal sphincter and then gently rotated to touch anal crypts. For vaginal specimens, excessive secretions or discharge were wiped away, and a swab was taken from the mucosa of the lower third of the vagina without using a speculum as per CDC recommendations.[1]

Exclusion criteria:

- Pregnant women who were on antibiotic treatment 2 weeks prior to recruitment.
- History of complicated previous or current pregnancy (abortion, premature rupture of membranes, premature delivery).
- Women with urinary tract infection or vaginal infection in the current pregnancy.

The study was approved by the research ethics committee of Alexandria University Hospital.

2.1. Demographic data collection

Demographic data was recorded, including the patient's age, gestational age, number of pervious pregnancies, number of antenatal visits, contraceptive method used.

2.2. Specimen collection and transport

- Each swab (vaginal or rectal) was inoculated directly onto each of chromogenic agar plate; CHROMagar™ StrepB (CHROMagar microbiology, France) and sheep blood agar plates (Oxoid, UK), just after sample collection.[22]Then swabs were dipped into 5 ml of selective enrichment broth; Lim Broth (Todd Hewitt, Oxoid, supplemented with 5% sheep blood with colistin (10 μg/ml) and nalidixic acid (15 μg/ml).

The inoculated plates and broths were transported immediately in ambient temperature to the Microbiology Laboratory of Alexandria Main University Hospital (AMUH) to be processed according to the recommendations of CDC.[1]

2.3. Specimen processing

- The inoculated CHROMagar™ StrepB and blood agar plates were incubated aerobically at 35–37 °C for 18–24 h in 5% CO_2.
- The inoculated selective broth was incubated for 18–24 h at 35–37 °C in 5% CO_2, then was subcultured onto CHROMagar™ StrepB, and sheep blood agar and incubated aerobically at 35–37 °C for another18–24 h in 5% CO_2.
- After incubation period, plates were examined. The suspected GBS colonies on CHROMagar™ StrepB (mauve colonies) and on blood agar (b-haemolytic and non-haemolytic) were picked and subcultured onto sheep blood agar and incubated aerobically at 35–37 °C for 18–24 h in 5% CO_2. Isolates were identified using conventional methods on the basis of colonial morphology, Gram staining, haemolysis, CAMP test, bile esculin, hippurate hydrolysis (Liofilchem, Italy), and latex agglutination test with specific antisera (Strepto B latex kit, Liofilchem, Italy).[23] (Fig. 1).
- The isolated identified strains were stored in Todd- Hewitt broth along with 15% glycerol at −70 °C until tested. The negative culture results were issued at 72 h, according to the CDC recommendations.

2.4. Antimicrobial susceptibility testing

- The susceptibilities of GBS isolates to different antimicrobial agents were tested using modified Kirby Bauer disc diffusion method. The following antimicrobial discs and concentrations were selected according to CLSI guidelines: Penicillin (P), 10 units; ampicillin (AMP), 10 μg; ceftriaxone (CRO), 30 μg; cefotaxime (CTX), 30 μg; cefepime (FEP), 30 μg; erythromycin (E), 15 μg; azithromycin (AZM), 15 μg; clindamycin (DA), 2 μg; tetracycline (TC), 30 μg; vancomycin (VA), 30ug; linezolid (LZD), 30ug; levofloxacin (LEV), 5 μg (Oxoid). *Streptococcus pneumoniae* ATCC 49619 was used as quality control strain as recommended by the CLSI.[24]
- Erythromycin-resistant isolates were further classified as having cMLSB (constitutive macrolide—lincosamide—StreptograminB resistance), iMLSB (inducible resistance), or M phenotype (macrolide—StreptograminB resistance and Lincosamide susceptibility) by double-disc approximation method. Blunting was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disc, indicating MLSB inducible methylation. Resistance to both erythromycin and clindamycin indicated MLSB-constitutive methylation. Resistance to erythromycin but susceptibility to clindamycin without blunting indicated an efflux mechanism (M phenotype).[25]

2.5. Molecular test (PCR)

DNA extraction from bacteria was performed by the method described by Schmitz et al.[26]The supernatant (three uL) was used

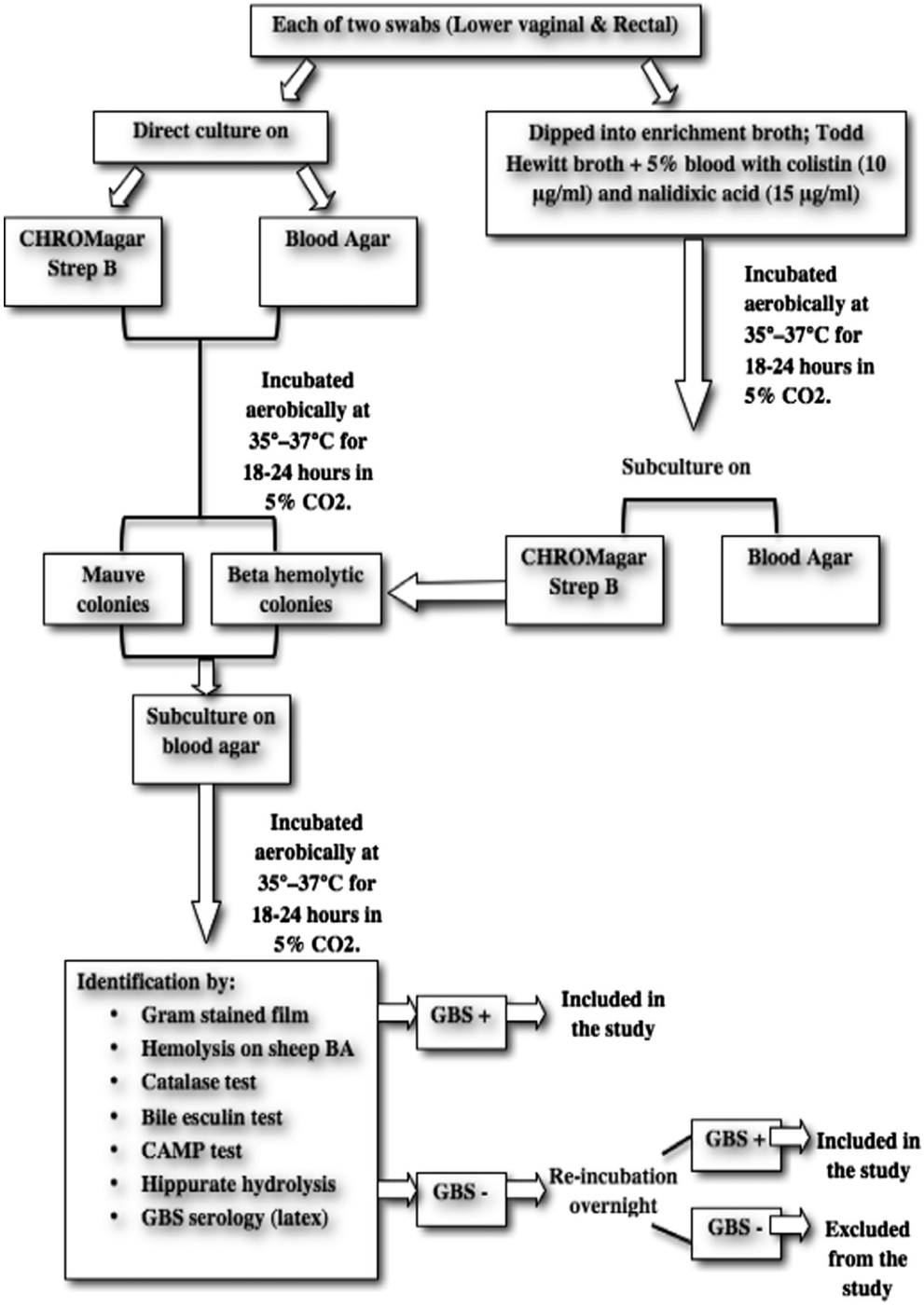

Fig. 1. Flowchart of laboratory processing of vaginal and rectal swabs for isolation and identification of GBS in the study.

as a template in the PCR reaction. Four separate PCR reactions were done. Each PCR reaction consisted of 12.5 µL MyTaq Red Mix, 2× (Bioline, UK), 25 pmol of primers, and PCR grade water to a final volume 25 µl. The sets of primers (Invitrogen by Life Technologies, Thermo Fisher Scientific Inc., USA) for the detection of genes encoding immunoglobulin A-binding β-antigen (bac), α-antigen (bca), C5a peptidase (scpB), and rib are listed in Table 1.

The conditions of the amplification reaction were: an initial denaturation step at 95 °C for 2 min, 35 amplification cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s, extension at 72 °C for 60 s, followed by a final cycle of extension at 72 °C for

Table 1
Primer sequences used in the study.

Gene	Primer sequence	bp
scpB	scp F: ACAACGGAAGGCGCTACTGTTC scp R: ACCTGGTGTTTGACCTGAACTA	255
bac	bac F: TGT AAA GGA CGA TAG TGT GAA GAC bac R: CAT TTG TGA TTC CCT TTT GC	530
bca	bca F: CAG GAG GGG AAA CAA CAG TAC bca R: GTA TCC TTT GAT CCA TCT GGA TAC G	183
rib	rib F: CAG GAA GTG CTG TTA CGT TAA AC rib R: CGT CCC ATT TAG GGT TCT TCC	369

10 min. Negative extraction and master mix controls were included in every reaction.[27,28]

2.6. Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean, standard deviation. Significance of the obtained results was judged at the 5% level.

3. Results

3.1. Prevalence

- Among the 200 pregnant asymptomatic women at 35–37 weeks of gestation, **53 (26.5%)** were identified as GBS carriers. Vaginal colonization was shown in all carriers (53; 100%). Out of the 53 vaginal carriers, four (7.5%) had combined recto-vaginal colonization. None of the carriers had rectal colonization only.

3.2. Demographic data results

- The mean age of pregnant women carriers for GBS was (25.4 ± 2.9). GBS colonization was common mostly in the age group (20–30) and this finding was statistically significant (p = 0.01). GBS colonization was statistically significantly higher at 37 weeks of gestation (p = 0.002), among women who had frequent antenatal visits (p=<0.001), and who adopted the condom contraceptive method (p=<0.001). On the hand, the prevalence of GBS colonization was high among the multigravida but this was not statistically significant (p = 0.296). The results of socio-demographic characteristics are summarized in Table 2.

3.3. Culture results

- Detection of GBS from vaginal and rectal specimens by direct plating onto CHROMagar strepB or blood agar was equally sensitive as detection after selective broth enrichment. The turnaround time was 18–24 h when using the direct plating, versus 48 h after selective broth enrichment. On CHROMagar strept B; all mauve colonies were confirmed to be GBS isolates. On the other hand, not all beta hemolytic colonies or small non-hemolytic catalase negative colonies proved to be GBS on sheep blood agar where it required more effort for picking and isolation of GBS colonies.

3.4. Antibiotic susceptibility results

- None of the isolates was resistant to penicillins such as penicillin and ampicillin. Furthermore, all isolates were susceptible to ceftriaxone, cefotaxime, cefepime, vancomycin, and linezolid. On the other hand, 23 (43.4%), 15 (28.3%), 12 (22.6%), and 8 (15%) isolates were resistant to levofloxacin, azithromycin,

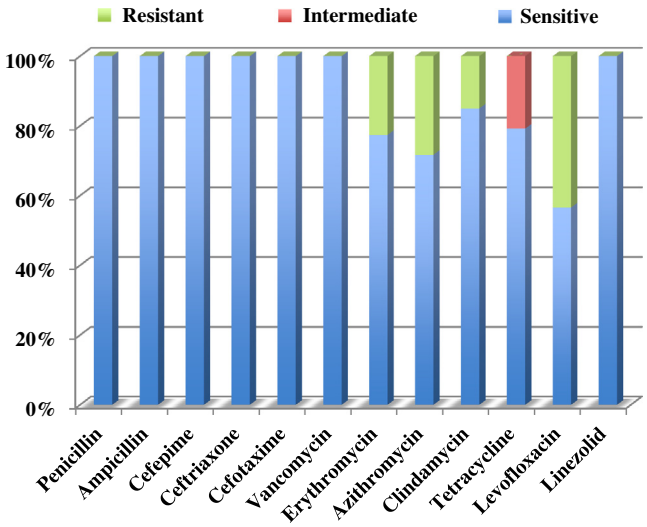

Fig. 2. The antibiotic sensitivity pattern of GBS carrier isolates.

Table 2
The socio-demographic factors associated with GBS colonization in the 200 pregnant women.

	Total n = 200	GBS positive N = 53		P value
		No	%	
Mean age[*]	26.02 ± 4.72	25.43 ± 2.90		0.467
<20	12	1	8.3	0.010
20–30	155	49	31.6	
>30	33	3	9.1	
Gestational age				
35 w	54	7	13.0	0.002
36 w	55	11	20.0	
37 w	91	35	38.5	
Number of previous pregnancies				
Primigravida	64	20	31.3	0.296
Multigravida	136	33	24.3	
Number of antenatal visits				
4 times	6	–	–	<0.001
5 times	37	7	18.9	
6 times	59	23	39.0	
7 times	56	23	41.1	
8 times	30	–	–	
9 times	12	–	–	
Contraceptive method used				
Condom	39	26	66.7	<0.001
Loop	57	7	12.3	
Oral contraceptives	3	–	–	
None	101	20	19.8	

[*] Age is presented in mean ± SD.

erythromycin, and clindamycin respectively. Eleven isolates (20.8%) of GBS were intermediately sensitive to tetracycline (Fig. 2).

- Concerning double disc approximation test for detection of erythromycin resistance phenotype, cMLSB mechanism was found in 50% (6/12) of erythromycin resistant isolates, while, iMLSB resistance was detected in two isolates (2/12; 16.7%). M phenotype was reported in four isolates (4/12; 33.3%).

3.5. PCR results

- All isolates possessed *scpB* gene (100%). The presence of *rib* gene was confirmed in 42 isolates (79.2%), bac in 19 isolates (35.8%). *ScpB*, *rib* and *bac* were identified in 19 isolates (35.8%). Twenty-three isolates (43.4%) were *scpB*, *rib* positive and *bac* negative. None of the isolates possessed the *bca* gene. A significant proportion (23 isolates; 43.3%) had the *scpB*, and *rib* genes simultaneously. The presence of these two genes characterized the prevalent virulence profile among our isolates. Nineteen isolates (35.8%) contained three virulence genes (*scpB*, *rib*, *bac*), while 11 isolates (20.7%) were positive only for a single gene (*scpB* gene) (Table 3, Fig. 3).

The virulence genes *scpB & rib* were detected in 15/15 (100%) and 4/15 (26.7%) of azithromycin-resistant isolates, respectively, in 12/12 (100%), and 4/12 (33.3%) of the erythromycin-resistant GBS isolates, respectively, and in 8/8 (100%), and 4/8 (50%) of the clindamycin- resistant isolates, respectively. While, the *scpB*, *rib*, and *bac* genes were identified in 23/23 (100%), 11/23 (47.8%), 8/23 (34.8%) of the levofloxacin resistant isolates. The *bac* gene was not detected in any of the erythromycin, azithromycin or clindamycin resistant isolates (Table 4).

Table 3
The frequency of virulence genes (*scpB*, *bac*, *bca* and *rib*) among GBS isolates.

Virulence genes	No	%
scpB	53	100
rib	42	79.2
bac	19	35.8
bca	0	0
scpB+/rib−/bac−/bca−	11	20.7
scpB+/rib+/bac−/bca−	23	43.4
scpB+/rib+/bac+/bca−	19	35.8

4. Discussion

In the present study, the rate of GBS among pregnant women was 26.5%, which is in agreement with other Egyptian studies[13,29] and higher than the rates reported in others.[30,31]According to a review article published in 2010, which consists of 76 original articles, the results demonstrated that Middle East are accounted for high risk countries for GBS colonization.[32]This worldwide variability is related to different sociocultural, geographic, climatic, biological and methodological determinants and this highlights the importance of individualizing preventive strategies according to local colonization rates.

Our results demonstrated that most GBS positive pregnant women were from 20 to 30 years old which is in accordance with previous studies conducted in Africa.[33,34]On the contrary, other

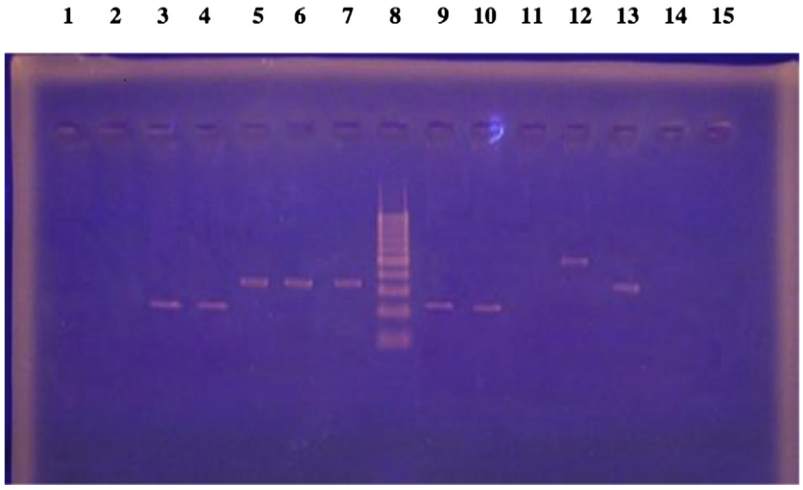

Fig. 3. Agarose gel electrophoresis of PCR of GBS virulence genes. Lane 8 = DNA ladder. Lane 1, 2, 11 = Negative samples. Lane 3, 4, 9, 10 = scpB positive isolates (255 bp). Lane 5, 6, 7, 13 = rib positive isolates (369 bp), Lane 12 = bac positive isolate (530 bp). Lane 14, 15 = Negative controls.

Table 4
The distribution of GBS isolates resistant to each antibiotic according to virulence genes.

Virulence factor	No of isolates resistant to each antibiotic (%)			
	Azithromycin	Erythromycin	Clindamycin	Levofloxacin
scpB gene	15/15 (100%)	12/12 (100%)	8/8 (100%)	23/23 (100%)
rib gene	4/15 (26.7%)	4/12 (33.3%)	4/8 (50%)	11/23 (47.8%)
bac gene	0	0	0	8/23 (34.8%)
bca	0	0	0	0
scpB+/rib−/bac−/bca−	11/15 (73.3%)	8/12 (66.7%)	4/8 (50%)	4/23 (17.4%)
scpB+/rib+/bac−/bca−	4/15 (26.7%)	4/12 (33.3%)	4/8 (50%)	11/23 (47.8%)
scpB+/rib+/bac+/bca−	0	0	0	8/23 (34.8%)
Total	15	12	8	23

studies reported increase in GBS positivity as the age increases.[10,35]The results of the current study found that GBS colonization is significantly higher among pregnant women who are at 37th week of gestation, who had frequent antenatal visits, and who use the condom as a contraception method. Our findings are not in agreement with those from other studies[33,36,37] that reported no significant differences in the GBS colonization rates when the sociodemographic and clinical obstetric variables including the risk factors for the newborn to develop infection were considered.

Taking into consideration that we did not take combined vagino-rectal swabs using the same swab, we can assume that combined vagino-rectal swabs would have been positive with all positive GBS cases in this study and that only vaginal swab is enough for screening of GBS. Our results are not in accordance with CDC recommendations to carry out rectovaginal sampling for GBS screening and are also different from many other studies that found rectovaginal sampling more appropriate than vaginal sampling only.[1,38]However, it should be mentioned that Nomura et al.[39]found no significant difference in detection rates between vaginal and rectal samples. Votava et al.[40]even found that the GBS detection rate using rectovaginal samples was only 16.9%, whereas the use of separate vaginal and rectal swabs yielded 22.7 and 24.1% GBS positive women, respectively. Gupta & Briski[41]- reported that although rectovaginal swabs are reported to increase the yield of GBS, this finding was not supported by their study. Also, several obstetric departments still use vaginal sampling only to assess GBS carriage.[38]The low count and often weakly hemolytic colonies of GBS within the large diverse population of rectal flora may explain the negative rectal samples in our study.

In the present study, the sensitivity of direct plating was comparable to that after selective TH broth enrichment. Similarly, El Aila et al.[38]reported comparable sensitivity of direct plating on chromID™ Strepto B agar (CA) and group B streptococcus differential agar (GBSDA) to that of plating on CA and GBSDA after Lim broth enrichment, whereby the latter enabled the detection of only one additional sample.

Although blood agar remains the agreed common reference for GBS culture, our results differed from the previous studies[42,43]as CHROMagar™ StrepB showed a similar performance as blood agar, however, the effort done to isolate suspected GBS colonies from blood agar was much bigger. The CHROMagar strepB allowed direct visual inspection of GBS colonies within 24 h. Moreover, the selection and picking of colonies was much easier (mauve colonies). It also supported growth of all GBS strains and produced typical colonies whatever their haemolytic properties in an aerobic environment.

Using the disc diffusion test, all tested isolates in this study were susceptible to penicillins and ampicillin, indicating that they would be effective as the first-line agents for IAP in our settings. No resistance was also reported for cefotaxime, ceftriaxone, cefepime, vancomycin and linezolid. Similar findings have been reported for GBS strains around the world including reports from Egypt.[6–9,13]On the other hand, the very high level of penicillin and ampicillin resistance (100%) was alarming when reported in Nigeria.[10]The authors justified this high rate by the ease of procurement of antibiotics in the developing country, the frequent use of antibiotics for therapy and prophylaxis, and other socio-economic factors.

Conversely, some women with penicillin allergy require other antibiotics for GBS prophylaxis such as clindamycin, erythromycin, or vancomycin, which are recommended in the CDC guidelines.[1]All isolates in the present study were susceptible to vancomycin. However, 22.6% and 15% of the tested isolates were resistant to erythromycin and clindamycin, respectively. Thus susceptibility testing should be performed before administering erythromycin or clindamycin in order to ensure activity against the isolate. Our results are slightly different from another study conducted in Egypt, which reported 13.15% resistance to erythromycin and 23.68% to clindamycin.[13]Resistance has been reported between 4 to 58.3% and 2.3% to 57.9% for erythromycin and clindamycin respectively in published literatures.[9,12–16]Consequently, the revised CDC guidelines in 2010[1]excluded erythromycin from the recommended alternative antibiotics.

In our study, cMLSB erythromycin resistant phenotype was found in six isolates (50%) of the 12 erythromycin non-susceptible isolates. While, 16.7% (2/12) of isolates showed iMLSB phenotype. Our results are in agreement with studies from Egypt, Portugal, and France[13,44,45], which reported that the majority of the erythromycin-resistant isolates showed cMLSB resistance. Therefore, in GBS isolates that are not susceptible to erythromycin, resistance to clindamycin should be suspected in about 2/3 of cases. The resistance of 66.7% (8 strains) of GBS strains to both antibiotics makes their use impractical as prophylactic antibiotic for pregnant GBS carriers in our hospitals. The M phenotype was detected in 33.3% (4/12) in the present study, but its occurrence has been reported among human GBS isolates at low rates.[28]

Some authors suggest that the presence of the *scpB* gene in human isolates is mandatory.[46,47]Others detected scpB in 97% of human isolates[28], but generally it is thought that only strains possessing scpB gene are infective for humans.[18]The scpB gene was detected in all isolates in the current study.

In the present study, *bac* gene was detected in 17 isolates (35.8%), It is generally a low rate when compared to a previous study.[18] On the other hand, Corrêa et al.[48] and Durate et al.[28] reported that none of their GBS carried the *bac* gene. Concerning the rib gene, it was unexpectedly detected in a very high percentage of our isolates; 42 isolates representing 79.2%. This was not consistent with a previous study conducted in Egypt which reported a rate of only 13%,[29] also in the study of Monica et al.[18], the rib gene was found in 35% of isolates. Previous studies noted that *bca* and *rib* genes were not present concomitantly in the same genome.[49,50] Rib protein encoded by the *rib* gene shares several properties with α-C protein encoded by *bca* gene. Both proteins are resistant to trypsin digestion and belong to the same family of bacterial surface proteins with repetitive structures showing a 47% identity, their N- terminal sequences are related and are 61% identical to each other. These properties suggest that both proteins may have a common origin.[51] This observation may explain the negative *bca* gene results in the present study. In addition, the detection of *rib and bac* genes in 79.2, 35.8%, respectively, of the isolates in this study suggests that a GBS vaccine containing these proteins would be less effective against Egyptian population.

All azithromycin, erythromycin, clindamycin and levofloxacin resistant GBS isolates in this study (100%) carried the *scpB* gene. While none of the azithromycin, erythromycin and clindamycin resistant isolates carried the *bac* gene. This finding could be of help when designing the vaccine.

The most important limitation of our study was the inability to follow up the positive GBS culture pregnant women to determine the rate of premature rupture of membranes and infantile infection. Another limitation is that serotyping of GBS was not done because of lack of antisera due to financial constraints. Knowledge about the prevalent serotypes in a given country is very important in development and implementation of effective vaccine.

5. Conclusion

The establishment of carriage status in pregnant women for GBS calls for a review of the present hospital policy to include rou-

tine screening and reporting of GBS colonization among pregnant women during antenatal visits. Rectal swab does not confer a clear benefit; so vaginal swab is enough for screening. Penicillin or ampicillin is still the antibiotic of choice for IAP. However, erythromycin or clindamycin susceptibility testing is still mandatory in case of penicillin allergy.

While the knowledge of the most prevalent virulence factors of GBS isolates from a given geographical area is essential to trace the epidemiological course of infections, surveillance of antimicrobial-resistance is relevant to guiding the design of more appropriate procedures for infection control and prevention. Moreover, further studies should be conducted to identify the most prevalent serotypes among Egyptian GBS isolates to guide in the design of appropriate vaccine.

6. Conflicts of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease. *Morb Mortal Wkly Rep*. 2010;19:1e36.
2. Murray PR, Rosenthal KS, Pfaller MA. *Medical microbiology*. 5th ed. USA; 2005: 247–50.
3. Schrag S, Phil D, Zell ER, Stat M, Lynfield R, Roome A. A population based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *N Engl J Med*. 2002;347:233–239.
4. Lopez SJB, Fernandez CB, Coto CGD, Ramos AA. Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. *Acta Paediatr*. 2005;94:451.
5. Capan M, Mombo-Ngoma G, Akerey-Diop D, et al.. Epidemiology and management of group B streptococcal colonization during pregnancy in Africa. *Wien Klin Wochenschr*. 2012;124:14.
6. Garland SM, Cottrill E, Markowski L, et al.. Antimicrobial resistance in group B streptococcus: the Australian experience. *J Med Microbiol*. 2011;60:230–235.
7. Dhanoa A, Karunakaran R, Puthucheary SD. Serotype distribution and antibiotic susceptibility of group B streptococci in pregnant women. *Epidemiol Infect*. 2010;138:979–981.
8. Murayama SY, Seki C, Sakata H, et al.. Capsular type and antibiotic resistance in *Streptococcus agalactiae* isolates from patients, ranging from newborns to the elderly, with invasive infections. *Antimicrob Agents Chemother*. 2009;53:2650–2653.
9. Allam AA, Bahgat MA. Phenotype and genotype of some clinical Group B streptococcus isolates resistant to erythromycin in Egypt. *Egypt J Med Microbiol*. 2006;15:71–77.
10. Onipede A, Adefusi O, Adeyemi A, Adejuyigbe E, Oyelese A, Ogunniyi T. Group B Streptococcus carriage during late pregnancy in Ile-Ife, Nigeria. *Afr J Clin Exp Microbiol*. 2012;13:135–143.
11. Kouji K, Noriyuki N, Yukiko N, et al.. Predominance of sequence type 1 group with serotype VI among group B streptococci with reduced penicillin susceptibility. *J Antimicrob Chemother*. 2011;66(11):2460–2464.
12. Shabayek S, Abdalla S. Macrolide- and tetracycline-resistance determinants of colonizing group B streptococcus in women in Egypt. *J Med Microbiol*. 2014;63:1324–1327.
13. Shabayek SA, Abdalla SM, Abouzeid AM. Vaginal carriage and antibiotic susceptibility profile of group B Streptococcus during late pregnancy in Ismailia. *Egypt J Infect Public Health*. 2009;2(2):86–90.
14. Abdelmoaty T, Wafaa Z, Kawthar M. Prevalence and antibiotic susceptibility of anogenital group B streptococci colonization in pregnant women. *Egypt J Med Lab*. 2009;18(2):105–111.
15. Joachim A, Matee MI, Massawe FA, Lyamuya EF. Maternal and neonatal colonization of group B Streptococcus at Muhimbili national hospital in Dares Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health*. 2009;9:437.
16. Quiroga M, Pegels E, Oviedo P, Pereyra E, Vergara M. Antibiotic susceptibility patterns and prevalence of Group B Streptococcus isolated from pregnant women in Misiones, Argentina. *Braz J Microbiol*. 2008;39:245–250.
17. Dore N, Bennetti D, Kaliszer M, Cafferkey M, Smyth CJ. Molecular epidemiology of group B streptococci in Ireland: associations between serotype, invasive status and presence of genes encoding putative virulence factors. *Epidemiol Infect*. 2003;131:823–833.
18. Lysakowska ME, Kalinka J, Bigos M, Prosniewska M, Wasiela M. Occurrence of virulence genes among S. agalactiae isolates from vagina and anus of pregnant women – a pilot study. *Arch Perin Med*. 2011;17:229–234.
19. Baron MJ, Filman DJ, Prophete GA. Identification of a glycosaminoglycan binding region of the alpha C protein that mediates entry of group B streptococci into host cells. *J Biol Chem*. 2007;282:10526–10536.
20. Jennings HJ. Capsular polysaccharides as vaccine candidates. *Curr Top Microbiol Immunol*. 1990;150:97–127.
21. Maione D, Margarit I, Rinaudo CD, et al.. Identification of a universal Group B streptococcus vaccine by multiple genome screen. *Science*. 2005;309:148–150.
22. Blanckaert H, Frans J, Bosteels J, Hanssens M, Verhaegen J. Optimisation of prenatal group B streptococcal screening. *Eur J Clin Microbiol Infect Dis*. 2003;22:619–621.
23. Tille PM. *Bailey & Scott's Diagnostic Microbiology*. 13th ed. Philadelphia, PA, USA: Mosby Elsevier; 2013.
24. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth Informational Supplement. CLSI Document M100-S25*, vol. 35. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2015: 3.
25. Desjardins M, Delgaty KL, Ramotar K, Seetaram C, Toye B. Prevalence and mechanisms of erythromycin resistance in group A and group B Streptococcus: implications for reporting susceptibility results. *J Clin Microbiol*. 2004;42:5620–5623.
26. Schmitz FJ, Steiert M, Tichy HV, Hofmann B, Verhoef G. Typing of methicillin resistant Staphylococcus aureus isolates from Dusseldorf by six genotypic methods. *J Med Microbiol*. 1998;47:341–351.
27. Manning SD, Ki M, Marrs CF, et al.. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. *BMC Infect Dis*. 2006;6:116.
28. Duarte RS, Bellei BC, Miranda OP, Brito MA, Teixeira LM. Distribution of antimicrobial resistance and virulence-related genes among Brazilian group B streptococci recovered from bovine and human sources. *Antimicrob Agents Chemother*. 2005;49:97–103.
29. Shabayek S, Abdalla S, Abouzeid AM. Serotype and surface protein gene distribution of colonizing group B streptococcus in women in Egypt. *Epidemiol Infect*. 2014;142:208–210.
30. Wali IE, Sorour AE, Abdalla MAH. Assessment of different methods for detection of Group B Streptococci carriage among pregnant females. *Egypt J Med Microbiol*. 2007;16:593–598.
31. Elbaradie SMY, Mahmoud M, Farid M. Maternal and neonatal screening for Group B streptococci by SCP B gene based PCR: a preliminary study. *Ind J Med Microbiol*. 2009;27:17–21.
32. Valkenburg-van den Berg AW, Houtman- Roelofsen RL, Oostvogel PM, Dekker PJ, Dörr PJ, Sprij AJ. Timing of group B streptococcus screening in pregnancy: a systematic review. *Gynecol Obstet Invest*. 2010;69:174–183.
33. Mohammed M, Asrat D, Woldeamanuel Y, Demissie A. Prevalence of group B Streptococcus colonization among pregnant women attending antenatal clinic of Hawassa Health Center, Hawassa, Ethiopia. *Ethiop J Health Dev*. 2012;26:36–42.
34. Mavenyengwa RT, Afset JE, Schei B, et al.. Group B Streptococcus colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstet Gynecol Scand*. 2010;89:250–255.
35. Donbraye-Emmanuel O, Okonko I, Donbraye E, et al.. Isolation and characterization of Group B Streptococci and other pathogens among pregnant women in Ibadan, Southwestern Nigeria. *J Appl Biosci*. 2010;5902:1781–1792.
36. Castellano-Filho DS, da Silva VL, Nascimento TC, de Toledo Vieira M, Diniz CG. Detection of Group B Streptococcus in Brazilian pregnant women and antimicrobial susceptibility patterns. *Braz J Microbiol*. 2010;41:1047–1055.
37. Jones N, Oliver K, Jones Y, Haines A, Crook D. Carriage of group B streptococcus in pregnant women from Oxford, UK. *J Clin Pathol*. 2006;59:363–366.
38. El Aila NA, Tency I, Claeys G, et al.. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. *BMC Infect Dis*. 2010;10:285.
39. Nomura ML, Passini Junior R, Oliveira UM. Selective versus non-selective culture medium for group B streptococcus detection in pregnancies complicated by preterm labor or preterm-premature rupture of membranes. *Braz J Infect Dis*. 2006;10:247–250.
40. Votava M, Tejkalova M, Drabkova M, Unzeitig V, Braveny I. Use of GBS media for rapid detection of group B streptococci in vaginal and rectal swabs from women in labor. *Eur J Clin Microbiol Infect Dis*. 2001;20:120–122.
41. Gupta C, Briski LE. Comparison of two culture media and three sampling techniques for sensitive and rapid screening of vaginal colonization by group B streptococcus in pregnant women. *J Clin Microbiol*. 2004;42:3975–3977.
42. Poisson DM, Chandemerle M, Guinard J, Evrard ML, Naydenova D, Mesnard L. Evaluation of CHROMagar StrepB: a new chromogenic agar medium for aerobic detection of Group B Streptococci in perinatal samples. *J Microbiol Meth*. 2010;82:238–242.
43. Charron J, Demandion E, Laudat P. *Détection rapide par culture de Streptococcus agalactiae dans les prélèvements génitaux sur un nouveau milieu chromogène CHROMagar StrepB. Poster 5508*. Paris: Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse; 2009.
44. 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;10:31–36.

45. De Mouy D, Cavallo J-D, Leclercq R, Fabre R. Antibiotic susceptibility and mechanisms of erythromycin resistance in clinical isolates of Streptococcus agalactiae: French multicenter study. *Antimicrob Agents Chemother*. 2001;45:2400–2402.
46. Franken C, Haase G, Brandt C, et al.. Horizontal gene transfer and host specificity of beta-haemolytic streptococci: the role of a putative composite transposon containing scpB and lmb. *Mol Microbiol*. 2001;41:925–935.
47. Dmitriev A, Suvorov A, Shen AD, Yang YH. Clinical diagnosis of group B streptococci by scpB gene based PCR. *Indian J Med Res*. 2004;119:233–236.
48. Corrêa AB, Oliveira IC, Pinto Tde C, Mattos MC, Benchetrit LC. Pulsed-field gel electrophoresis, virulence determinants and antimicrobial susceptibility profiles of type Ia group B streptococci isolated from humans in Brazil. *Mem Inst Oswaldo Cruz*. 2009;104:599–603.
49. Hannoun A, Shehab M, Khairallah MT, et al.. Correlation between group B streptococcal genotypes, their antimicrobial resistance profiles, and virulence genes among pregnant women in Lebanon. *Int J Microbiol*. 2009. 2796512.
50. Kong F, Gowan S, Martin D, James G, Gilbert GL. Molecular profiles of group B streptococcal surface protein antigen genes: relationship to molecular serotypes. *J Clin Microbiol*. 2002;40:620–626.
51. Wastfelt M, Stalhammar-Carlemalm M, Delisse AM, Cabezon T, Lindahl G. Identification of a family of streptococcal surface proteins with extremely repetitive structure. *J Biol Chem*. 1996;271:18892–18897.