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Full Length Research Paper

Phenotypic traits and comparative detection methods of vaginal carriage of Group B streptococci and its associated micro-biota in term pregnant Saudi women

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Group B streptococcus (GBS) is a significant cause of serious infections in neonates and the composition of the vaginal micro-biota also affect neonatal outcome. To investigate vaginal carriage of Group B streptococcus in 217 term Saudi pregnant women, the direct antigen detection method in selective enrichment broth, Todd-Hewitt broth with 8 µg/ml gentamicin and 15 µg/ml nalidixic acid, using 2 commercial kits was used and compared to Centers for Disease Control and Prevention, CDC-gold standard method where following enrichment conventional means for identifying GBS is through isolation on subculture to blood agar plates, which requires 72 h before a final result is achieved. Phenotypic traits of Group B streptococcus serotypes distribution, antibiotic susceptibility patterns, biofilm formation and its associated vaginal micro-biota were also studied. The CDC-gold standard method positivity rate of Group B streptococcus was 23%, with no significant correlation with any of the studied demographic factors. In comparison, direct antigen detection method in boiled selective enrichment broth growth revealed 52.2 and 100% sensitivity and 100% specificity after growth amplification for 4 and 18 h incubation, respectively. Of 50 Group B streptococcus isolates, serotype II was the most predominant (42%), followed by serotype IV (16%). No resistance to ampicillin, cefepime, ceftriaxone or vancomycin was found, but 10, and 6%, of the strains were resistant to erythromycin and clindamycin, respectively. Overall 54% of Group B streptococcus strains were biofilm producers, but (100%) among serotype Ib, III, or V. Of several coexistent, *Escherichia coli and Enterococcus feacalis* were the most frequently Group B streptococcus -associated organisms, but with no statistical correlation.

Key words: Group B Streptococcus, GBS-serotypes, antibiotics susceptibility, GBS-biofilm, vaginal micro-biota, *Escherichia coli, Enterococcus feacalis*.

INTRODUCTION

Infection by *Streptococcus agalactiae* (Group B Streptococcus, GBS) is still the most frequent cause of neonatal diseases, pneumonia, septicemia, and meningitis, early onset Group B streptococcal diseases (EOGBSD) occurring within neonates aged less than 7 days of life (Tazi et al., 2008; Verani et al., 2010). The composition of the human vaginal microflora is affected by several host factors, including, black race, Hispanic ethnicity, age, high parity, health care workers, high Body Mass Index, chronic diseases as diabetes, sexual activity, pregnancy and the use of contraceptives, antibiotics, or spermicides, as well as individual habits such as antiseptic-douching hygiene (Stapleton et al.,

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2005; El Aila et al., 2009; Khan et al., 2011). None of these risk factors, however, would certainly be predictive of positive pregnant women with GBS colonization. It is noteworthy that women with one of these risk factors but who have negative prenatal screening cultures are at relatively low risk for neonatal EOGBSD compared with women who were colonized prenatally but had none of the risk factors (Verani et al., 2010). The overall impact of these factors, might at least, explain the discrepancy among reported incidence of GBS –maternal colonization worldwide (6.5 to 36%), as well as to type specimens and methods used for its detection. On the other hand, GBS persistent colonization may possibly be correlated with its ability of biofilm-formation which correlates with a specific pili formation but varies among GBS serotypes (Kaur, 2009; Rinaudo et al., 2010).

The Centers for Disease Control and Prevention, (CDC) recommends universal bacteriological screening of mothers for vaginal and rectal GBS colonization at 35 to 37 weeks of gestation, followed by selective intrapartum antibiotic prophylaxis for all screen-positive women, is the strategy currently recommended to reduce incidence of colonization in neonates and to prevent EOGBSD (Tazi et al., 2008; Verani et al., 2010). Due to these measures, incidence of neonatal GBS sepsis has decreased from 2.2/1000 to 0.4/1000 births after chemoprophylaxis (Elbaradie et al., 2009; Khan et al., 2011). The current CDC-gold standard method, (GSM) for GBS detection is incubation in a STHB [Todd-Hewitt broth with 8 µg/ml gentamicin and 15 µg/ml /ml nalidixic acid (SPML–K.S.A)] followed by subculture onto a blood agar plate as, direct plating of swabs yields false-negative culture results in as many as 50% of GBS colonized women (Verani et al., 2010). Although widely utilized, this procedure may require up to 72 h before a final result is achieved. Therefore several alternative methods have emerged with the goal of reducing laboratory costs and decrease turnaround time while improving sensitivity and specificity (Rallu et al., 2006; Tazi et al., 2008; Peltroche-Llacsahuanga et al., 2010; Verani et al., 2010; Poisson et al., 2011). Regardless of the method selected to identify GBS, the use of an enrichment broth improves detection substantially, but lengthens the time to obtain a final result. However, for antenatal testing, the accuracy of results is much more important than timeliness (Towers et al., 2010; Verani et al., 2010).

In Saudi Arabia, King Khalid University Hospital (KKUH) is an 800 bed facility with all general and sub-specialty medical services. The hospital provides primary, secondary care services for Saudi patients and King Saud University staff. It also provides tertiary care services to all Saudi citizens on referral bases. In KKUH the CDC strategy of screening all term pregnant women was approved and routinely implemented only recently (2010). Accordingly, in the attempt to enrich the local contemporary laboratory data in Saudi Arabia regarding the current (GBS) colonization, the present study deals with direct antigen detection method (DADM) in STHB of vaginal carriage of GBS, and its prevalence correlation to demographic factors, its phenotypic traits of serotypes distribution, antibiotic susceptibility patterns, biofilm formation as well as its associated vaginal micro-biota among term pregnant Saudi women.

MATERIALS AND METHODS

Subjects

The Current study was conducted at KKUH, Riyadh, Saudi Arabia. Low vaginal swabs were collected by a health care provider, from a total of 217 consecutive healthy term (>35-37 weeks) pregnant Saudi women visiting antenatal outpatient clinic at KKUH, Riyadh, from September 2010 through March 2011. The study was approved by KKUH Ethical–committee, and a written informed consent and questioner for the different characteristics was filled up and taken from each of all women who agree to participate in the study.

Vaginal swabs, culture and growth media

Vaginal swabs were collected circumferentially from the distal third of the vagina without using speculum. Amies Agar Gel-TS (MLM-K.S.A.) was used as a transport medium for vaginal specimens to be cultured (Fraile et al., 2005).Upon receipt of specimens in the laboratory, within 4 h of collection, one low vaginal swab-specimen taken from each subject was directly streaked (one side) onto a surface dried MacConcky agar (MLM-K.S.A.) plate, incubated aerobically at 37°C for 24 h (to recover GBS-associated possible Gram negative bacteria),then the same swab was transferred into STHB followed by subculture on non selective sheep blood agar (MLM-K.S.A.), to inhibit Gram negative bacteria (Mandell et al., 2005) and to recover GBS and other associated Gram positive organisms including yeasts. In parallel, the direct GBS antigen co-agglutination detection using 2 commercially kits (Mastastrep latex co-gglutination test, MAST diagnostic, MAST Group ltd. Bottle, UK), and Phadebact (Phadebact streptococcus test, Boule diagnostic, AB, Huddings, Sweden), was also performed on a boiled [to exclude false positive mostly due to possible resistant coliform bacteria (El-Kersh et al., 2002)] part of growth- amplified in STHB after both 4 and 18 h period of incubation to asses heavily and lightly GBS-colonization in comparison to the GSM, sub-cultured on none-selective sheep blood agar incubated aerobically for 24 h at (37°C) with CO_2 (5%). Typical colonies suggestive for GBS with or without b-haemolysis were identified by Gram staining, negative catalase reaction, growth without aesculin hydrolysis on bile-aesculin agar and positive standard Christie-Atkins-Munch-Petersen (CAMP) reaction and hippurate hydrolysis (Winn et al., 2006). Negative plates were re-incubated for an additional 24 h before being discarded. GBS identity confirmation was completed by group B-specific latex agglutination (Mastastrep and Phadebact test). A positive result was recorded when a co-agglutination visible to the naked eye was formed within one minute. In parallel all GBS possible associated gram negative organisms growing on MacConkey agar plates and Gram positive organisms growing on blood agar subculture plates were also purified and isolated by sub-culturing on respective media for confirmatory testing. Biochemical standard methods and appropriate API kits were used for identification of all isolates to the species level parallel with standard lab references strains.

GBS-serotyping

GBS-Serotyping was determined by slide co-agglutination with bacterial suspension from fresh growth on blood agar and the specific antisera of the available polysaccharide antigens of Types Ia, Ib, II, III, IV and V (Denka Seiken Co, Ltd. Tokyo, Japan). Test was performed as previously described (Al-Huseini et al., 2000).

The susceptibility testes

All 50 GBS strains were tested for their in vitro susceptibility against 9 antibiotics on Muller Hinton agar (Oxoid, UK) supplemented with 5% sheep RBCs(SPML–K.S.A), using antibiotic disk diffusion method. Susceptibility was performed, and results interpreted according to Clinical Laboratory Standard Institute CLSI guidelines. Each strain had its susceptibility classified as sensitive, intermediate or resistant to each one of these antibiotics (Morales et al., 1999; Clinical and Laboratory Standards Institute (CLSI), 2007; Fu et al., 2004). The antibiotic tested were, 10 µg Ampicillin, 30 µg Cefepime, 30 µg ceftriaxone, 2 µg clindamycin, 15 µg erythromycin, 10 µg gentamicin, 300 µg nitrofurantoin, 30 µg Tetracycline and 30 µg vancomycin. All antibiotic disks were obtained from Oxoid ltd, Basingstoke. Hampshier, England.

Biofilm assay

Each of the 50 GBS strains was grown in Brain Heart infusion broth (Oxoid, UK) under static conditions at 37°C 18 h, then culture was diluted to 1:200 in fresh broth. An aliquot of 200 µl of the resulted suspension was transferred into 96 well tissue culture plates (flat bottom). Four plates were made; one was used for growth assessment and the other 3 were used to take the average reading of biofilm formation. The plates were incubated for 48 h at 37°C under 5% CO_2. For the growth assessment plate growth was assessed by measuring the absorbance at 490 nm (A_{490}). For the other 3 microtitter plates; the planktonic cells were aspirated, and the wells were washed thrice with sterile saline. The plates were then inverted on filter paper and were allowed to dry for 1 h at room temperature. For biofilm quantification, 200 µl of 0.25% aqueous crystal violet solution (Sigma, USA) was added to each well, and the plate was allowed to stand for 15 min at room temperature. The wells were subsequently washed thrice with sterile saline to wash off excess crystal violet. A volume of 230 µl of 95 % ethanol was added to each well and absorbance was measured at 550 nm (A_{550}) (Kaur et al., 2009). The experiment was repeated in all of the three plates. As a control, the background crystal violet binding was measured for wells exposed only to the medium with no bacteria, and the background optical density (OD) was subtracted from the OD values of test wells. Those values above 0.2 were considered as high biofilm producers. Values below 0.081 were categorized into low or non-biofilm producers. OD values between 0.081-0.2 were taken as moderate biofilm producers (Head and Yu, 2004; Upadhyaya et al., 2011).

RESULTS

The 217 pregnant females where screened for GBS, and 23% of them were found to be colonized with GBS. To study the effect of demographic factors on GBS carriage, the pregnant females were grouped according to the presence or absence of GBS (Table 1). Mean age between the two groups was 28 years old, while the mean week of gestation was 36 weeks and the mean Body Mass Index (BMI) was 22 in both groups. As statistically analyzed by SPSS statistical package software version 17.0 and the student T-test for independent groups; results indicated that the mean of age, week of gestation and BMI between the two groups was with no statistical difference with P value >0.05 (Table 1). The effect of other culture source and patients characteristics illustrated in Table 1 was also studied in both groups. The Chi-square test, and Fisher's exact test where used and P value was always greater than 0.05.indicating that the rate of GBS colonization was found to be 23%, regardless of age, medical condition (including diabetes), number of abortion, parity, BMI, or level of education (Table 1). Of the 217 pregnant females evaluated in the study, 23% showed a positive culture for GBS from the subculture of THSB on blood agar (GSM). Likewise 23% were positive for GBS by rapid antigen testing of the mixed enriched culture of THSB after 24 h incubation. While only 12% specimens were positive for GBS by rapid antigen testing of STHB after 4 h incubation. It is noteworthy that the specimens that gave positive DADM after 4 h incubation of STHB had heavy growth of GBS in the subculture of GSM. While the ones that showed positive DADM only after 18 h incubation of STHB had very scant growth of GBS on the blood agar plate subculture. Results of DADM after only 4 h incubation seems to indicate that over 50% of our positive GBS subjects are heavily colonized with GBS but lightly GBS colonization was only detected after 18 h incubation. As tested on a boiled part of STHB, both kits used for DADM after 4 h incubation has a sensitivity of 52.2%, and a specificity of 100%. For the same test the negative predictive value was 87.5%, the positive predictive value was 100%. While the same test have a sensitivity of 100% and specificity of 100% after 18 h incubation (Table 2). Of the total 50 GBS strains isolated, serotype II was the most commonly encountered (42%), followed by serotype IV and Ia which accounted of 16 and 12%, respectively. While serotype III constituted only 8% of the strains as did serotype Ib (8%), but serotype V was rarely (4%) encountered (Figure 1). The obtained results on the susceptibility profiles of the tested 50 GBS isolates revealed that all strains were sensitive to ampicillin, cefepime, ceftoriaxon, nitrofurantoin and vancomycin. On the other hand the majority of the GBS strains were resistant to tetracycline (92%). Also some resistance was seen toward erythromycin and clindamycin, 10 and 6%, respectively (Figure 2).

Out of the 50 GBS strain tested, 54% formed biofilm (Figure 3). Photographs for phase contrast images of GBS in the microtitter plate is shown in Figure 4.The level of biofilm formation was found to be variable amongst the GBS strains. Attempt to find a correlation between a biofilm formation and serotype of GBS; revealed that 100% of isolates with serotype Ib, III and V were biofilm-forming strains. While 50% of GBS with serotype Ia and IV were biofilm forming stains. The percent of biofilm

Table 1. Demographic characteristics of pregnant Saudi female and their relation with GBS colonization. SPSS statistical package software version 17.0 was used to measure P value. The table shows the total number of female with the specific characteristic, the number with characteristic and GBS colonization and the percentage. Also the P value is shown.

Characteristic	Total no of women with characteristic	No of women with GBS	%	P value
Tooth condition				
Good	141	30	21.3	0.462
Not Good	76	13	17	
Gum Condition				
Gingivitis	9	4	44.4	0.079
No gingivitis	208	39	18.75	
Medical condition				
None	191	40	20.9	0.612
Yes	26	4	15.4	
Abortion				
None	172	35	20.34	0.835
Yes	45	8	17.8	
No of born children				
None	92	13	14.1	0.071
One or more	125	30	24	
BMI				
Under weight	48	4	8.3	0.100
Normal	116	28	24.13	
Overweight	36	8	22.2	
Obese	17	2	11.76	
Level of education				
None	9	2	22.2	0.061
Elementary	37	2	5.4	
High school	61	17	27.9	
Collage degree	110	22	20	

Table 2. Sensitivity, specificity, and predictive Value of direct latex co-agglutination from lim broth both after 4 and 24 h of incubation, respectively.

Type of the test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Rapid antigen testing from lim broth after 4 h enrichment	52.2	100	100	87.5
Rapid antigen testing from lim broth after 24 h enrichment	100	100	100	100

forming strain for serotype II and the nontypeable were 38 and 40%, respectively (Table 3).

The present study also revealed that only 9% GBS isolates were isolated solely, without association with any other organism(s). While 56.5% were associated with one organism, prominently *E. coli*. The recovery of two or

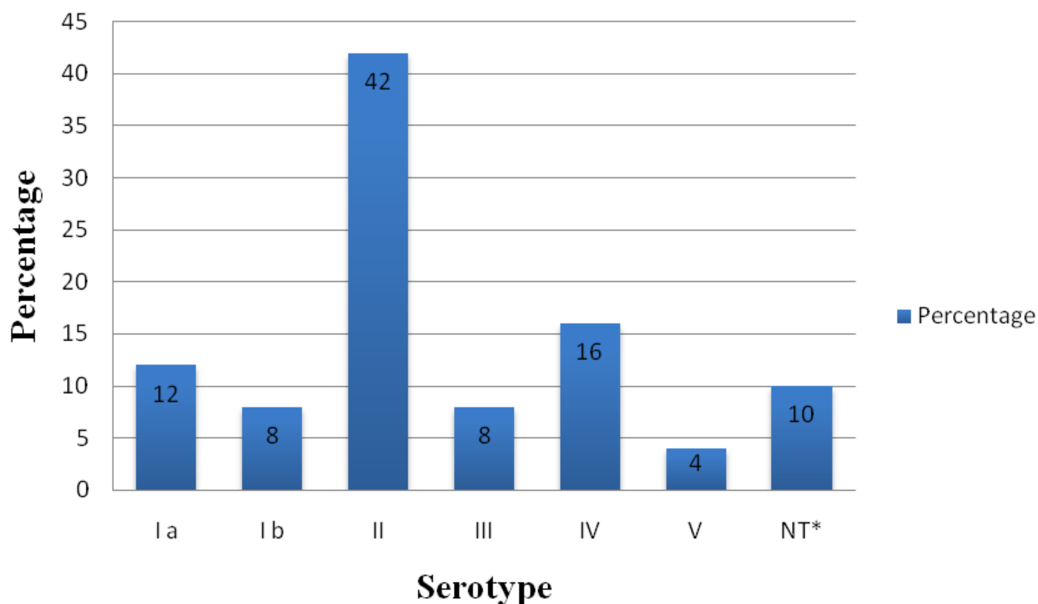

Figure 1. Serotype distribution of GBS isolated from Saudi pregnant female at later pregnancy.

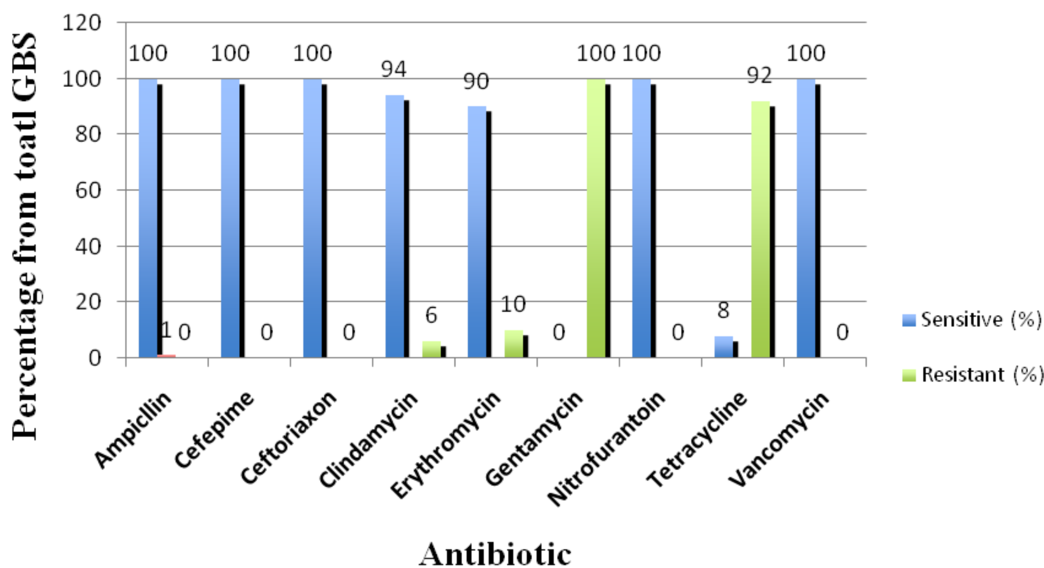

Figure 2. Distribution of GBS isolates according to susceptibility to various antimicrobial agents tested.

more organisms in association with GBS accounted for 17.4 and 17.4%, respectively (Figure 5). Proximity Matrix analysis was done to find the relationship between GBS and associated organisms. Figure 6 shows a dendrogram that was made using average linkage between groups (Hierarchical Cluster analysis, Cluster Method: Nearest neighbor, Binary Squared Euclidean Distance). From the dendrogram it was clear that there is proximity between GBS and *E. coli.*

DISCUSSION

GBS is present worldwide and the prevalence of GBS carriers varies in relation to the geographical area and/or a number of demographic factors *per se*. Accordingly, information of the epidemiological situation of a defined area is crucial to implement a screening program and to evaluate the cost-effectiveness of such a strategy. In this study, the prevalence rate of GBS colonization in late

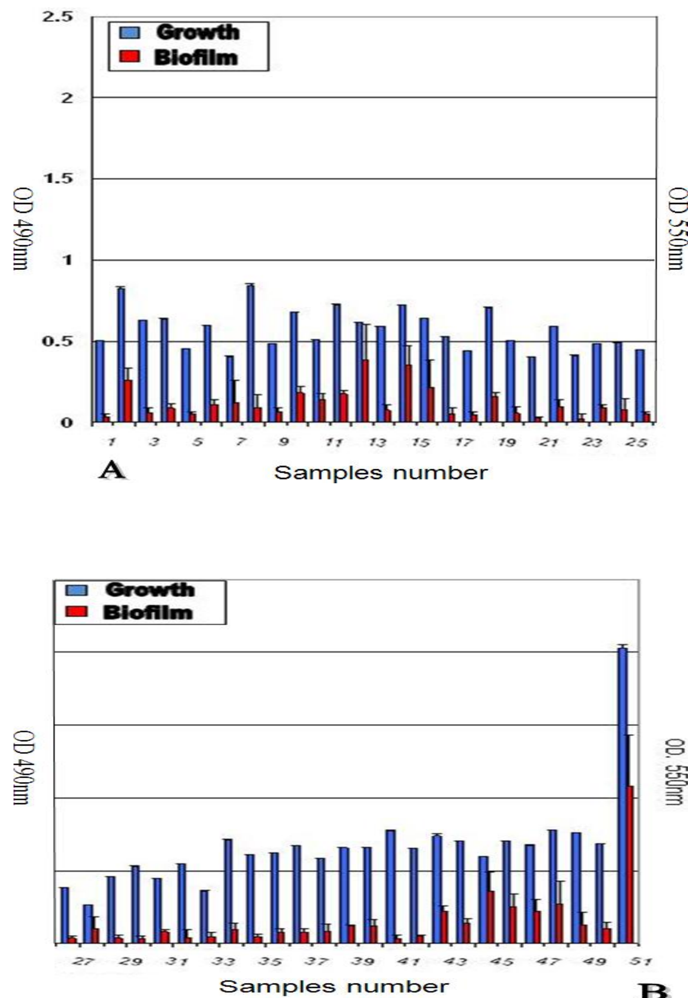

Figure 3 (A and B). The blue line represents the absorbance of growth wells read at (490 nm). And the red line represents the absorbance of crystal violet stained wells read at (550 nm). The sample code (1-50) represent the (50) GBS isolates, and sample code 51 represent the control organism (Staphylococcus epidermidis ATCC 12228). Strong biofilm formation can be seen in sample code (2, 13, 15, 16, 43, 45, 46, 47 and 48).

pregnancy was 23% regardless of any of the studied demographic factors illustrated in Table 1.

These findings are consistent with those previously reported from Iran (Akhlaghi et al., 2009), India (Dechen et al., 2010), Nigeria (Donbraye-Emmanuel et al., 2010), Netherlands (Valkenburg-van et al., 2006), as well as Jeddah, Saudi Arabia (Milyani and Abu, 2011). However, Akhlaghi et al. (2009) and Milyani and Abu (2011) reported a significantly high GBS colonization rate among diabetic pregnant–women. This discrepancy is most likely due to sampling sites and/or to the limited number of diabetic pregnant-women enrolled in this study. This holds also true for the reported increased GBS carriage with increased age and/ or multi-parity (El-Kersh et al., 2002; Aali et al., 2007). Stoll and Schuchat (1998) reported that maternal GBS colonization rates in developing countries ranged from 12 to 22%. In Europe, GBS vaginal colonization rates ranged from 6.5 to 36%, with one third of the studies reporting rates of 20% or greater (Barcaite et al., 2008). Therefore in comparison, the magnitude of 23% positive GBS obtained in this study is relatively high and thereby constitutes a group of women whose infants are at great risk of EOGBSD which conceivably justify and strengthen the recently adopted CDC screening program at our institute KKUH Riyadh.

This conclusion is compatible with that previously drawn by Eisenberg et al. (2006) in Israel in 2006, who observed a significantly higher maternal colonization rate of 13.7 vs. 5.4% previously found in 1984; therefore the authors recommended routine prenatal screening for GBS in their perinatal population. A noteworthy that in the present study we tested only vaginal specimen-cultures,

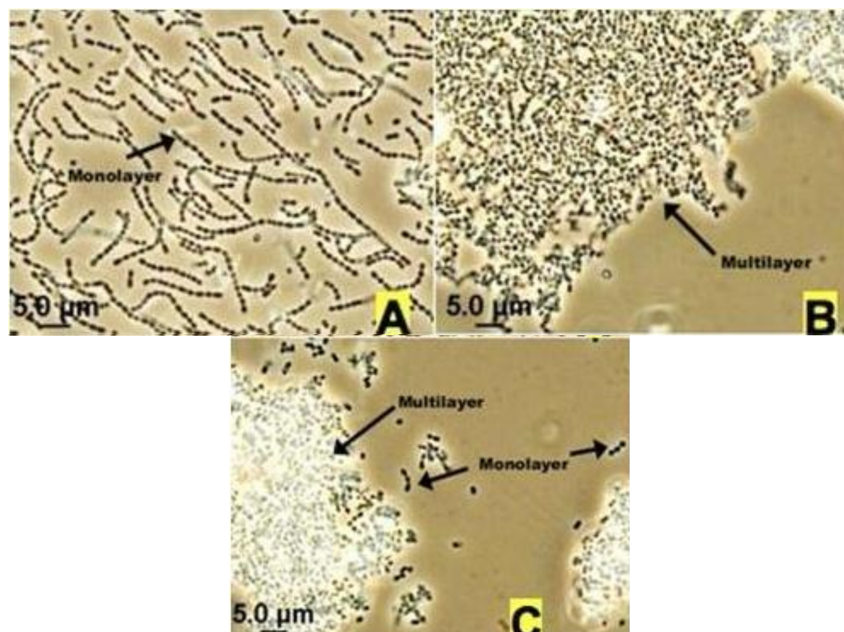

Figure 4. Phase contrast image of GBS after incubation at 30°C in microtiter plate for 48 h. (A) Only monolayer of cells seen without formation of biofilm. (B) Multilayer of GBS is seen which indicates biofilm formation. (C) Both monolayer and multilayer of cells seen.

Table 3. Distribution of different serotypes among the biofilm and non-biofilm forming GBS isolates.

Serotype	Number of biofilm forming GBS	Number of non-biofilm forming GBS	% of biofilm forming strains
Serotype Ia	3	3	50
Serotype Ib	4	0	100
Serotype II	8	13	38
Serotype III	4	0	100
Serotype IV	4	4	50
Serotyep V	2	0	100
Nontypeable	2	3	40

hence the observed GBS colonization rate of 23% would have been increased by at least 18% by the inclusion of also rectal cultures (that is, reaching > 27% as previously found by El-Kersh et al. (2002), since several previous reports indicated that GBS colonization of rectal samples is 18 to 24% higher than that of vaginal samples (El Aila et al., 2010). However, Nomura et al. (2006) found no significant difference in detection rates between vaginal and rectal samples and Gupta and Briski (2004) reported a similar detection rate of 23.8% of GBS when using rectovaginal and vaginal sampling. Votava et al. (2001) 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. Interestingly, Acikgoz et al. (2003) found that about 5.74% of GBS-carriers were only detected from cervico-vaginal samples directly inoculated onto nonselective human blood agar. Also according to El Aila et al. (2010) several obstetric departments still use vaginal sampling only to assess GBS positivity, probably to avoid discomfort of the experienced pain during rectal swabbing (Orafu et al., 2002; Jamie et al., 2004). The findings of the current study confirm the previous ones of Park et al. (2001), El-Kersh et al. (2002) and Rallu et al. (2006) that DADM of GBS was found to be as sensitive and specific as the CDC- GSM, and moreover, the assay is a fast, reliable, reproducible, and cost effective method that is not labor intense and is easy to perform in all obstetrical centers which have access to the most basic of diagnostic microbiology services. In the present study 2 factors apparently contributed to our observed high specificity

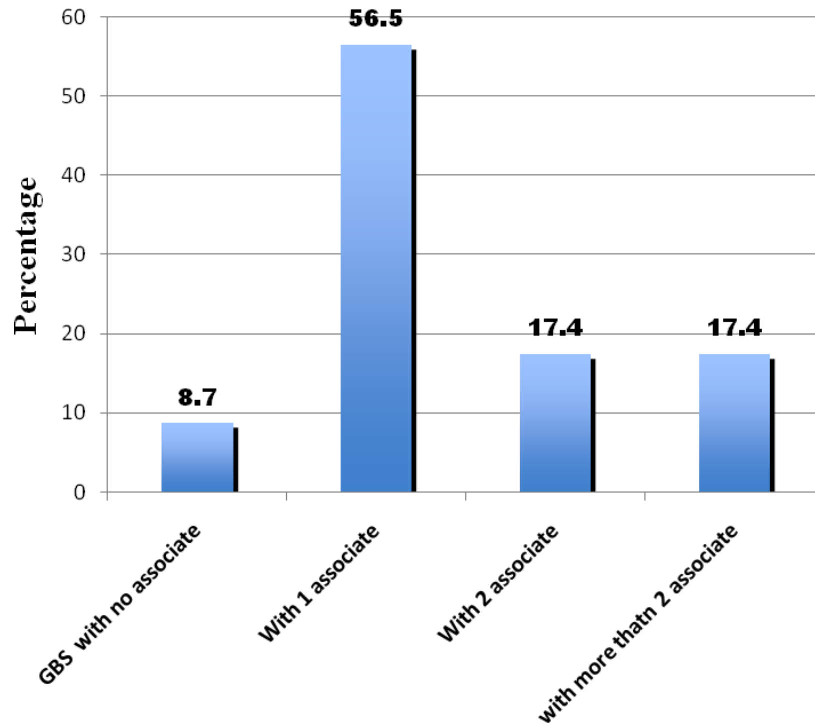

Figure 5. Association of GBS with other organisms isolated from Saudi pregnant female in late pregnancy.

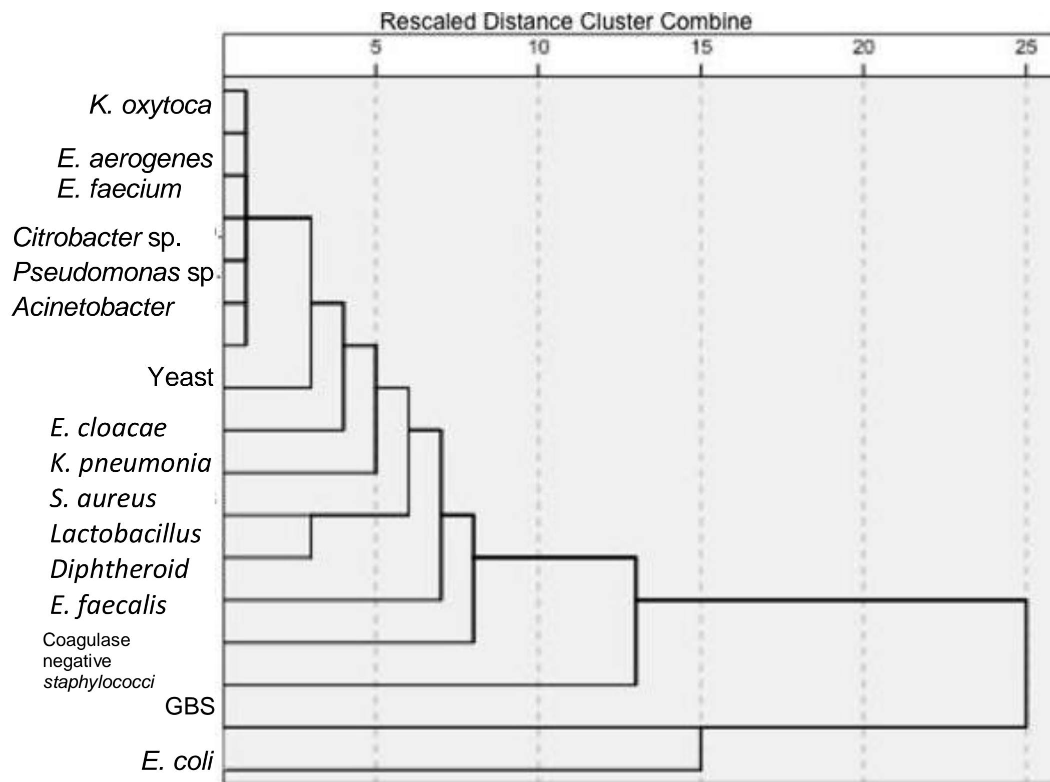

Figure 6. Dendrogram using average linkage (between groups). It shows the proximity between GBS and associated organisms that are isolated from Saudi pregnant females at late pregnancy.

and sensitivity of the DADM namely:

i) The use of a boiled part of the STHB, thereby eliminating any possible cross reaction by resistant Gram-negative bacterial envelopes (El-Kersh et. al., 2002).
ii) The use of gentamicin/nalidixic acid rather than colistin/nalidixic acid Lim broth, thereby attenuating possible *Enterococus fecalis* overgrowth/ inhibition of GBS strains (Park et al., 2001).

Therefore, in accordance with Rallu et al. (2006) we believe that antigen detection on incubated STHB should replace the CDC- GSM for screening for GBS carriage at 35 to 37 weeks of gestation, especially in regions where facilities for PCR assays are limited ,and its high cost would preclude its use (Tazi et al., 2008). Furthermore the GBS capsular polysaccharides are not only important virulence factors and epidemiological serotype markers but also the target of vaccine development efforts. As expected the distribution of GBS-serotypes in our country differs from that reported in other countries as serotype II accounted for 42% of GBS strains, while serotypes IV and Ia accounted for 16 and 12%, respectively. Serotype III GBS has been associated with a high percentage of maternal infection, a substantial proportion of early-onset neonatal human GBS infections and almost all late-onset neonatal infections (Martinez et al., 2004). Serotype III accounted for only 8% in this study, and it has been reported to be generally of low prevalence in the Middle East and Eastern Europe (Ippolito et al., 2010). In the United States the predominant GBS serotypes are Ia, III, Ib, and V, while serotypes VIII and VI are most prevalent in Japan (Martinez et al., 2004; Ippolito et al., 2010). Obviously the prevalence of a given GBS serotype varies according to geographical location, type of clinical specimens, and time of study conductance (Simoes et al., 2007; Ippolito et al., 2010)

The GBS antibiotics susceptibility patterns obtained in this study, agree with those previously reported (Morales et al., 1999; Martinez et al., 2004; Edwards, 2006; Shabayek et al., 2009), and confirm its predictable empiric susceptibility to penicillin and cephalosporin at least in Saudi Arabia. Thus all GBS isolates were sensitive (100%) to Ampicillin, Cefepime, Ceftoriaxon, and Vancomycin, implying their possible use for empiric prophylaxis. While 5 and 10% of the isolates were resistant to clindamycin and erythromycin, respectively, reflecting their still rational use *per se* in Saudi Arabia (AL Huseni et al., 2000). The GBS strains-resistance trend toward these antibiotics is important, since erythromycin is considered first-line prophylaxis for those with allergy to penicillin. In Brazil, however, Castellano-Filho et al. (2010) reported 22.7 and 50% of GBS isolates were resistant to erythromycin and clindamycin, respectively, while in Taiwan, Fu et al. (2004) and Simoes et al. (2007) reported that as much as 39.9% of GBS isolates were resistant to penicillin G. This highlights, the importance of ongoing population-based monitoring for trends in antimicrobial resistance (Edwards, 2006; Barcaite et al., 2008). The fact that most of our GBS isolates were resistant to gentamicin or tetracycline is in agreement with literature data (Morales et al., 1999; Al Huseini et al., 2000; Edwards, 2006; Shabayek et al., 2009) and thereby emphasizing GBS- intrinsic resistance to these antibiotics (Al Huseini et al., 2000). On the other hand, biofilm formation was observed in human GBS isolates that have been isolated from different sources like vagina or anorectal region of pregnant women, neonatal skin swabs, diabetic foot, pus and blood samples. Thus biofilm formation could be an important property in infections caused by GBS (Kaur et al., 2009; Sekhar et al., 2010). This study revealed that 54% of the GBS isolates were biofilm –formers.

In a similar study, Kaur et al. (2009) reported that 76.5% of GBS strains isolated from asymptomatic carriers were biofilm producers. The present study also confirmed that GBS serotypes Ib, III, and V were distinctly (100%) related to biofilm formation than other serotypes (Kaur et al., 2009; Sekhar et al., 2010), implying their biofilm contribution to their frequent invasive infections. Of note, serotype V has been associated with invasive infections in the elderly and with higher rates of antimicrobial drug resistance (Martinez et al., 2004; Kothari et al., 2009). With respect to vagino-perirectal micro biota, 56.5% of our GBS isolates were isolated in association with another single organism primarily *E. coli* as expected. Nevertheless, based on Proximity Matrix analysis, this relationship could not be proven statistically in support of similar finding previously reported by Davies et al. (2010). Though these organisms my reflect a possible contamination of the vaginal tract by rectal microorganisms the colonization with especially *E.coli* is also a predisposing factor for urinary tract infection in women, and it has been documented that EOD caused by *E. coli* is increasing (Dechen et al., 2010).

In summary,this work has confirmed that GBS can be detected by DADM in STHB (boiled) as reliably as the gold standard cutlure method that is recommended by CDC but with the advantage of being rapid and cheaper than PCR. Also confirmed the empric GBS full susceptibility to beta lactam antibiotics and vancomycin but susceptibility to erythromycin and/ or clindamycin needs lab verifications, larger studies are required to evaluate the risk of maternal-fetal infections (including those of *E. coli*) and to improve current prophylaxis measures. Studies are also warrented to further elucidate the epidemiology and clonal diversity of GBS in Saudi Arabia.

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