



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

Diagnostic performance of two chromogenic media for *Streptococcus agalactiae* screening in pregnant women

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ARTICLE INFO

Article history:

Received 30 June 2022

Received in revised form 19 July 2022

Accepted 20 July 2022

Keywords:

Group β Streptococcus
Antimicrobial resistance
Chromogenic media
Granada agar
ChromID strepto B

Abstract

Background: Group β Streptococcus (GBS) colonization among pregnant females is common and is regarded as a substantial cause of neonatal diseases if not treated properly. Thus, we aimed to evaluate the diagnostic performance of two chromogenic media (Granada agar and ChromID StreptoB agar) for screening of GBS in pregnant women between 35-37 weeks of gestation. In addition, we determined their susceptibility profile for guiding the antimicrobial prophylaxis for cases of GBS colonized pregnant women. **Method:** This study included 112 vagino-rectal swabs collected in duplicates from 112 pregnant women between 34-37 weeks of gestation. All swabs were incubated in enrichment broth for 18 hours followed by subculture on blood agar, ChromID strepto B, and Granada agar. Growth of confirmed GBS isolate was subjected to antimicrobial susceptibility testing (AST) to penicillin, vancomycin, clindamycin, erythromycin, and cefotaxime by disc diffusion method. **Results:** The frequency rate of GBS among Egyptian pregnant females was (25.89%). Granada agar was the most accurate among the tested media (98.21%) versus ChromID (96.4%). We observed a high resistant rate for all the tested antibiotics. **Conclusion:** The examined chromogenic media showed promising results and proved to have the potential to be implemented as a screening method for GBS in pregnant women. Regarding the antibiotics' resistance pattern, our results can be an indicator that it is no longer suitable to use the antibiotics empirically without testing. As antibiotic treatment failure is likely, it became inevitable to perform AST before starting any antibiotic to identify the most appropriate treatment for colonized pregnant women.

Introduction

Group β Streptococcus (GBS) is known for its ability to colonize both gastrointestinal and genitourinary tracts. The significance of GBS is higher in pregnant women as it is regarded as a substantial cause of neonatal diseases. It may lead to dreadful consequences in newborns as it might lead to sepsis, pneumonia, and meningitis [1].

The incidence rates of GBS infections in newly born are not reassuring. Early-Onset GBS Infection (EOD GBS) disease incidence ranged from 0.7 cases/1000 live births in 1997 to 0.21–0.25 cases/1000 live births in 2014 and 2015. The highest rates of EOD cases are reported in Africa. Late-Onset GBS Disease (GBS LOD) incidence was stable from 2006 to 2015, with an average

DOI: 10.21608/MID.2022.148315.1338

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incidence of 0.31 cases/1000 live births. Bacteremia is the most common infection and accounts for 93% of GBS LOD followed by meningitis in 31.4% of cases [2-4].

Neonatal invasive GBS disease mortality rates range from 1%–8.4% in term infants to 5%–20% in preterm infants. The mortality rates of EOD range from 5% in developing countries to 27% in Africa. For LOD, the case fatality rate is about 7%. In addition, in utero GBS disease accounts for approximately 1% of stillbirths worldwide and up to 4% of stillbirths in Africa. So, vertical transmission of GBS still represents an emergency worldwide [2-4].

The Centers for Disease Control and Prevention (CDC) recommends performing prenatal GBS screening by swabs taken from the vaginal introitus and perianal area from all pregnant women of 35-37 weeks of gestation [2].

Intrapartum antibiotic prophylaxis minimizes the vertical transmission of GBS as well as the early onset of neonatal sepsis. Penicillin or ampicillin is the first option but with mild allergic reactions they can be substituted with cefazolin. While in case of severe reactions, clinicians usually resort to vancomycin or clindamycin as an alternative [5].

Owing to the high susceptibility of GBS to beta-lactams and the absence of resistance against penicillin, penicillin is the mainstay of treatment of GBS infections. Thus, the current guidelines do not advise antibiotic susceptibility testing routinely for pregnant women colonized with GBS. Nevertheless, it ought to be performed in cases of past grave anaphylactic reactions due to the mounting resistance against the valid alternatives [2]. So, CDC recommends antibiotic susceptibility testing if erythromycin or clindamycin is demanded to prohibit neonatal GBS infection owing to their high resistance rate [6].

The conventional diagnosis of GBS colonization involves culturing vagino-rectal specimens in a selective enrichment medium, as Todd-Hewitt broth containing colistin and nalidixic acid, then subculturing on sheep blood agar. Yet, it takes a minimum of 48 hours to fully identify GBS. CDC prioritizes the research for the development of novel chromogenic media to reveal the existence of GBS. This guarantees accurate results of prenatal cultures and enables their processing at laboratories with limited resources. It includes a novel

chromogenic agar, i.e. chromID Strepto B (formerly Strepto B ID) agar or Chrom Agar, which spots GBS as red colonies after aerobic incubation [7].

Thus, this study aimed to evaluate the diagnostic performance of two chromogenic media (Granada agar and ChromID StreptoB agar) in comparison to the conventional culture technique for screening of GBS in pregnant women between 35-37 weeks of gestation. In addition, we aimed to determine their susceptibility profile for guiding the antimicrobial prophylaxis for cases of GBS colonized pregnant women.

Materials and Methods

Study design and study population

The study was conducted on 112 vagino-rectal swabs collected in duplicates from 112 pregnant women attending the out-patient Obstetric clinic of Ain Shams University Hospitals (ASUH) and submitted for routine culture and sensitivity in the main microbiology laboratory of ASUH. The swabs were collected during the period between December 2017 and August 2018.

The vagino-rectal were selected fulfilling the following inclusion criteria: pregnant women between 35th to 37th weeks of gestation. Exclusion criteria were as follows; any pregnant woman with premature rupture of membrane, any pregnant woman known to have diabetes mellitus or gestational diabetes, and any pregnant woman on current antibiotic treatment. This research was approved by Ethical Research Committee, Faculty of Medicine, Ain Shams University. (ethical approval number: FWA 000017585, December 2017), an informed consent was obtained from each patient before enrollment.

Microbiological workup for the included vagino-rectal swabs

One swab of the 112 duplicate vagino-rectal swabs was subjected to direct Gram stained film. The second swab was incubated for 18 hours in a selective enrichment broth medium (Todd Hewitt broth with antibiotics) (BioMérieux, Marcy l'Etoile, France).

Subculture was then performed using the streak plating technique on each of the following media; blood agar plates (Oxoid, England), ChromID strepto B (BioMérieux, Marcy l'Etoile, France), and Granada agar (BioMérieux, Marcy l'Etoile, France) [8]. Plates were incubated overnight at 37

°C under aerobic conditions for 18-24 hours. The plates were examined for presumptive positives which were defined as; beta hemolytic colonies on blood agar, pale pink to red colonies on ChromID strepto B, and orange or red pigmented colonies on Granada agar [9, 10].

Phenotypic identification of the suspected colonies by: Gram stain, Catalase test, Bacitracin test, and CAMP test. Our reference phenotypic method for diagnosis of GBS was the combined positivity for all the following; β hemolysis on blood agar, bacitracin resistance, and positive CAMP test [11]. The identification of all GBS isolates were further confirmed by vitek2C system (BioMérieux, France).

Susceptibility testing

Antibiotic susceptibility by disc diffusion was done to all isolates proved to be GBS according to the clinical and laboratory standard institute recommendations (2018) [12] using the following discs: penicillin (10ug), vancomycin (30ug), clindamycin (2ug), erythromycin (15ug), and cefotaxime (30ug). *Streptococcus pyogenes* (ATCC 19615) and *Staphylococcus aureus* (ATCC 24923) were used as a quality control.

Statistical analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 20). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

For the descriptive statistics, frequency and percentage of non-numerical data were calculated. As for Analytical statistics: Pearson chi-square test or Fisher's exact test were performed to examine the relationship between two qualitative variables. A statistically significant difference was considered at p value ≤ 0.05 .

The diagnostic performance of the different media was detected by the following parameters; sensitivity, specificity, PPV, NPV, and accuracy. Kappa statistics was used to compute the measure of strength of agreement between two investigational methods. The closer the Kappa to 1.0, the better the agreement.

Results

Results of different methods of identification

Twenty-nine isolates (29/112) (25.89%) fulfilled the criteria and were confirmed to be GBS positive. As regards the results of blood agar, Granada agar, Chrom ID StreptoB agar, and CAMP test 33/112

(29.5%) of the isolates exhibited β hemolysis on blood agar. Twenty-nine beta-hemolytic isolates were CAMP test positive. All the twenty-nine GBS isolates were confirmed by the Vitek2c system. The results revealed a 100% agreement between the Vitek2c and the phenotypic identification. As regards the Granada agar, 29/112 (25.9%) of the isolates showed positive results. On the other hand, 31/112 (27.7%) showed positive results on Chrom ID StreptoB agar. The two (2) patients with positive ChromID StreptoB agar other than the 29 fulfilling the criteria of confirmed positivity showed also B-hemolysis but without bacitracin resistance so CAMP test was done and results were negative confirming that these two (2) results were false positive (**Figures 1, 2**).

Table 1 shows that there was a highly significant agreement between GBS by reference method and Granada agar results where the two markers agreed in 100% (Positive 27.68% + Negative 72.32 %) of cases and kappa = 1. Also, there was a significant agreement between GBS by reference method and ChromID results where the 2 markers agreed in 98.21% (Positive 25.68% + Negative 72.32 %) of cases and kappa = 0.9545.

Table 2 shows the sensitivity and the specificity of Granada agar and ChromID StreptoB agar according to the reference blood agar. Both Granada agar and ChromID StreptoB agar had the same sensitivity 100% but Granada agar had 100% specificity whereas ChromID StreptoB agar 97.59% which resulted in more accuracy of Granada agar (100%) than ChromID StreptoB agar (97.59%). No significant difference was found between the two types of media (p value= 0.155).

Results of antibiotic susceptibility testing

Twenty-nine isolates (29/112) (25.89%) fulfilled the criteria and were confirmed to be GBS positive. All colonies proved to be GBS positive were subjected to antibiotic susceptibility test by disk diffusion method using the following discs: penicillin, vancomycin, clindamycin, erythromycin, and cefotaxime.

The results of the antibiotic susceptibility of the tested GBS isolates by the disc diffusion method are shown in **table (3)**. The highest level of resistance among GBS isolates was reported against cefotaxime where 19/29 (65.5%) were resistant. No macrolide-lincosamide-streptogramin B (MLS) pattern of resistance was noted in any of the GBS isolates.

Table 1. Agreement between GBS by reference method versus Granada agar and Chrom ID.

		Group B strept		Total	Agreement			
		Negative	Positive		%	Kappa	p value	Sig.
Granada	Negative	83 (72.32%)	0 (0%)	83 (72.32%)	100%	1	<0.001	S
	Positive	0 (0%)	29 (27.89%)	29 (27.89%)				
Total		83 (72.32%)	29 (27.68%)	112 (100%)				
		Group B strept		Total	Agreement			
		Negative	Positive		%	Kappa	p value	Sig.
Chrom-ID	Negative	81 (72.32%)	0 (0%)	81 (72.32%)	98.21%	0.9545	0.000	S
	Positive	2 (1.79%)	29 (25.89%)	31 (27.68%)				
Total		83 (74.11%)	29 (25.89%)	112 (100%)				

* Poor: if $k < 0.20$, Fair: if $0.21 < k < 0.40$, Moderate: if $0.41 < k < 0.60$, Substantial: if $0.61 < k < 0.80$, Good: if $k \geq 0.81$.

Table 2. Sensitivity, specificity and accuracy of Granada and Chrom ID StreptoB agar to detect GBS.

	Sensitivity	Specificity	PPV	NPV	Accuracy	Pairwise comparison	
						p value	Sig.
Granada	100%	100%	100%	100%	100%	0.155	NS
Chrom-ID	100%	97.59%	97.59%	100%	98.21%		

*NPV=negative predictive value. *PPV=positive predictive value.

Table 3. The results of antibiotic susceptibility of the positive GBS isolates by disc diffusion method.

	Resistant		Sensitive	
	N	%	N	%
Penicillin	13	44.8%	16	55.2%
Vancomycin	14	48.3%	15	51.7%
Cefotaxime	19	65.5%	10	34.5%
Clindamycin	14	48.3%	15	51.7%
Erythromycin	13	44.8%	16	55.2%

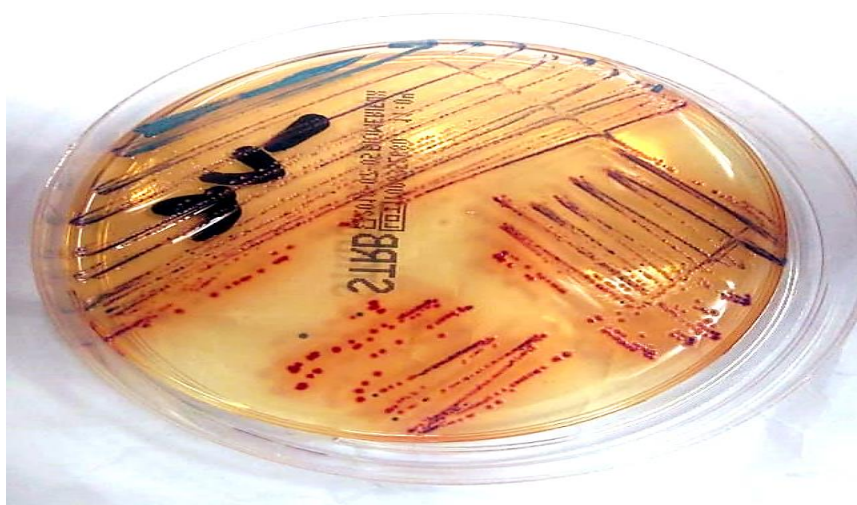
Figure 1. Chrom ID StreptoB agar showing the red colonies characteristic of Streptococcus agalactiae.

Figure 2. Granada agar showing the orange colonies characteristic of positive *Streptococcus agalactiae* on the right side of the plate. The white colonies on the left side indicates negative for *Streptococcus agalactiae*.



Discussion

Group β *Streptococcus* remains the chief cause of neonatal disease and death in both developed and developing countries. Usually, it is too late to treat the newly born with GBS-early onset disease who are septicaemic at birth. So, prevention is mandatory to decrease the incidence of neonatal GBS infections. Although GBS in colonized pregnant women is mostly asymptomatic, they can suffer from diverse infections like chorioamnionitis, puerperal sepsis or other critical conditions and increased incidence of stillbirths and premature delivery [13].

In the current study, we found that 25.89% (29/112) of the pregnant women who underwent the screening were colonized with GBS. These results were concordant with other studies carried out in Egypt such as the study done in Benha university hospital by **Tash and co-workers** (2019) which showed a prevalence rate of 28% [14], also another study was carried out by **El Shahaway and colleagues** (2019) at Zagazig university hospital which showed a prevalence rate of 23.5% [15]. Similarly, another study performed by **Sadaka and his team** (2017) at Alexandria reported a prevalence rate of 26.5% for GBS [16]. Also, at Ismailia a research was conducted by **Shabayek and colleagues** showed a prevalence rate of 25.3% [17].

Other researchers from different countries also found similar results to ours that ranged from 22.4% to 28.8% [10,18-20].

On the other hand, other studies reported lower prevalence rate as **Clouse and colleagues** (2019) from Jordan and **Mohammed and coworkers** (2020) from Saudi Arabia who found prevalence rates of (19.5%, 15%) respectively [21, 22].

The lower prevalence rate reported in these two studies could be attributed to using different methods of detection as the latex agglutination method was used in both studies and the second study used the PCR method besides.

It is worthy to note that in a study done by **Vieira and colleagues** (2019) they obtained three different prevalence rates of maternal GBS colonization according to the technique used (51.1% by qPCR, 30.7% by Xpert GBS, and 14.3% by cultures) [23]. These findings can draw our attention to the possibility of existing potential higher prevalence rate of GBS.

As for the results of the diagnostic performance of the different chromogenic media, the present study showed that Granada agar had a sensitivity and specificity of 100%. Whereas, ChromID StreptoB agar had a sensitivity of 100% and 97.59% specificity. These results were in concordance with a study carried in France that showed that Granada agar had a sensitivity of 94.3% and a specificity of 100%, and ChromID StreptoB agar had a sensitivity of 100% and specificity of 98.8% [24].

Also, a study was performed in Australia reported high false positive results of ChromID StreptoB agar with specificity and sensitivity of 73% and 100% respectively [25].

On the other hand, different results were found in a study conducted in Brazil on 31 GBS isolates -recovered from 110 pregnant women-testing Granada agar in comparison to PCR technique. They showed that Granada displayed a sensitivity of 76.6% versus PCR of 86.6% sensitivity. While both methods displayed 100% specificity [19], and another study in Japan that detected a total of 319 GBS isolates showed 1 false negative result from culture on ChromID StreptoB agar with 20 false negative results from culture on blood agar [10].

Different results could be due to different culture methods, different methods of specimen collection or different laboratory quality control [26].

The results of the current study showed that chromogenic media can be a more accurate, convenient and practical choice for screening GBS colonization. It could be evaluated by junior microbiologist easily and avoid misdiagnosis with other organisms with no overlapping so it can be implemented routinely in clinical laboratories.

In this study antibiotic sensitivity testing was done for the GBS collected specimens by the disc diffusion method which resulted in high resistant rate for all the used antibiotics as for, penicillin 44.8%, cefotaxime 65.5%, clindamycin 48.3%, erythromycin 44.8% and vancomycin 48.3%.

Mengist and his coworkers (2017) from Ethiopia reported closely similar results to ours with higher resistance rate (77.3%) towards penicillin, and erythromycin intermediate resistance 36.4% and resistance of 22.6%. However, their isolates exhibited lower resistance towards clindamycin (18.2%) while none of their isolates showed resistance to vancomycin [27].

Also, another study in Kenya conducted by **Jisuvei and colleagues** (2020) reported that among the positive 60 GBS isolates recovered from 292 pregnant women, resistance was detected for penicillin G in 42/58 (72.4%) isolates, ampicillin in 32/58 (55.2%) isolates, clindamycin in 14/46 (30.4%) isolates, and vancomycin in 14/58 (24.1%) isolates [28].

A study conducted in Zimbabwe on 43 GBS isolates were obtained from 420 vaginal samples reported increased resistance towards all their tested antibiotics. The resistance rates towards penicillin G, clindamycin, ceftriaxone, erythromycin and vancomycin were (69.8%, 55.8%, 46.5%,30.2%, and 30.2%) respectively [29].

On the other hand, other studies had results with very big difference from this study such as a study by **Tash et al.**, (2019) from who isolated 70 GBS isolates from 250 pregnant women with 100% susceptibility to each of penicillin, cefotaxime and vancomycin. Also, their isolates showed lower resistance rate towards clindamycin (17.2%) while resistance to erythromycin was similar to that detected in the current study (42.8%) [14]. This much less resistance pattern recorded against most of their tested antibiotics could be due to different isolates serotypes, patient's demographic data, underlying diseases and co-morbidities. Unfortunately, we did not determine GBS serotype in the current study, which is a critical information in view of the upcoming vaccines. Identification of different GBS serotypes is of paramount importance as various serotypes have the potential to cause different diseases. For example, virulent serotype III has been associated with neonatal invasive disease and meningitis. Also, detection of the predominant serotype/s in a specified country can aid in vaccine development as an effective preventive strategy [3].

Also, **Mohamed and his team** (2020) from Saudi Arabia who conducted their research on 400 pregnant women and recovered 60 GBS isolates from them. They reported that none of their isolates showed resistance to penicillin G, ampicillin, linezolid, daptomycin, and vancomycin. Their isolates displayed lower resistance than ours against erythromycin (16.7%) and clindamycin (15%) [22].

Those unexpected results of increased resistance against most of the available choices of treatment are probably the consequence of the cumulative uncontrolled misuse of over the counter antibiotics especially in developing countries like Egypt. Also, the overuse of antibiotics for farm animals and delayed hospitalization due to high costs for the patient in developed countries are possibly blamed to a great extent in this untamed situation of increased antibiotics resistance. This

ensures the importance of increasing awareness against the devastating results of antibiotics mal-use [30].

Our study is one of few studies in Egypt that reported such a high resistance against β -lactams -especially penicillin- and vancomycin. This increasing pattern in antibiotic resistance was proven in many studies as **Malik and coworkers** (2019) who stated that a recent report indicates that the global antibiotic consumption increased by 65% between 2000 and 2015 [30]. This caused a high resistance Gram-positive cocci (such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, methicillin resistant *Staphylococcus aureus*) to the most common antibiotics in Africa. The high rate of antimicrobial resistance was also proven by other more recent studies in our continent Africa as a study carried in Nigeria by Lakoh and colleagues (2020) which stated that antibiotic resistance among the gram-positive pathogens with Penicillin resistance rate of 100% for *Enterococcus faecium* and *Staphylococcus aureus* [31].

Also, the high rate of resistance could be attributed to multiple resistance genes which unfortunately were not studied in our research. A study by **Mudzana and his team** (2021) found a link between some of the resistance gene exhibited by their isolates and their multidrug-resistance pattern detected in their study [29].

Despite the current recommendation of not performing AST for any colonized pregnant woman with GBS, the results in this study showed that it is no longer suitable to give antibiotics like penicillin and cephalosporins; that were once considered the standard treatment without testing. The results have proven that the phenomenon of multidrug resistance has extended to include GBS and hence it is mandatory to perform AST to optimize the treatment choices and to reduce the mal-use of antibiotics.

It is recommended to further study the prevalence of GBS colonization in a larger sample of Egyptian females with determining the GBS serotypes, which is a critical information in view of the upcoming vaccines. Also, characterization of antimicrobial susceptibility pattern and types of resistance has become an essential requirement to formulate local guidelines of therapy.

This study recommends the dissemination of awareness among clinicians about the problem of

antimicrobial resistance and the importance of the rational use of antimicrobial prophylaxis.

Conclusions

In conclusion, the examined Chromogenic media in the present study showed promising results and proved to have the potential to be implemented effectively for screening of GBS in pregnant women. Based on the obtained results we deduce that it is now crucial not only to implement perinatal screening of pregnant females but also, to conduct antimicrobial susceptibility testing to ensure effective therapy.

Declaration of interest: The authors report no conflicts of interest.

Funding information: none received.

Author contributions

NAF and SAE designed the study. MBR collected the samples. NAF and MBR did the microbiological experiment. NAF, MBR and SAE performed the statistical analysis. NAF and NNS wrote the first draft of the manuscript. NAF and SAE provided critical suggestions on study design and manuscript writing. All authors contributed to the revision of the manuscript and approved the final version.

Acknowledgments: None.

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