Comparison of various methods for Group B Streptococcus Identification and Cost Comparison

RP Nagassar[1], K Daniel[1], RJ Bridgelal-Nagassar[2]

[1]The Department of Microbiology, Sangre Grande Hospital, The Public Health Observatory, The Eastern Regional Health Authority, Trinidad
[2]Country Health Administration, St George Central, The North West Regional Health Authority, Trinidad

Corresponding Author:
Dr. Rajeev P. Nagassar
Department of Microbiology,
Sangre Grande Hospital,
Eastern Regional Health Authority
Email: rpnagassar@gmail.com

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ABSTRACT

Objectives

To verify the utility, cost and feasibility of various methods for Group (Gp) B *Streptococcus* (GBS) identification; To elucidate the prevalence and resistance patterns of GBS in a clinic population.

Design and Methods

Isolates were collected from pregnant patients by culturing lower vaginal swabs (LVS) and rectal swabs (RS) from May to September 2015 at Sangre Grande Hospital, Trinidad. These were screened in Carrot Broth (CB), Gram stained and isolated on Blood Agar (BA) and *Streptococcus* Selective Agar (SSA) simultaneously. Identification was done simultaneously with the Microscan Autoscan® and Streptex® - Streptococcal Grouping kit, to establish concordance. The Microscan Autoscan® panel identified various *Streptococcus* spp. and Streptex® identified Lancefield Gps A-G. Antimicrobial susceptibility was determined with the Microscan Autoscan® for Gp B *Streptococcus* only. Discordant isolate identifications between Microscan and Streptex were retained for further analysis. Gram staining was also carried out on negative CB. The total cost of identification of isolates was calculated in Trinidad and Tobago dollars.

Results

36 LVS & RS samples were collected: 16 Gp B, 1 Gp C, 11 Gp D & 8 with no *Streptococci* Gp identification.
Prevalence of Gp B *Streptococci*: 44.4%.
Concordance between CB and other methods was 86.1%.
Sensitivity: 100%; CI (72% - 100%), Specificity: 80%; CI (59% - 93%). Accuracy: 86.1%; CI (70% - 96%).
Microscan Autoscan® and Streptex® identified 100% of isolates correctly.
Penicillin resistance was 12.5%, Vancomycin and Clindamycin sensitivity were 100% each.
The costs for isolation media plates were - SSA: $ 26 per plate and BA: $18 per plate. *Streptococcus* identification and sensitivity using Microscan Autoscan® Panel 33: $114 per isolate (with blood agar). Streptococcus identification using Streptex®: $193 per isolate (with blood agar) and Carrot Broth: $49 per isolate.

Conclusion

Microscan Autoscan® Panel was cheaper than Streptex® for final identification and sensitivity testing. With total costs considered, CB combined with Microscan Autoscan® and blood agar culturing is cost effective, giving rapid identification and susceptibility results.

Key Words: Group B *Streptococci*, Carrot Broth, Microscan, Streptex, SSA, Cost

INTRODUCTION

In a systematic review and meta-analysis by Russel *et al,* it was found that maternal Group (Gp) B *Streptococcus* (GBS) colonization worldwide was 18%.[1] Regional variation was noted from 11% to 35% with a lower prevalence in Southern Asia and Eastern Asia. Studies by Orrett *et al* have found the prevalence to be approximately 31 – 32% in Trinidad and Tobago.[2,3]

Reliable methods to detect Group B *Streptococci* are needed, as this has implications in pregnancy.[4] GBS can infect neonates during passage through the vagina at birth resulting in the sequalae of neonatal infection. Conventionally, Blood Agar (BA) is used followed by identification and susceptibility testing.[4] However, Strep B Carrot Broth (Hardy Diagnostics, Santa Clara, CA) may also be used for rapid identification of this group of pathogens. This is a chromogenic, pigmented enrichment broth/media and a colour change indicates a positive result for the presence of beta-haemolytic GBS. Thus, this broth is also used as an identification system which we have evaluated in this study. Once this broth is positive, further identification is not needed. The broth should be sub-cultured to an appropriate agar plate if no colour is detected and then further tested, or the broth can be tested directly to determine if GBS is present.[4] Selective Streptococcal agar (SSA) can be used to selectively grow *Streptococci*, ensuring its isolation over other flora in the vaginal canal.[5] Once the *Streptococci* are grown, they must be identified (once no identification by the Carrot Broth is obtained), for example, by use of the Lancefield grouping system.[4] In addition, automated or semi-automated machines such as the Microscan Autoscan® may be used for identification and susceptibility testing. This study focused on the cost effectiveness of screening, identification and susceptibility testing for *Streptococcus agalactiae* or GBS using Carrot Broth, various growth media and identification techniques in the Obstetrics clinic at the Sangre Grande Hospital, Trinidad. It also looked at the prevalence of Group B *Streptococci* in the clinic.

METHODS

Isolates were collected from pregnant patients by culturing lower vaginal swabs (LVS) and rectal swabs (RS) from May to September 2015 at Sangre Grande Hospital (SGH), Trinidad. This was not done in a randomized manner but by convenience sampling. Samples were received following a call for LVS and RS samples for Group B *Streptococci* identification, in pregnancy. No patient identifiers were used and demographic data was also excluded as this study was only based on the isolates.

These LVS and RS were screened in Carrot Broth (CB). The swabs were placed in the broth and assessed after 24 hours. A colour change to pink indicated a positive test. A loop full of carrot broth was streaked onto Blood Agar (BA) [Oxoid®] and *Streptococcus* Selective Agar (SSA) [Oxoid®] simultaneously for culturing and comparison of the media. Cultures were incubated at 37°C for 24 hours. The CB was evaluated primarily for its performance in identification of Gp B *Streptococci* (GBS), not for its enrichment properties. The enrichment property was a secondary outcome.

The isolate identification was compared with the Microscan Autoscan® (Siemens, Sacramento, California) and Streptex (Thermo Scientific, Texas) - Streptococcal Grouping kit, for concordance simultaneously. The Microscan Autoscan® system identified a panel of *Streptococci* while the Streptex® system identified the Lancefield Groups A-G. Gram staining was done on negative CB to rule out bacteria which were not Gram-positive cocci and thus would not fit the criteria for further testing.

Discordant Results

A discordant result was one that did not compare or match with Microscan® and Streptex® kits, these isolates were saved at – 20° C for further molecular testing.

Susceptibility Testing

Antimicrobial susceptibility was done with the Microscan Autoscan® for GBS.

Quality Control

American Type Culture Collection (ATCC) Control 12401,

for *Streptococcus agalactiae* was used as the positive control. *Escherichia coli* ATCC® 25922 was used as the negative control.

Cost Comparison

The cost of microbiological testing was calculated as follows:

a. Cost of Carrot Broth Identification: Cost of CB Media (one vial is used per isolate)

b. Cost of Microscan Identification: Cost of CB plus Cost of Media plus cost of Microscan panel (one panel is used per isolate)

c. Cost of Streptex Identification: Cost of CB plus Cost of Media plus cost of one Card (one card is used per isolate)

Data Analysis

SPSSv22 was used for analysis of the data. Confidence intervals were used for analysis of performance characteristics of Carrot Broth. Descriptive statistics were used for prevalence of antibiotic resistant isolates and for comparing total costs among the different identification methods.

RESULTS

Thirty-six (36) LVS and RS samples were collected: 16 GBS, 1 Gp C, 11 Gp D and 8 which had no *Streptococci* Gp identification but were Gram negative or Gram positive bacilli by Gram stain.

Prevalence

Prevalence of GBS *Streptococci* was 44.4%.

Performance of Carrot Broth

The agreement between CB and other methods was 86.1% for identification of GBS. Sensitivity was 100%: confidence interval [CI] (72% - 100%), Specificity was 80%: CI (59% - 93%) and accuracy was 86.1%: CI (70% - 96%).

Concordance

Selective *Streptococcal* agar (SSA) and Blood Agar (BA) showed no difference in isolation of *Streptococci*. However, SSA was more selective for *Streptococci* than other species.

Microscan Autoscan® and Streptex® identified 100% of the isolates correctly and thus, there was no discordance in results and no need for further confirmation of identity.

Antibiotic Sensitivity

Penicillin resistance was 12.5%, Vancomycin and Clindamycin sensitivity was 100%.

Cost

SSA cost was $26 per agar plate and BA was $18 per agar plate. *Streptococci* identification and sensitivity using Microscan Autoscan® Panel 33 plus the cost of BA and CB were $114 per isolate. *Streptococci* identification and sensitivity using Microscan Autoscan® Panel 33 plus the cost of SSA and CB were $122. *Streptococci* identification using Streptex® plus BA and CB was $193 per card. Streptococci identification using Streptex® plus SSA and CB was $ 201 per card. Carrot Broth was $49 per vial. See Table 1 for cost comparison.

DISCUSSION

Carrot broth (CB) had acceptable agreement with other identification tests and was inexpensive. This test has an acceptable sensitivity and can be used for screening and identification of GBS. Importantly, a negative CB test is always followed by identification and testing with the Microscan Autoscan®. Thus, the recommended algorithm for using CB is to always follow with BA and Microscan Autoscan® identification and sensitivity or BA and Streptex®, for identification only.[4] Streptex® testing will have to be followed by manual sensitivity testing making it even more expensive than presented in the results. Microscan Autoscan® identification and sensitivity are

Table 1 Cost Comparison

CB*	SSA**	BA***	Microscan plus BA plus CB	Microscan plus SSA plus CB	Streptex plus BA plus CB	Streptex plus SSA plus CB
$ 49	$ 26	$ 18	$ 114	$ 122	$ 193	$ 201

*CB – Carrot Broth *SSA - Streptococcal Selective Agar *** BA - Blood Agar

**** Cost was in Trinidad and Tobago Dollars ($)

thus preferred as shown in this study under the setting of the Sangre Grande Hospital especially if CB is negative. If a hospital needs a more selective agar for *Streptoccci* then SSA can be used instead of BA. Use of SSA may make the process of *Streptococci* identification more streamlined and reduce the other contaminating bacteria.

This study shows that there is a small amount of resistance of GBS to penicillin. Banno *et al* have noted resistance in GBS.[6] Thus, although minimally prevalent globally, the possibility of resistance exists. This pilot study at the Sangre Grade Hospital shows a small amount of phenotypic resistance and thus further molecular studies should be done.[6] In a study by Mousavi *et al* 93.5% of GBS showed reduced penicillin susceptibility.[7] Gizachew *et al* in their study published in 2019, noted resistance to penicillin in Africa.[8] Thus, the small amount of resistance seen in this study is congruent with the emerging resistance seen globally in GBS. In the event of allergies or treatment failure, clindamycin or vancomycin can be used, respectively.

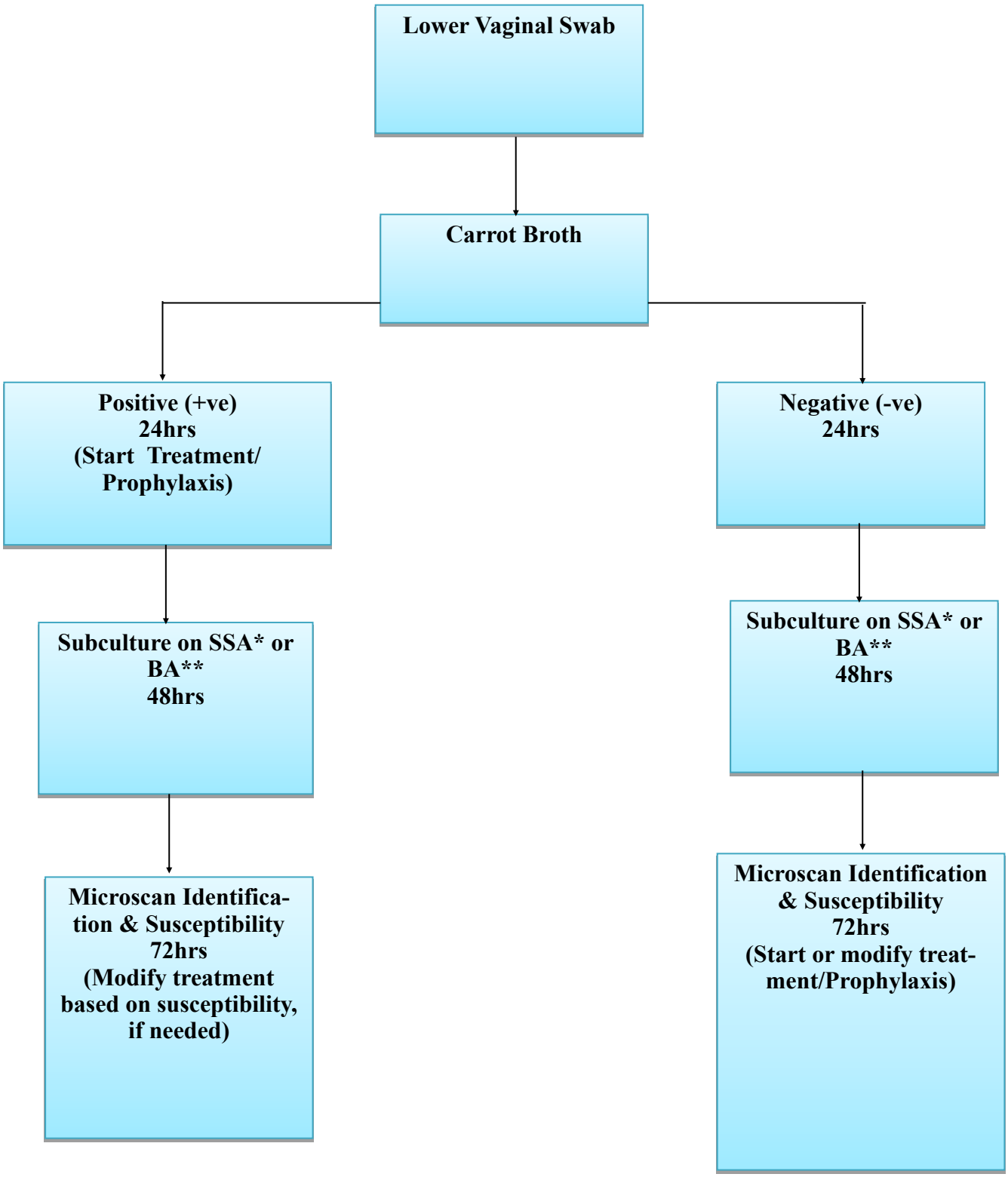
The prevalence of GBS is high at 44.4 %, however this has been seen in previous studies by Orrett *et al* at 31.4% and 32.9% in 1994 and 2003, respectively.[2,3] In the systematic review and meta-analyses by Russel *et al* in 2017, it was noted that the prevalence in the Caribbean region, is one of the highest in the world at an average of 34%.[1] Thus, the prevalence is higher than that found in the literature. This high prevalence indicates that pregnant women at the Sangre Grande Hospital, should be screened for group B *Streptococci* in pregnancy. There may have been a bias in the calculation of prevalence due to the small sample size and this was just a clinic-based study and not a population-based study.

In a publication in 2019, the American Academy of Paediatrics along with the American College of Obstetricians and Gynaecologists recommended that antenatal microbiology-based testing for the detection of maternal GBS colonization should be done.[10] This is to facilitate appropriate administration of intrapartum antibiotic prophylaxis. These authors also note that colonization with GBS is associated with both early onset and late onset GBS neonatal meningitis.[9] The American College of Obstetricians and Gynaecologists recommend universal GBS screening between 36 and 37 weeks of gestation.[10] Figure 1 highlights the suggestive use of carrot broth for identification and treatment or prophylaxis decision making.

The CB is the cheapest identification system evaluated and can identify GBS in less than 24 hours allowing rapid clinical decision making to be possible. The Microscan Autoscan® system was cheaper than the Streptex® and also allowed susceptibility results to be obtained. It is thus preferred based on the costs presented. The BA is cheaper to use but it is not as selective in identification of *Streptococci* spp. as SSA. Thus, in terms of cost if selectivity is not needed, BA can be used for culturing and then identification and susceptibility testing.

This study has several limitations, including the small sample size. It is a pilot study and lays the ground work for additional studies. The Microscan Autoscan® is a semi-automated machine and manpower time, electricity and other indirect costs will further increase the costs. This was not a population prevalence study but it focused on the prevalence in a clinic. Further studies are needed on the molecular characteristics of GBS with reduced penicillin susceptibility or resistance in Trinidad and Tobago and the Sangre Grande Hospital. This study did not evaluate the enrichment properties of CB versus other media for enrichment for example LIM Broth but focused on the identification properties of the CB. We also did not have external funding for the study. Lastly, the study did not assess the number of neonates who developed early onset or late onset neonatal meningitis.

Figure 1: Flow Chart of Positive (+ve) vs. Negative (–ve) CB Screening



*Streptococcal Selective Agar (SSA) ** Blood Agar (BA)

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