Use of a Rapid Diagnostic Test for Simultaneous Identification of Group B Streptococcus and Determination of its Susceptibility to Clindamycin

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ABSTRACT

Objectives: To examine the ability of a newly-developed Group B streptococcus diagnostic test to determine susceptibility to clindamycin within 6.5 hours.
Methods: 358 patients were screened between 35-37 weeks. Vaginal-rectal swabs were collected, and as a separate study, GBS was identified by a newly described rapid culture-based test, and the performance of this test in detection of GBS has been reported separately. In addition to processing for identification of GBS in the vaginal-rectal specimen, samples were simultaneously processed for susceptibility to clindamycin. Breakpoint concentrations for clindamycin were determined through use of disc-diffusion and E-Test, and the GBS detection assay was modified by the addition of 2 micrograms of clindamycin per blot. Blots were then incubated for 6 hours at 37 degrees Celsius, and then after washing with sample buffer, HRP-conjugated antibody was added and bound GBS was detected.
Results: 358 patients were screened, with a GBS prevalence of 29.5% as determined by an in-house culture. Of positive samples, 28% were found to be resistant by culture with either disc-diffusion or E-Test. Processing of these samples by the rapid assay revealed that 86% of the samples showing resistance to clindamycin by routine methods were resistant by the rapid test. When examined independently, the rapid assay provided an overall level of clindamycin resistance of 43%.
Conclusions: This newly developed assay for detection of GBS provides the novel advantage of allowing for simultaneous determination of clindamycin susceptibility, but a large number of false positives are observed.
Learning Objective: Learners will be able to review screening guidelines for GBS prophylaxis, qualities of a screening test, and resistance levels of GBS to clindamycin.

INTRODUCTION

•Screening for GBS is recommended between 35-37 weeks gestation[1]
•Routine culture requires 24-48 hours, with determination of antimicrobial susceptibility requiring an additional 24-48 hours[2]
•Per 2010 CDC Guidelines, identification of GBS by polymerase chain reaction (PCR) may be done after an enrichment step, allowing for a more rapid identification of GBS[3]
•To determine antibiotic resistant strains of GBS, specific primers must be utilized.

-HOWEVER

•Use of an enrichment step prevents this test from being done in a truly rapid fashion
•Identification of GBS by PCR technology may not be suited for detection of strains showing de novo resistance (Primers not yet known/sequenced)

METHODS

Three vagina-rectal swabs were collected per patient at 35-37 weeks gestation.
The first swab was sent for routine processing, and any decision made to provide treatment in labor was based on the results obtained from processing of this swab by a commercial lab.
The second swab was processed by an in-house culture, after an overnight enrichment in LIM broth, and then subcultured on CNA agar. Further subculture was performed in the presence of clindamycin, and resistance/susceptibility was determined by either disc diffusion or E-Test.
The third swab was processed directly by the rapid test as follows:
The swab was placed in 1 ml sterile phosphate-buffered saline (PBS), and then 20 microliters was placed on a nitrocellulose membrane (NCM) previously coated with a polyclonal anti-GBS antibody, and then blocked with either bovine serum albumin or StartingBlock. After a 30 minute inoculation, NCM was washed with PBS, and then placed over CNA agar.
Plates were then incubated for 6 hours at 37 degrees Celsius, either in the presence or absence of clindamycin.
After the incubation, NCM was removed, washed with PBS, and then an HRP-conjugated polyclonal antibody against GBS was added, and NCM was incubated for 20 minutes at room temperature.
Excess antibody was removed by washing with PBS, and a positive signal generated by reaction of DAB with bound HRP was recorded.

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RESULTS

Commercial processing revealed that 23.6% of patients were GBS positive.[4]
In-house culture of specimens obtained from the same 358 patients revealed a colonization rate of 29.5%. (Rates of colonization ranged from 16% in Asians to 40% in African Americans.[4])
Processing directly by the 6-hour test revealed a GBS colonization rate of 36.8%.[4]
When resistance to clindamycin was determined, we observed that 28% of GBS isolates were resistant to clindamycin by routine culture performed by our in-house assay. Of the 110 positive GBS isolates, 86% of these were found to be resistant by the rapid test. Of the 358 total patients, we observed a level of resistance to clindamycin in 43% of the GBS isolates.

Detection of GBS from Vaginal-Rectal Specimens in 358 Antepartum Patients (N)	
Commercial Test	23.6% (84)
In-House Culture	29.5% (110)
Rapid Test	36.8% (131)

Resistance Rates of 110 GBS Isolates	
In-House Culture	28% (31)
Rapid Test	43% (47)
Concordance Between the Two Tests	86%

CONCLUSIONS

•Identification of GBS by this 6-hour culture is accurate, and allows for a reduction in the total time required to obtain a result[4]

•Determination of antimicrobial resistance may also be determined as the specimen is being cultured, allowing for simultaneous identification of GBS and determination of its susceptibility to a clinically relevant antimicrobial agent

•Improvements in the accuracy of the test may allow for this test to be used in the processing of clinical isolates used in antepartum patients