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Streptococcus agalactiae: Identification methods, antimicrobial susceptibility, and resistance genes in pregnant women

Introduction

1. Definition of the Bacterium:
    - **Streptococcus agalactiae**, known as **Group B Streptococcus**, is a bacterium that inhabits the gastrointestinal and genital tracts as part of the normal microbiota.
2. Clinical Importance:
    - This bacterium is clinically significant due to the risk of infections in neonates.
3. Infection Prevalence:
    - There has been an increase in reported GBS **(Group B Streptococcus)** infections among non-pregnant adults.
4. Risk of Infection in Neonates:
    - GBS **(Group B Streptococcus)** colonization in pregnant women during childbirth represents the main risk for developing infections in newborns.
5. Colonization Rates:
    - The estimated prevalence of GBS **(Group B Streptococcus)** colonization in pregnant women ranges from 10% to 35% in various countries, including Brazil.
6. Systematic Reviews:
    - A recent systematic review found an average colonization rate of 20%, with the highest rates reported in African countries.
7. Transmission of Infection:
    - Vertical transmission occurs in approximately half of newborns from colonized mothers, with an infection rate estimated between 1% and 4% when antibiotics are not used.
8. Prevention Methods:

- Prevention includes identifying colonized pregnant women through vaginal/rectal swabs between the 35th and 37th weeks of gestation.

9. Cultivation Methods:
    - A specialized culture medium, such as Todd Hewitt broth, is used to enhance bacterial growth.
10. Isolation and Identification Methods:
    - Isolation methods include the CAMP test **(Christie–Atkins–Munch-Peterson)**, serogrouping, chromogenic media, and molecular tests.
11. Treatment of Infection:
    - Penicillin is the preferred treatment, with no known resistance, while erythromycin and clindamycin are used for penicillin-allergic pregnant women.
12. Bacterial Resistance:
    - An increase in resistance among GBS **(Group B Streptococcus)** strains to erythromycin and clindamycin has been observed, associated with specific genes.
13. Epidemiological Impact:
    - GBS **(Group B Streptococcus)** infections in neonates represent a significant epidemiological problem, with rising antibiotic resistance.
14. Study Objectives:
    - The study aims to compare identification methods, verify the susceptibility profile, and determine the resistance genes of GBS strains isolated from pregnant women in prenatal care in Vitória da Conquista, Bahia State, Brazil.

Materials and methods

Study design, period, region, and population

A- Study design : cross- sectional study.
B- Period : from January 2017 to February 2018.
C- Region : the county of Vitória da Conquista, in Bahia State, Brazil.
D- Population : 186 vaginal and rectal secretion samples from pregnant women.

Inclusion and exclusion criteria

A- Inclusion criteria:
1- Pregnant women with a 32-to-40 gestational age.
2- lives in the urban area of the county.

B- Exclusion criteria:
if the participants had used antibiotics in the last 7 d before collection.

Samples collection

A- Item used: single vaginorectal swabs (without using speculum)
B- Area: from the lower middle third region of the vagina and from perianal region.
C- Sample packaging: were packaged in Stuart transport media.
D- Sample transfer to: the Clinical Analysis Laboratory of the Multidisciplinary institute of Health of the Federal University of Bahia (within 8 h after collection).

Bacterial isolation and identification

A- Isolation process:
1- The samples were seeded by depletion technique onto Streptococcus chromIDTMStreptoB chromogenic medium.
2- inoculated in Todd Hewitt broth.
3- placed at a 35°C-37°C bacteriological incubator for 24 h.
4- subcultured in chromogenic agar and kept in the incubator at 37°C for 24 h.

B- Identification:
- All pink or red colonies (characteristic for GBS identification) underwent legitimized identification tests.
- All serogrouping-confirmed isolates were forwarded for antibiogram and an aliquot of each of them was cryopreserved at -20oC in a Brain Heart Infusion (BHI) broth with 15% glycerol.

Antimicrobial susceptibility testing

A- Testing type: disk diffusion antimicrobial susceptibility test.
B- Antibiotics used for testing:
1- Penicillin (10 units).
2- Ampicillin (10 µg).
3- Cefotaxime (30 µg).

4- Erythromycin (15 µg).
5- Clindamycin (2 µg).
6- Vancomycin (30 µg).

Determination of erythromycin resistance phenotypes

- A D-test was performed for each erythromycin and/or claritromycin-resistant sample.
- The clindamycin and erythromycin resistances were considered indicators of constitutive MLSB phenotype

Procedure:

1- Comercial Erythromycin (15 µg) and Clindamycin (2 µg) discs were placed 12 mm apart on Mueller Hinton agar plates supplemented with 5% blood.
2- kept at a 35- 37oC, 5% CO2, incubator for 24 h with subsequent observation of the bacterial growth inhibition halo.

Result:

1- Reduction of the bacterial growth inhibition halo around the clindamycin disc in the region close to the erythromycin disc, which defined the inductive MLSB phenotype.
2- Isolated sensitivity to clindamycin indicated the M phenotype and the sensitivity to erythromycin alone defined the L phenotype.

Determination of the resistance genetic profile

1- The presence of resistance genes in erythromycin and/or clindamycin resistant strains was assessed by PCR
- Resistance genes for Erythromycin: ermB, ermTR, and mefA
- Resistance gene for clindamycin: linB

2- The bacterial DNA for the PCR performing was extracted by boiling method
3- The PCR results were analyzed according the presence or absence of bands in 2% agarose gel in 1x TAE buffer solution, stained with ethidium bromide and visualized on UV transilluminator after electrophoretic run.

Result

Item	Result
Number of analyzed sample	186
Number of GBS-positive sample	32 (17.2% among pregnant women)
Culture method	CRO1: 26 positive, 1 unconfirmed
	CRO2: 32 positive, 1 unconfirmed
Cross analysis result	1 positive only in CRO1
	24 positives in both method
	7 positives only in CRO2
CRO2 performance	Sensitivity: 96.9%
	Positive predictive value (PPV) 96.9%
	Negative predictive value (NPV): 99.3%
Antibiotic susceptibility	All strains susceptible to penicillin, ampicillin, cefotaxime, and vancomycin
Antibiotic resistance	6 strains (18.8%) resistant to clindamycin
	8 strain (25.0%) resistant to erythromycin
	All resistant strains had negative D-test result
Resistance phenotypes	2 strains (25%) with M phenotype
	6 strain (75%) with cMLSB phenotype
Resistant genes	4 isolates (44.4%) carrying emrB genes
	4 isolates (44.4%) carrying mefA genen
	1 isolate (11.1%) carrying ermTR gene
	7 isolates (87.5%) had a single resistance gene
	1 isolate (12.5%) had two resistance genes (mefA+ermB)
	No strains carried linB gene

Discussion

❖ GBS Colonization Prevalence:

- Study Finding: 17.2% prevalence of GBS colonization.
- Consistency: Aligns with previous studies (Oliveira, Borger) and CDC's reported range of 10%-30%.
- International Data:
  - Taiwan: 21.8%
  - Italy: 25.5%
  - Ethiopia: 19.0%
  - Pakistan: 17.0%
- Global Estimate: 18% maternal colonization, varying from 11% to 35%.
- Significance: Highlights the importance of GBS screening to manage and prevent infections.

❖ Importance of GBS Screening:

- Current Situation: No routine GBS screening in the studied county.
- Risk: High GBS prevalence could lead to 1%-2% invasive infections in newborns from colonized mothers.
- Recommendation: Implement a GBS screening protocol to reduce risks.

❖ CDC Recommendations and Advances for GBS Identification:

- Current CDC Recommendations:
  1. Enrichment: Use Todd Hewitt broth.
  2. Cultivation: Grow on 5% sheep blood agar.
  3. Identification: CAMP test for presumptive identification, serogrouping for confirmation.

  Note: Direct seeding without enrichment may increase false negatives.

- Advancements:

  Chromogenic Media: Newer media, such as ChromID Strepto B which use in this study, enable the detection of both hemolytic and non-hemolytic strains, addressing the limitations of older media that could not identify non-hemolytic strains associated with more severe infections.

❖ Comparison of GBS Identification Methods:

Method	Sensitivity	Specificity	Advantages	Disadvantages
CRO1	78.1%	96.3%	Fast, easy visualization, lower cost	Lower sensitivity; may miss some positives
CRO2	96.9%	99.3%	High sensitivity and specificity; detects both hemolytic and non-hemolytic strains	Slightly more complex due to enrichment step
CAMP	100%	N/A	High sensitivity; good for presumptive identification	Not suitable as sole confirmatory test; possible false positives
SERO	N/A	N/A	Confirmatory test for GBS	Requires additional tests; time-consuming

CRO2 is the most effective method for GBS screening due to its high sensitivity, specificity, and ability to minimize false negatives.

❖ Resistance Rates and Phenotypes in GBS Strains:

- Resistance Rates:
  - Erythromycin: 25%
  - Clindamycin: 18.8%

- Comparison of Resistance Rates with other studies

Study	Erythromycin Resistance	Clindamycin Resistance
Current Study	25%	18.8%
Melo et al.	8.1%	5.9%
Dutra et al.	4.1%	3.0%
Borger et al.	9.4%	6.2%
Bolukaoto et al. (South Africa)	21.1%	17.2%
Mengist et al. (Ethiopia)	6.5%	3.2%
Frohlicher et al. (Switzerland)	14.5%	8.2%
Pinheiro et al. (Portugal)	15%	9.6%

- Resistance Phenotypes

Aspect	Details
Resistance Phenotypes	cMLSB: (75%) M: (25%)
Associated Genes	cMLSB: ermB, ermTR M: mefA Combination of mefA and ermB Genes: Found in some samples, indicating multiple mechanisms of resistance.
Resistance Types	cMLSB: Macrolides, Lincosamides, Streptogramins B M: Macrolides
D-test Results	100% of erythromycin-resistant strains did not show reduced sensitivity to clindamycin.
Comparison with Other Studies	Dutra et al. (Brazil): Did not detect mefA gene Bolukaoto et al. (South Africa): Found cMLSB, iMLSB, M, and L phenotypes.

This study found higher macrolide resistance in GBS compared to some earlier research. It highlights the importance of:

- Susceptibility Testing: Crucial for selecting the right treatment, especially for those with penicillin allergies.
- Penicillin Allergy: Rare but requires close monitoring of resistance due to macrolide resistance genes.
- Resistance Monitoring: Regular testing is needed as resistance can spread between bacteria.

Conclusion:

This study represents the first comparison of identification methods and the analysis of the antimicrobial susceptibility profiles of GBS strains in the specified county. The findings may contribute to the development of future screening and antibiotic prophylaxis protocols designed to prevent GBS infections in newborns.

Abbreviation:

GBS	Streptococcus agalactiae
CRO1	Biological samples directly seeded in chromogenic media
CRO2	Biological samples seeded in chromogenic media after growth in Todd Hewitt broth
CAMP	Christie, Atkins, and Munch-Petersen test
SERO	Serogrouping
ChromID StreptoB	A chromogenic medium developed by BIOMERIEUX, specifically designed for the isolation and identification of Streptococcus agalactiae
cMLSB	Constitutive Macrolide-Lincosamide-Streptogramin B
iMLSB	Inducible Macrolide-Lincosamide-Streptogramin B
M phenotype	Macrolide-resistant phenotype
L phenotype	Lincosamide-resistant phenotype
mefA	Macrolide efflux gene A
ermB	Erythromycin ribosome methylation gene B
ermTR	Erythromycin ribosome methylation gene T and R