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Research Article

A comparative study of isolation, identification and grouping of streptococcal isolates from throat swabs of school going children applying different methods of extraction for antigen

Mohana S. J[*a], Sasikumar C[b] and Yasodha S[c]

[a*]*Department of Plant Biotechnology, Gurunanak College, India*
[b]*Department of General Engineering, Jeppiaar Institute of Technology, India*
[c]*Royal Bio Research Centre, Chennai, India*

QR Code

***Correspondence Info:**
Dr. Mohana S. J
Department of Plant Biotechnology,
Gurunanak College, India

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Abstract

Objective: The objective of this research was to collect the throat swabs from symptomatic and asymptomatic children and microbiologically positive sample were further taken up for grouping.

Methods: Extraction of streptococcal group antigen by various methods namely enzyme extraction, fullers extraction, Lancefield extraction. Comparing the antigen extraction methods in identifying the sero groups of BHS.Comparative studies between conventional and enzymatic methods of extraction. Which one is ideal method of extraction and which one is common among the children.

Results: Throat swabs were collected in the age group of 5-12 both symptomatic and asymptomatic children. Symptomatic such as rashes, throat pain, fever, cold, cough etc. Totally 361 throat swabs were collected of which 19 were BHS.18 isolates were typeable and 1 was non typeable. In enzyme extraction is not effective in the extraction of group D streptococci may be extracted with mutanolysin only after 4H incubation although effective for the extraction of some strains of group D streptococci. In fullers extraction it was seen to give an important clue to the structure of cell wall of gram positive bacteria in lance field extraction was easily performed specific and sensitive method.

Conclusion: Group A streptococcal isolates were just 2 in no which shows that group A is not the predominant strain in our study. There by denoting the prevalence of rheumatic fever and RHD are becoming less. There was no cross reaction among groups were lancefield and fullers method were applied. In enzymatic method there was slight cross reaction noted between A and D in one isolate,F and G in one isolate and C and G in another isolate.

Keywords: Streptococcal, Throat swab, children, enzymatic study.

1. Introduction

Streptococcus is a genus of spherical Gram-positive bacteria belonging to the phylum Firmicutes and the lactic acid bacteria group. Cellular division occurs along a single axis sin these bacteria, and thus they grow in chains or pairs, hence the name. In addition to strep throat, certain *Streptococcus* species are responsible for many cases of meningitis, bacterial pneumonia, endocarditis, erysipelas and necrotizing fasciitis (the 'flesh-eating' bacterial infections); Based on specific carbohydrates in the bacterial cell wall. These are named Lancefield groups A to T, although some species, such as *S. pneumoniae*, do not express Lancefield antigens. In the medical setting, the most important groups are the alpha-hemolytic streptococci, *S. pneumonia* and *Streptococcus* Viridan. S-group and the beta-hemolytic streptococci of groups A and B (also known as "Group A Strep" and "Group B Strep"). The streptococci possess a typical gram – positive cell wall consisting of peptidoglycan and teichoic acid. Most streptococci, except for many of the viridans group, have a layer of a group of common c carbohydrate (polysaccharide), which can be used to classify an isolate serologically. A schematic

diagram of the streptococcal cell wall is shown in figure 15-2. Other cell-wall antigens are present in specific c carbohydrate groups and are explained in the discussions of the individual groups. Some species can produce a type-specific polysaccharide capsule as well.

Several different approaches to classification of the catalase-negative, gram – positive cocci have been used. Four commonly used classification schemes are (1) hemolytic pattern on sheep red blood cell agar; (2) physiologic characteristics; (3) serologic grouping or typing of c carbohydrate (lancefield classification), capsular polysaccharide, or surface protein, such as the m protein of streptococcus pyogenes; and (4) biochemical characteristics. The identification process for a streptococcal isolate in the clinical laboratory may use features from each scheme.

The clinical laboratory scientist often makes an initial classification of the streptococci based on the hemolytic pattern of the isolate grown on sheep blood agar (sba). The types of hemolysis possible are outlined in table 15-1. When lysis of the red blood cells (rbcs) in the agar surrounding the colony is complete, the resulting area is clear; this is referred to as β – hemolysis partial lysis of the rbcs results in a greenish discoloration of the are surrounding the colony and is termed ∝ hemolysis

When the RBCS immediately surrounding the colony are unaffected, the bacteria are termed nonhemolytic. Some references term this result hemolysis. Because no lysis of the RBCS occurs, however, the term hemolysis is confusing and is not recommended. Some isolates belonging to the viridans group produce what is called wide-zone or ∝ prime hemolysis. The colonies are surrounded by a very small zone of no hemolysis and then a wider zone of β hemolysis. This reaction may be mistaken for β hemolysis at first glance. The use of a dissecting microscope or the scanning objective shows the narrow zone of intact RBCS and the wider zone of complete hemolysis. Streptococci have also been classified according to physiologic characteristics. This classification divides the species into four groups: pyogenic streptococci, lactococci, enterococci, and viridans streptococci. Organisms in group a possess the same antigenic c carbohydrate; those in group b have the same c carbohydrate, and so on. The classic lancefiled serologic grouping has been most significant in classifying and identifying β hemolytic streptococci. During the past several decades, however, since DNA relatendness has been applied to the classification and identification of ∝ - hemolytic streptococcai species, investigators have found no correlation between genetic relationships and streptococcal group antigens. Contrary to group B β hemolytic streptococci, in which there is only one species identified, ∝ hemolytic streptococci as a whole are phenotypically and genotypically diverse and therefore difficult to characterize. Streptococcal species other than those that produce β hemolysis possess C carbohydrate. Some are found as normal flora in animals or as animal pathogens, and others may be found in both humans and animals. The lancefiled groups seen in human infections are A, B, C, D, F, G, and N, although not all members of these groups cause human infection. Biochemical identification can be performed even by small laboratories. Although definitive identification requires a large number of biochemical characteristics or perhaps serologic methods, presumptive identification can be accomplished relatively easily with a few key tests and characteristics Presumptive identification, in the great majority of cases, possesses a high enough rate of accuracy to be useful to the clinician and does not require the exhaustive additional tests that are needed to meet the criteria for definitive identification, especially for species in groups A, B, and D, as well as streptococcus Pneumoniae and enterococcus, speciation of the viridans streptococci, however, does require a considerable increase in the number of tests. In selecting an identification scheme or kit, the clinical laboratory scientist must evaluate the needs of the clinicians and patient population served, the cost of an expanded identification scheme, the resources and abilities of the laboratory, and the usefulness of the data obtained.

. Some stains of *S. Pyougenes* cause a red spreading rash, referred to as scarlet fever, caused by streptococcal pyrogenic extoxins (Spes), formerly called erythrogenic toxins. The three immunologically distinct types are SpeA, Spe B, and Spe C. These proteins share the ability to stimulate T. Lymphocyte cell proliferation by interaction with class II Major histocompatibility complex (MHC) molecules on antigen presenting cells and specific V B – Chains of the T-Cell receptor. This interaction results in the production of interleukin-1, tumor necrotizing factor, and other cytokines that appear to mediate the disease processes associated with these toxins.

2. Materials and methods

2.1 Sources of Specimen

361 throat swabs were collected from symptomatic and asymptomatic children in schools and hospitals at the age of 5 to 12. Symptoms such as fever, throat pain, rashes, cough, etc., were looked for specimens were collected by using sterile cotton swabs in the tonsillar area and the posterior pharynx. Children were asked to gargle their throat with sterile saline to get rid off the commensals.

2.2 Isolation and Identification

The specimens were streaked in to bap and cap and incubated at 37º c. On next day colonies were observed for presence of bhs and identified by gram's staining and

catalase test. After isolation and identification of streptococci they were stored in RCM. streptococcal antigens extraction was done with formamide, Hcl and enzyme extraction method.

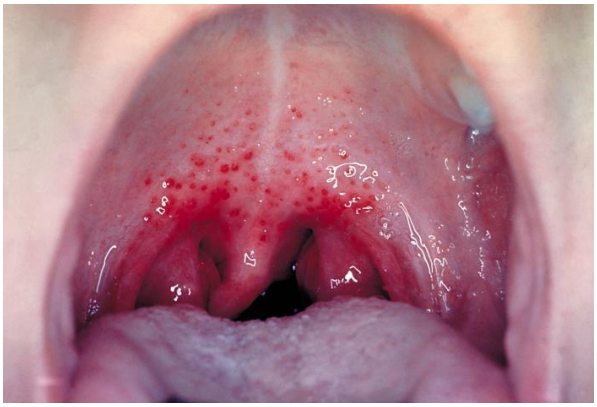

Figure 1: Strep Throat

2.3 Presumptive identification

Bacitracin disc test

The high sensitivity group of GABHS to bacitracin is used as a screening method for differentiating between group a and non group a streptococci.bhs strains showing zones of inhibition around special bacitracin sensitivity discs, containing 0.05u of bacitracin are considered, presumptively, to be gas. Some investigators have also reported good success using discs containing 0.11u of bacotracin. However others have reported that this concentration increased the number of non group astrains exhibiting zones of inhibition. The concentrations of bacitracin mentioned produce at least some zone of inhibition in practically all strains of gas since a small fraction of group c and group g streptococci display a similar sensitive to bacitracin. The concentration is critical discs containing higher concentrations of bacitracin should not be used.

The bacitracin should be used only on pure culture of streptococci, not on primary cultures on non selective agar media.as with any antibiotic sensitivity test many factors can influence the outcome in particular use of too light an inoculum or of bap that are too thin may results in over sized zones of inhibition.

2.4 Methodology

Enzyme Extraction method

Cultures: Colonial characteristics hemolysis and cell morphology before staining the test. Ensure that the organisms to be tested are gram positive and catalase negative .Any BAP culture yielding 2-6 well separated colonies may be used, they should have been inoculated from a pure culture of organism.

Principle: Streptococci carry group specific carbohydrate antigens in their cell walls .after extraction by a specifically developed enzyme preparation these antigens will agglutinate latex particles will agglutinate latex particles coated with the corresponding antibody. The latex remains in smooth suspension in the absence of group specific antigen.

Method: Using a sterile bacteriological loop. Pick 6 colonies of strep (avoiding other types of colony on the plate) and emulsifying them in 0.4 ml extraction enzyme). Incubate the mixture in water bath at 37^0C for 10 minutes shake the tubes vigorously after 5 minutes incubation. Resuspended the latex reagents by gently agitation. Dispense 1 drop of each latex onto a circle on the test slide. Add one drop of the extract from a Pasteur pipette (or another device delivering appropriate 50 microliters) to each drop of latex reagent and mix the contents of each circle with a separate mixing stick. Rotate the slide for not longer than 1 minute then observe for agglutination.

Fullers Method (Formamide Extraction)

Equipment and supplies

Centrifuge

Water bath heater adjustable to 160˚c

Reagents

THB culture of the strain to be extracted, incubated for 17-24 hours at 37˚c

Formamide.

Acid alcohol (1ml of 36%HCL, 99ml of 95% ethanol)

Acetone

Saline, 0.85%

Phenol red pH indicator solution. Dissolve 0.1 g phenol red in 28ml sodium hydroxide

0.01 mol/l, and adjust the volume to 250ml with dis-Sodium hydroxide, 0.2mol/l

Methodology

1. Centrifuge (approximately 1500 g for 30 minutes) 5 ml of the Todd-Hewit: both culture and discard the supernatant.
2. Resuspend the pellet in 0.1ml of formamide and mix weil.
3. Heat at 160 C in the oil-bath for 10 minutes or until almost completely dissolves.
4. Cool, add 0.25ml of acidic alcohol and shake.
5. Centrifuge (at least 650g for 30 minutes) to remove the precipitate and collect the supernatant.
6. Add 0.5-1.0ml of acetone (the amount depends on formation of precipitate) to the clear supernatant fluid.
7. Centrifuge (at least 650 g for 30 minutes) to collect the precipitate (group-specific polysaccharide).
8. Discard the supernatant and dissolve the precipitate in 0.3-0.4 ml of saline.
9. Add 1 drop of phenol red solution and adjust the ph to 7.2 with NAOH 0.2mo/l
10. Add one drop of the extract from a Pasteur pipette (or another device delivering appropriate 50 microliters) to

each drop of latex reagent and mix the contents of each circle with a separate mixing stick.

11. Rotate the slide for not longer than 1 minute then observe for agglutination.

Figure 2: Supernatant

Figure 3: Sreptococci Extract

Lancefield Method (Hydrochloric acid extraction)

Equipment and Supplies

Centrifuge, water bath at 100c

Reagents

TDH culture of the strain to be extracted, incubated over night at 35-37c

HCL, 0.2mol/l(may be prepared in 0.85% saline,)

NAOH.1.0mol/l

NAOH, 0.2mol/l

Phenolred ph indicator solution

Method

1. Centrifuge (650-1500g for 30 minutes)30 -40ml of the TDH culture and discard the supernatant .If a firm ,secure pellet is not obtained, the higher speed or longer
2. Centrifuging items will be required.
3. Resuspend the pellet in 0.35ml of HCL,
4. Heat this suspension in a boiling water bath at 100c for 10 minutes
5. cool the suspension to RT and add one drop of phenolred solution
6. Adjust the pH initially with NAOH 1mol/l.as the endpoint is approached. Centrifuge above and collect the supernatant fluid (lancefield extract)
7. Add one drop of the extract from a Pasteur pipette (or another device delivering appropriate 50 microlitres) to each drop of latex reagent and mix the contents of each circle with a separate mixing stick.
8. Rotate the slide for not longer than 1 minute then observe for agglutination.

Positive Result

The test should be considered positive when agglutination occurs within 1 minute

Negative Result

A negative result is obtained if no agglutination occurs after 1 minute.

Positive Control

Dispense 1 drop onto a separate circle of the card. Dispense 1 drop of control onto each of the 5 circles. Spread each mixture over the entire area of the circle, using a separate stick for each test. Rock the card manually for 1 minute. Each of the five test antisera suspensions should demonstrate obvious agglutination.

Negative Control

Dispense 1 drop of negative control and add the given solution onto a separate circle of the card .No agglutination occurs.

3. Results and discussion

Throat swabs were collected in the age group of 5-12 both symptomatic and asymptomatic children's. Totally 361 throat swabs were collected of which 19 were BHS. 18 isolates were typeable and one was non typeable.

Table 1: Sex wise distribution of sample

No. of sample	Male	female
361	150	211

Table 2: Age wise distribution

Age group	No. of sample
5-6	51
6-7	40
7-8	37
8-9	41
9-10	58
10-11	69
11-12	65

Throat swabs were collected from both symptomatic and asymptomatic childrens, symptomatic such as rashes, throat pain, fever, cold, cough, etc.

Table 3: Sampling distribution

No. of sample	Symptomatic	Asymptomatic
361	249	112

19 positives were extracted by three different methods. 18 were typeable and one was nontypeable.

Table 4: Group distribution

Positive sample	Typeable	Non-typeable	Symptom-matic	Asympto-matic
19	18	1	14	5

Three different methods of extraction are enzyme, fullers and lancefield method.

Enzyme Extraction

Malcolm *et al* described enzyme solution will extract the serogroup antigens from single colonies of groups A, B, C, F, G streptococci in 1 MIN at R.T. this enzyme extraction is not effective for the serogrouping of all group D streptococcus species .enzyme extracts can be used with latex or co agglutination reagents. Enzyme pronase is not effective in the extraction of group D streptococci, may be extracted with muatanolysin only after 4 H incubation archomopeptidase, although effective for the extraction of some strains of group D streptococci. In our results cross reaction occurred in enzyme extraction. Group A, C, F cross reaction occurred in the group G in enzyme extraction. When compared to other extraction strongest answer was opted.

Table 5: Extraction of enzyme method distribution as follows

Group distribution	No. of isolates	Percentage of isolates	Positive samples
Group A	3	16.66%	18
Group B	5	27.77%	18
Group D	1	5.55%	18
Group F	2	11.11%	18
Group G	7	38.88%	18

Cross reactions more in the enzyme extraction method.

Figure 4: Results of Enzyme Extract

Fullers Extraction

Gooder H And Maxted W R Vol.No.182;808-9;1958 Rebecca Lancefields Hot Acid Extraction Method For Serogrouping Haemolytic Streptococci .Many Years Later, However It Was Seen to give an important clue to the structure of the cell wall of gram positive bacteria. Fuller's method tended to be reserved for use with strains that gave unexpected negative or equivocal results in lancefield grouping. An important feature of the fuller procedure was the sequential treatment of the extract with acid alcohol and acetone, which remove much of the cross reactivity material and concentrated the group polysaccharide. In our results the distribution of serogrouping was just same as enzymatic extraction and form amide extraction was almost in accordance.

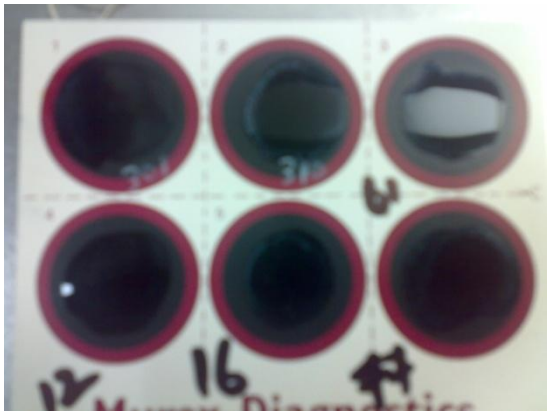

Figure 5: Results of Fullers Extract

Table 6: Extraction of Fullers Method Distribution

Group distribution	No. of isolates	Percentage of isolates	Positive samples
Group A	3	16.66%	18
Group B	5	27.77%	18
Group D	1	5.55%	18
Group F	2	11.11%	18
Group G	7	38.88%	18

No cross reaction were observed in fullers extraction

Lancefield Extraction

Waleria *et al* described two new methods for serological grouping of BHS the nitrous acid extraction and the slide agglutination method of Christensen *et al* were compared with the lancefield hot HCL extraction method. It was easily performed, specific, and sensitive when highly potent antisera were used .for the Christensen method these highly potent antisera had to be diluted to avoid cross reactions between group A and C and group B and G, respectively. A few strains, most of them group B could not be grouped by the latter method. Using these 3 grouping methods 2 sets of commercial sera were compared with the more potent sera supplied by R.C. Lancefield .the low antibody content of these commercial sera especially anti-group B and antisera, contributed to the inferior results obtained in some of the grouping reactions.

In our results group A that which was identified as enzymatic and formaline that reacted for group D. there is no accordance possibility of so cross reactivity occurs.

Table 7: Extraction of hot acid method

Group distribution	No. of isolates	Percentage of isolates	Sample positives
Group A	2	11.11%	18
Group B	5	27.77%	18
Group D	2	11.11%	18
Group F	2	11.11%	18
Group G	7	38.88%	18

4. Conclusion

Throat swabs of the 361 children 150 were boys and 211 girls. It was observed that BHS were isolated from 18 children (4.98%) out of these 361.There is evidence that asymptomatic throat infection caused by GAS may lead to acute rheumatic fever. Asymptomatic infections with subsequent rise in streptococcal antibody have also been reported in patients with previously diagnosed rheumatic fever. The prevalence of asymptomatic carriage of BHS has been reported between 11-47% from various countries. However they observed that the prevalent of BHS in throat of asymptomatic school children was 18.8% and group A was the predominant strain. Various studies conducted in south India have reported a predominance of group c over group A organisms. In our study the prevalence of BHS in throat of asymptomatic school children was % and group G was most predominant one. Pharyngitis and scarlet fever result in complications of streptococcal infections but are rare in asymptomatic carriers. Antibiotics have been shown to reduce the severity of acute symptoms and the shorten the duration of the illness by about one day .another important reason for treating uncomplicated streptococcal pharyngitis is to markedly reduce the incidence of subsequent RF or its recurrence. Antibiotics such as penicillin, ceftizoxime, cephalosporins, amoxicillin are used for the treatment. Rapid streptococcal tests utilize antibodies to detect streptococcal antigens .there are four different direct streptococcal antigen detection platforms available in a wide number of different commercial products these are latex agglutination, optical immunoassay & immunochromatographic detection. The majority of rapid assays use today are based on double antibody sandwich & /or immunochromatgraphy because these techniques do not require mixing, multiple reagent, addition or washing. In our results group A that which was identified as enzymatic and formaline that reacted for group D. There is no accordance possibility of so cross reactivity occurs. The distribution of serogrouping was just same as enzymatic extraction and formamide extraction was almost in accordance.

Conflict of interest

All authors have none to declare.

References

[1]. Ryan K J ;Ray CG., Sherris Medical Microbiology (4th edition)MC Graw Hill –2004; ISBN 0 -8385-85 29-9

[2]. Facklam R. What Happened To The streptococci overview of what happened to the streptococci: overview of taxonomic and nomenclature changes". *Clin. Microbiol. Rev.* 2002; 15 (4): 613–30.

[3]. Patterson MJ. Streptococcus. In: Baron's Medical Microbiology (Baron S *et al*, eds.) (4th ed.). Univ of Texas Medical Branch. ISBN 1996; 0-9631172-1-1.

[4]. Facklam R. "What happened to the streptococci: overview of taxonomic and nomenclature changes". *Clin Microbiol Rev* 2002; 15 (4): 613–30. doi:10.1128/CMR.15.4.613-630.2002.

[5]. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. "Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC". *MMWR Recomm Rep* 2002; 51 (RR-11): 1–22.

[6]. Huang PY, Lee MH, Yang CC, Leu HS. Group B streptococcal bacteremia in non-pregnant adults. *J Microbiol Immunol Infect*. Jun 2006; 39(3):237-41.

[7]. Sendi P, Johansson L, Norrby-Teglund A. Invasive group B Streptococcal disease in non-pregnant adults : a review with emphasis on skin and soft-tissue infections. *Infection.* Mar 2008; 36(2):100-11.

[8]. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, *et al*. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA.* May 7 2008; 299(17):2056-65.

[9]. Gardam MA, Low DE, Saginur R. Group B streptococcal necrotizing fasciitis and streptococcal toxic shock-like syndrome in adults. *Arch Intern Med.* 1998; 158:1704-8.

[10]. Wu HM, Janapatla RP, Ho YR, Hung KH, Wu CW, Yan JJ, *et al*. Emergence of fluoroquinolone resistance in group B streptococcal isolates in Taiwan. *Antimicrob Agents Chemother*. May 2008; 52(5):1888-90.

[11]. Bayer AS, Chow AW, Anthony BF. Serious infections in adults due to group B streptococci. Clinical and serotypic characterization. *Am J Med.* 1976; 61:498-503.

[12]. Colford JM Jr, Mohle-Boetani J, Vosti KL. Group B streptococcal bacteremia in adults. Five years' experience and a review of the literature. *Medicine (Baltimore).* Jul 1995; 74(4):176-90.

[13]. Dworzack DL, Hodges GR, Barnes WG. Group B streptococcal infections in adult males. Am J Med Sci. 1979; 277:67-73.

[14]. Farley MM, Harvey RC, Stull T. A population-based assessment of invasive disease due to group B Streptococcus in nonpregnant adults. *N Engl J Med.* 1993; 328:1807-11.

[15]. Gallagher PG, Watanakunakorn C. Group B streptococcal bacteremia in a community teaching hospital. *Am J Med*. May 1985; 78(5):795-800.

[16]. Harrison LH, Ali A, Dwyer DM. Relapsing invasive group B streptococcal infection in adults. *Ann Intern Med.* 1995; 123:421-7.

[17]. Jackson LA, Hilsdon R, Farley MM. Risk factors for group B streptococcal disease in adults. *Ann Intern Med*. 1995; 123:415-20.

[18]. Lerner PI. Meningitis caused by Streptococcus in adults. *J Infect Dis*. 1975; 131 Suppl: S9-16.

[19]. Lerner PI, Gopalakrishna KV, Wolinsky E. Group B streptococcus (S. agalactiae) bacteremia in adults: analysis of 32 cases and review of the literature. *Medicine (Baltimore)*. 1977; 56:457-73. .

[20]. Opal SM, Cross A, Palmer M. Group B streptococcal sepsis in adults and infants. Contrasts and comparisons. *Arch Intern Med*. 1988; 148:641-5.

[21]. Persson E, Berg S, Bergseng H, Bergh K, Valsö-Lyng R, Trollfors B. Antimicrobial susceptibility of invasive group B streptococcal isolates from south-west Sweden 1988-2001. *Scand J Infect Dis*. 2008; 40(4):308-13.

[22]. Schuchat A. Group B streptococcus. *Lancet*. Jan 2 1999; 353(9146):51-6. .

[23]. Schwartz B, Schuchat A, Oxtoby MJ. Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *JAMA*. 1991; 266:1112-4.

[24]. Trivalle C, Martin E, Martel P. Group B streptococcal bacteraemia in the elderly. *J Med Microbiol*. 1998; 47:649-52.

[25]. Hickman M.E., Rench M.A., Ferrieri P., Baker C.J. Changing epidemiology of group B streptococcal colonization. *Pediatrics* 1999; 104:203-9.

[26]. Moyo S.R., Mudzori J., Tswana A.S., Maeland J.A. Prevalence, capsular type distribution, Anthropometric and obstetric factors of group B Streptococcus (Streptococcus agalactiae) colonization in pregnancy *Cent Afr J Med* 2000; 46: 115-20.

[27]. Rocha M.L.F., Beltrame L.M.R., Guedes S.C. Prevalência do Streptococcus agalactiae na flora vaginal de gestantes do último trimestre. *Rev Bras Patol Clin* 1984; 20:110-2.

[28]. Smânia Jr.A., Benchetrit L.C., Smânia E.F.A., Fracalanzza S.E.L. Isolamento de estreptococos do grupo B, de gestantes e neonatos, em Florianópolis, Santa Catarina. *Rev Bras Anal Clin* 1986; 18:103-8.

[29]. Mocelin C.O., Carvalho D.A.F., Brites C., *et al*. Isolamento de Streptococcus agalactiae de gestantes na região de Londrina, PR. *Rev Bras Ginecol Obstet* 1995;17:915-8.

[30]. Miura E., Martin M.C. Group B streptococcal neonatal infections in Rio Grande do Sul, Brazil. *Rev Inst Med Trop São Paulo* 2001; 43:243-6.

[31]. Vaciloto E., Richtmann R., Costa H.P.F., *et al*. A survey of the incidence of neonatal sepsis by group B streptococcus during a decade in a Brazilian maternity hospital. *Braz J Infect Dis* 2002; 6:55-62.

[32]. Beraldo C., Brito A.S.J, Saridakis H.O., Matsuo T. Prevalência da colonização vaginal e anorretal por Estreptococo do grupo B em gestantes do terceiro trimestre. *Rev Bras Ginecol Obstet* 2004; 26:543-9.

[33]. Baker C.J., Edwards M.S. B streptococcal infections. In: Remington J., Klein J.O. (eds.). Infectious diseases of the fetus and newborn infant. 4th ed. Philadelphia: WB Saunders, 980-1054.

[34]. Torres M.O., Sánchez-Pérez H.J., Nazar-Beutelspacher A., *et al*. Fatores asociados a la colonización por Streptococcus del grupo B en mujeres embarazadas de Los Altos, Chiapas. *Salud Pública de Méx* 2000; 42:1-15.