Evaluation of Group B Streptococci Among Egyptian Labouring Women and Their Neonates

Thesis
Submitted for Partial Fulfilment
of the Master Degree in
Gynecology and Obstetrics

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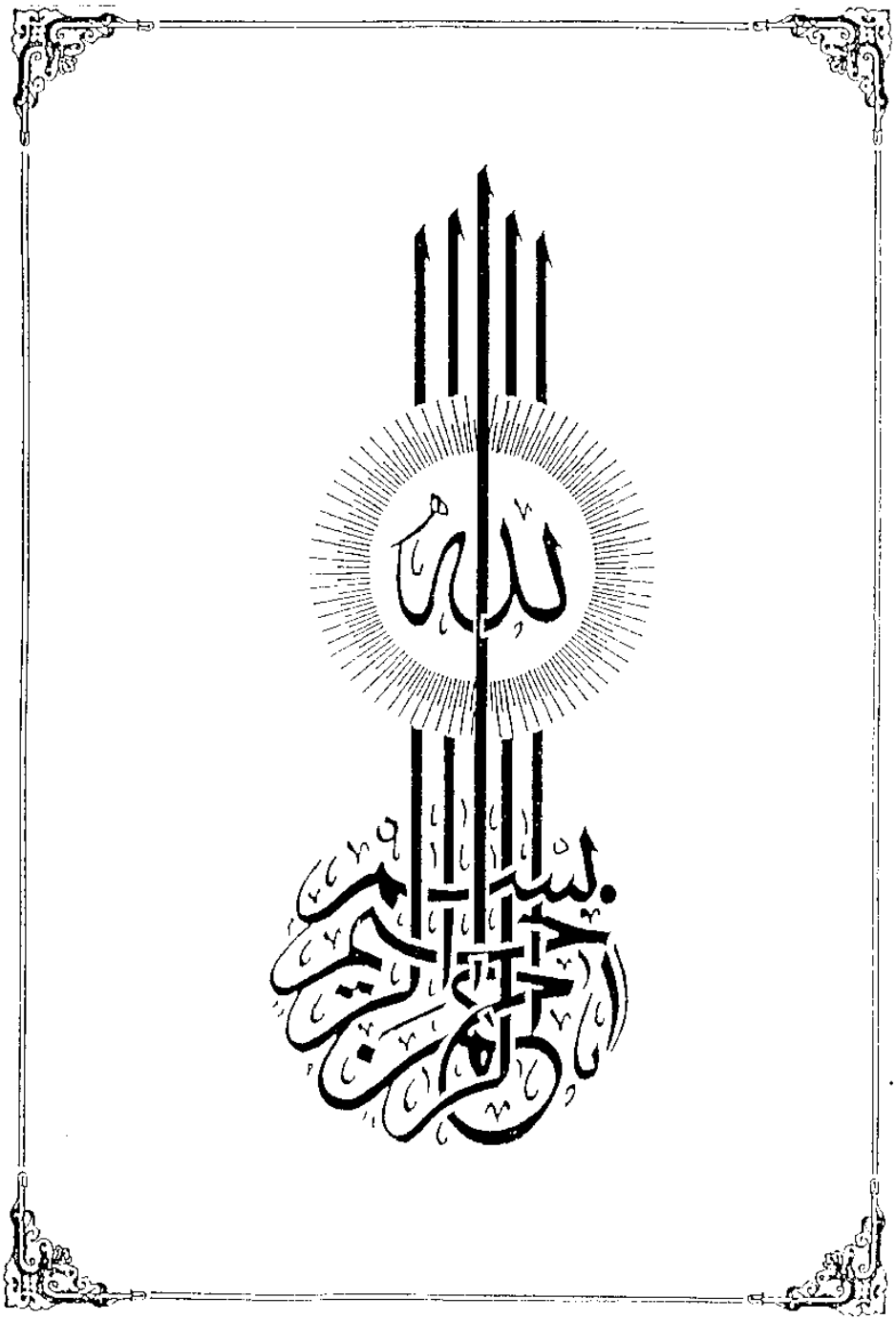
ACKNOWLEDGMENT

I would like to present my cardial gratitude to Prof. D.M. Ekram Shoukry for his fruitful directions and advise that helped me too much in the presentation of this work.

Special thanks to Dr. Mohamed Yehia for his continuous sharing, opinions and advise during writing this thesis.

I would like to present my special thanks to Dr. M. Abdel-Halim Hafez for his help in my work at Central Research Medical Laboratory.



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INTRODUCTION

Group B streptococcus (GBS), called streptococcus agalactide, first recognized by Billeroth in 1873, is classified into order Eubacteriales family Lactobacilleae, class schizomycetes and genus streptococcus on the basis of its biochemical and physiological charcteritics. It is subdivided into types Ia, Ib, Ic, II, III, X and R on the basis of carbohydrate and protein antigens present on its wall. Bovine strains of GBS are found in the bovine teat, while human strains are present in the femal vagina, the orophorynx, anorectum and the external auitory canal of newborns. It could be transmitted vertically from mother to child in utero and during parturition, cross infection by the nursery staff could also occur during the immediate postpartum period. Two types of diseases are caused in the newborn: The early onset disease occuring within a week of birth and the Late disease presenting during the late neonatal period.

The former usually presents in the form of septicaemia while the later presents as meningitis. Adults infections include puerperal sepsis, pyelonephritis and a wide range of other infections. Usually malignancy, diabetis mellitus and sickle all disease, the organism is sensitive to penicillin

which is the drug of choice in treating established infections by GBS. Control measures are based on treatment of cases, eradication of vaginal colonization and chemo-prophylaxis of infants at risk. An effective vaccine may become avaliable in the near future (Onile B.A., 1985).

Group B streptococci infection is an important cause of neonatal morbidity and mortality. Effective therapeutic intervention has been prevented to date by our inability to rapidly detect vaginal colonization (Reardon et al, 1983). Baker et al (1976) showed that the use of selective broth medium is the most accurate means of identifying group B streptoccocci.

Noble et al (1983) in vitro work, found the production of carotenoid pigment in serumstarch broth to be 99.5% to 100% specific for rapidly detecting beta hemolytic group B streptococci, yielding the typical change in color within 4 hours in 50% of cultures and in 100% in 18 hours. So, by applying this method of detection to our women and neonate, we can save time by this rapid bedside method rather than laboratory

plating methods of detection. This may be of benefit in selecting the neonates that might receive penicillin prophylaxis within 2 hours of birth (Lloyd et al, 1979).

AIM OF THE STUDY

To study the presence of group B streptococci among parturient women and their neonates.

To evaluate the effectiveness of a rapid method of detection, GBS.

Bacteriological Review

Streptococci

Streptococci are gram positive cocci arranged in Paris, in short or long chains. They are non sporing, generally non motile and catalase-negative and some may produce haemolysin. Many streptococci have the ability to haemolyse red cells in vitro complete haemolysis around the colony is called Beta haemolysis, incomplete lysis with the formation of green pigment is termed.Alpha. Haemolysis and no haemolysis for some reason reffered to as gamma haemolysis. Most species are aerobic or facultative anaerobes and a small group are obligatory anaerobes. Many of the streptococci have a cell wall poly saccharide antigen called C substance which when extracted by hydrochloric acid or formamide or lysed by enzymes, forms an antigen used for serological grouping (Lancefield's groups). These groups can be further subdivided into types (Griffith's types). Griffith, by agglutination with absorbed type specific sera and specially prepared "smooth" suspensions subdivided the group A haemolytic streptococci into more than 30 antigenic types. A few strains of other groups were also typed, the technique requires experience and is propably only of epidemiological value.

Presumptive Identification of GBS

Definitive identification from a single colony of a putative B streptococcus can be accomplished only by extraction of the group carbohydrate antigen and serological identification of the extracted antigen with specific antibody. Because the inherent delay from initial culture to final seroidenti fication, both presumptive bio-chemical methods and rapid serological technique have been developed to provide information to the physician.

The colonial appearance of group B streptococcus on sheep blood agar at 24 h is distinct from that of group A or D, the common human streptococcal pathogens. The colony is usually gray soft, and mucoid, often larger than 2mm and surrounded by small hazy zone of beta hemolysis (Braunstein et al, 1969). Although the group B haemolysin appears unrelated to the oxygen labile streptolysin (O) (Buchanen, R.E., and N.E. Gibbson, 1974) determination of hemolysis is usually performed by microscope examination of sub surface or incubation under reduded oxygen tension (Facklan et al, 1974). Non hemolytic isolates occur rarely isolation of non hemolytic of group B streptococcus from a human neonates has been documented (Wilkinson Et al, 1971).

Pigment production potentiated by prolonged incubation at room temperature, anaerobic incubation, or the incorporation of starch into the culture medium is an unstable property (Buchanan and Gibbons, 1974).

A unique combination of biochemical reactions allow presumptive identification of the putative group B colony as shown in the table:

Procedure	Identification			
	Group A	Group B	Not Group A, B, D	Group D
Hemolysis	Beta	Beta	Beta	Beta, Alpha or none
Bacitracin susceptibility	+(99.5)	-(6.0)	-(7.5)	-(1.1)
Hydrolysis of Esculin in presence of 40% bile	-(0)	-(0)	-(0.3)	+(99.5)
Tolerance to 65% Nacl	-(1.9)	±(79.2)	±(15.4)	±(79.5)
Hydrolysis of sodium hippurate	-(0)	+(99.6)	-(0.3)	-(5.4)

From (Facklam Et al, 1974

An ancillary factor for the identification of group B streptococci, long utilized in veterinary microbiology is the CAMP factor, named for the scientists originally describing the phenomenon (Christie et al, 1944). The CAMP factor is thought to be a diffusible, heat stable extra cellular protein of group B organisms that enhances rapid hemolysis of sheep erythrocytes by staphalococcal beta hemolysis (Brown et al, 1974 - Esselveld et al, 1958) classically the reaction is produced by streaking the staphylococcal stains across the diameter of a sheep blood agar plate, and the streptococcal strains to be assayed perpendicular to the staphylococcal streak. Positive reactions are indicated by an arrow pattern of hemolysis after over night incubation at $37c^{o}$ and the reaction is enhanced by anaerobiosis (Christie et al, 1944). Even some of the non hemolytic group B streptococci can potentiate this hemoly ic phenomenon (Romers and Wilkinson, 1974).

"Serological Identification"

Definitive identification of streptococci rests upon immunological reactivity with specific antisera prepared against the group and type antigentic moieties. The prototype precipitin test, is the classic diagnostic technique

Romers and Wilkinson (1974) have described a rapid florue-scent antibody test for use on smears of suspected colonies (Romers and Wilkinson, 1974). Use of counter immuono electro phoresis (CIEP) also provides a more rapid alternative to the capillary preceipitin assay for extracted carbohydrate antigen. Edwards and Larson have demonstrated that CIEP possesses grea-ter sensitivity (Edward and Larson, 1973).

Rapid Methods of Identifying Group B Streptococci

Rapid methods of identifying lancefield group B strepto-cocci were compared to the standard Fuller's Extraction method Such tests as sodium hippurate hydrolysis, bile tolerance, aesculin hydrolysis, pyruvate fermentation, CAMP factor, pig-mentation, and bacitracin haemolysis were tested on both rou-tine clinical specimens and national collection of type culture strains. The results show that pigmentation on Islam's medium was the most definitive test available rapidly to identify group B streptococci (Sheenaa Waitkins, 1980).

The results show that not rapid methods of identifying streptococci are reliable. If the laboratory were to use sodium hippurate hydrolysis as a means of recognising group B organisms, then serious misidentification would certainly occur. Alternative methods such as bacitracin resistance, although, useful may again confuse the identification, particularly with group D streptococci. Neither sodium hippurate nor bacitracin could be used alone as presumptive test. Elimination of group D could be achieved by utilising the puruvate fermention, bile tolerance, and aesculin hydrolysis. But all of these methods increase the workload of the lab and are unsatisfactory since they prove the identification of group B by exclusion rather than by positive means.

One test that could identify positively is the CAMP reaction, since only group B streptococci are positive, but as a rapid screeing procedure, it proved to be too time consuming and labour intensive to perform. The most straight forward and easy method was the pigmentation of group B on starch medium; both Fallon's and Islam's methods were exclusive to group B, Islam's giving better results than Fallon's medium (93% : 77%). The inoculation procedure is simple and could

be included in primary cultivation from clinical specimens; the bright orange pigmentation is distinctive and easy to recognise. Both Islam's and Fallon's media are easy to prepare and can be stored in the refrigerator and results can be read after overnight anaerobic incubation, it must be noted, however, that 7% of group B streptococci did not pigment on Islam's medium and 23% did not pigment on Fallon's (this seems a little excessive, but the strains tested were therefore essential that if either of these tests were referred ones and may behave abnormally). It is therefore, essential that if either of these tests is used as screeing method, then non pigmented strains should be further tested by serological means.