Comparing Urine Samples with Vaginal Swabs in Detecting *Streptococcus agalactiae* Carriage in Pregnant Women Using Chromogenic Media

Thesis

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ABSTRACT

This study was conducted on 50 Egyptian pregnant females in their late third trimester (35-37 weeks of gestation).

One vaginal swab and one urine sample were taken from each female and they were inoculated onto selective lim broth and incubated for 18-24 hours followed by subculture on TSA and two types of chromogenic media; GRAN and STRB. The plates were incubated for 24 hours and if culture was negative, incubation was extended to 48 hours. GBS growth confirmation was done using latex agglutination test.

Over the duration of the study, 19 (38%) out of the 50 examined pregnant women were found to be colonized by GBS in either the genital tract, the urinary tract or both.

There was no difference regarding the sample types in detecting GBS as 17 (34%) of the vaginal swabs and 17 (34%) of the urine samples showed positive cultures on at least one of the three media, and on each medium there was no statistically significant difference between vaginal swabs and urine samples in detecting GBS.

KEY WORDS:

Comparing Urine Samples with Vaginal Swabs in Detecting *Streptococcus agalactiae* Carriage in Pregnant Women Using Chromogenic Media

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Introduction

Streptococcus agalactiae, usually termed group B *Streptococcus* (GBS), is one of the most important causes of early-onset neonatal infection ***(Dagnew et al., 2012; Camacho-Gonzalez et al., 2013)***. Approximately 10–30% of pregnant women are colonized with GBS in the vagina or rectum ***(Campbell et al., 2000)***. Centers for Disease Control and Prevention (CDC) guidelines, 2010, recommend screening vaginal or rectovaginal GBS colonization in pregnant women at 35–37 weeks of gestation. For pregnant women colonized by GBS, intrapartum administration of antibiotics is recommended to prevent GBS transmission to the newborn during delivery. As a result of prevention efforts, incidence of GBS has declined dramatically over the past 15 years, from 1.7 cases per 1,000 live births in the early 1990s to 0.34–0.37 cases per 1,000 live births in recent years.

Maternal GBS bacteriuria, including pure and predominant growth of GBS in the urine, has been associated with GBS colonization and an increased risk for early-onset GBSdisease (EOGD) ***(Liston et al., 1979; Moller et al., 1984; Heath et al., 2009)***. Women with GBS isolated from the urine at any time during the current pregnancy should receive intrapartum antibiotic prophylaxis and do not need third trimester screening for GBS colonization ***(CDC, 2010)***.

One of the most important requirements for a screening protocol for GBS during pregnancy is the minimization of false negatives which, in this case, can produce a high risk of EOGD. Blood agar is widely used around the world for the culture of hemolytic GBS. As blood agar is a non-selective agar, two or more species of bacteria in recto-vaginal samples can make detection of GBS difficult ***(Morita et al., 2014)***. The performance of microbiological methods for GBS screening has been greatly improved by the use of selective chromogenic media which undergo color change in the presence of GBS ***(El Aila et al., 2010).*** Granada agar (GRAN), is a selective differential chromogenic medium used to screen for GBS in pregnant women, on which GBS colonies appear orange, while other bacteria are either inhibited or form white colonies. GRAN is a good medium for detecting GBS in urine specimens ***(Tamayo et al., 2004)***. ChromID Strepto B (STRB), is another selective chromogenic agar that was developed to screen for GBS in pregnant women, but it has not been tested for urine samples yet. GBS colonies on it appear pink or red, while other bacteria are either inhibited or form colonies in other colours ***(Morita et al., 2014)***.

Aim of the work

This study was conducted to:

- Compare urine samples with vaginal swabs in detecting GBS colonization in pregnant females.
- Compare the performance of chromogenic media; GRAN and STRB, with that of a conventional medium; Tryptic soy agar with 5% sheep blood (TSA), in detecting GBS colonization in pregnant females.

STREPTOCOCCI

Streptococci are Gram-positive spherical or ovoid cells, arranged in chains or pairs. All species are non-motile, non-spore forming and catalase negative, with complex nutritional requirements. All are facultatively anaerobic. All species fail to reduce nitrate. They ferment glucose with the production of lactic acid but no gas. Many species are members of the commensal microflora on mucosal membranes of humans or animals, and some are highly pathogenic. The genus is named according to its typical morphology; from the Greek *streptos*, pliant; coccos, a grain or berry **(*Kilian, 1998)*.**

In 1986, only 7 genera of facultatively anaerobic Gram-positive cocci were listed: *Aerococcus, Leuconostoc, Micrococcus, Pediococcus, Staphylococcus, Streptococcus* and *Stomatococcus* ***(Schleifer, 1986).*** After that *Streptococcus* genus was split into 3 genera; *Enterococcus, Lactococcus* and *Streptococcus* ***(Schleifer and Klipper-Balz, 1987).***

New genera of Gram-positive cocci that form chains have been established and the majority of these genera were split off the *Streptococcus* genus by genetic and phenotypic information. In 2002, 17 different genera of Gram-positive cocci were listed ***(Facklam, 2002; Fairlie et al., 2013).***

Historical Classification

The earliest attempt at differentiating the streptococci was probably made in 1903 by Shottmuller, who used blood agar to differentiate strains that were β-hemolytic from those that were not ***(Shottmuller, 1903)***. Before 1933, fermentation and tolerance tests were the only tests used for differentiating many of the streptococci. In 1934, Lancefield reported the

technique of demonstrating specific carbohydrate "group" antigens associated with the β-hemolytic strains. It is a serotype classification with 20 described serotypes named Lancefield groups A to V, excluding I and J ***(Lancefield, 1934).*** In 1937, Sherman proposed a scheme for placing the streptococci into 4 categories. These categories were organized by hemolytic reaction, group carbohydrate antigens and phenotypic tests; primarily fermentation and tolerance tests. Sherman's four divisions were the pyogenic division, the viridans division, the lactic division and the enterococci ***(Sherman, 1937)***.

In 1974, the genus *Streptococcus* was divided into four groups (I-IV) containing 21 named species ***(Deibel and Seeley, 1974)***. Although these authors omitted Sherman's original designations 'pyogenic', 'viridans', 'lactic' and 'enterococcus' divisions because some of the newly recognized species cut across these divisions, nevertheless growth and tolerance tests, haemolytic reactions and Lancefield grouping, supported by biochemical reactions, were retained as major criteria in the classification scheme and species groups formed therein ***(Hardie and Whiley, 1997)***.

According to Kilian in 1998, the genus includes 37 species and subspecies (table 1) ***(Kilian, 1998).***

One of the most useful tools applied to the revision of the classification system for *Streptococcus* genus is the application of 16S ribosomal ribonucleic acid (rRNA) gene sequencing. There are currently over 50 species recognised in *Streptococcus* genus (figure 1) ***(Facklam, 2002; Cole et al., 2008; Krzysciak et al., 2013).***

Table (1): Species and subspecies of the genus *Streptococcus.* Serological and hemolytic reactions, peptidoglycan type and main habitat.

Species	Lancefield group	Serotype antigen	Hemolysis	Peptidoglycan type	Main habitat
Pyogenic group					
S. pyogenes	A	M and T (protein)	β	Lys-Ala_{1-3}	Humans
S. agalactiae	B	Capsular polysaccharide	β (CAMP+)	Lys-Ala_{1-3} (Ser)	Humans, cattle
S. equi					
subsp. Equi	C		β	Lys-Ala_{1-3}	Horses, donkeys
subsp.zooepidemicus	C		β	Lys-Ala_{2-3}	Many animals
S. dysgalactiae					
subsp. Dysgalactiae	C, L		α, β, -	Lys-Ala_{1-3}	Pigs, cattle
subsp. Equisimilis	C, G		β	Lys-Ala_{1-3}	Humans
S. canis	G		β(CAMP+)	Lys-Thr-Gly	Many animals
S. iniae	-		β, α	Lys-Ala_{1-3}	Freshwater dolphins
S. poreinus	E, P, U, V		β(CAMP+)	Lys-Ala_{2-4}	Pigs
S. uberis	-, E		α	Lys-Ala_{1-3}	Cattle
S. parauberis	-, E		α	ND	Cattle
S. hyointestinalis	-		α	Lys-Ala(Ser)	Pigs
Anginosus group					
S. anginosus	-, F, A, C, G		α, β	Lys-Ala_{1-3}	Humans
S. constellatus	-, A, C		α, β	Lys-Ala_{1-3}	Humans
S. intermedius	-, F, G		α, β	Lys-Ala_{1-3}	Humans
Mitis group					
S. mitis	O, K, -		α	Lys-direct	Humans
S. oralis	-		α	Lys-direct	Humans
S. pneumonia	-	CPS	α	Lys-Ala_2(Ser)	Humans
S. gordonii	H1, H2		α	Lys-Ala_{1-3}	Humans
S. sanguis	H1, -		α	Lys-Ala_{1-3}	Humans
S. parasanguis	-		α	ND	Humans
S. crista	-		α	ND	Humans
Salivarius group					
S. salivarius	H2, K, -		-	Lys-Ala_{2-3} Lys-Thr-Gly	Humans
S. vestibularis	-		-	ND	Humans
S. thermophilus	-		-	Lys-Ala_{2-3}	Milk, dairy product
Bovis group					
S. bovis	D		-, α	Lys-Thr-Ala	Cattle, sheep, pigs, humans, dogs, pigeons
S. equines	D		α	Lys-Thr-Ala	Horses, other animals
S. alactolyticus	D		α, -	ND	Pigs, chickens
Mutans group					
S. mutans	-, E	c, e, f	-, β, α	Lys-Ala_{2-3}	Humans
S. cricetus	-	A	-, α	Lys-Thr-Ala	Hamster, rats, humans
S. sobrinus	-	d, h, g (or) cell wall carbohydrate	α, -	Lys-Thr-Ala	Humans
S. dowreii	-	H	-	Lys-Thr-Ala	Monkeys
S. rattus	-	B	-	Lys-Ala_{2-3}	Rats, humans
S. macacae	-	C	-	ND	Monkeys
S. ferus	-	C	-	Lys-Ala_{2-3}	Rats

(Kilian, 1998)

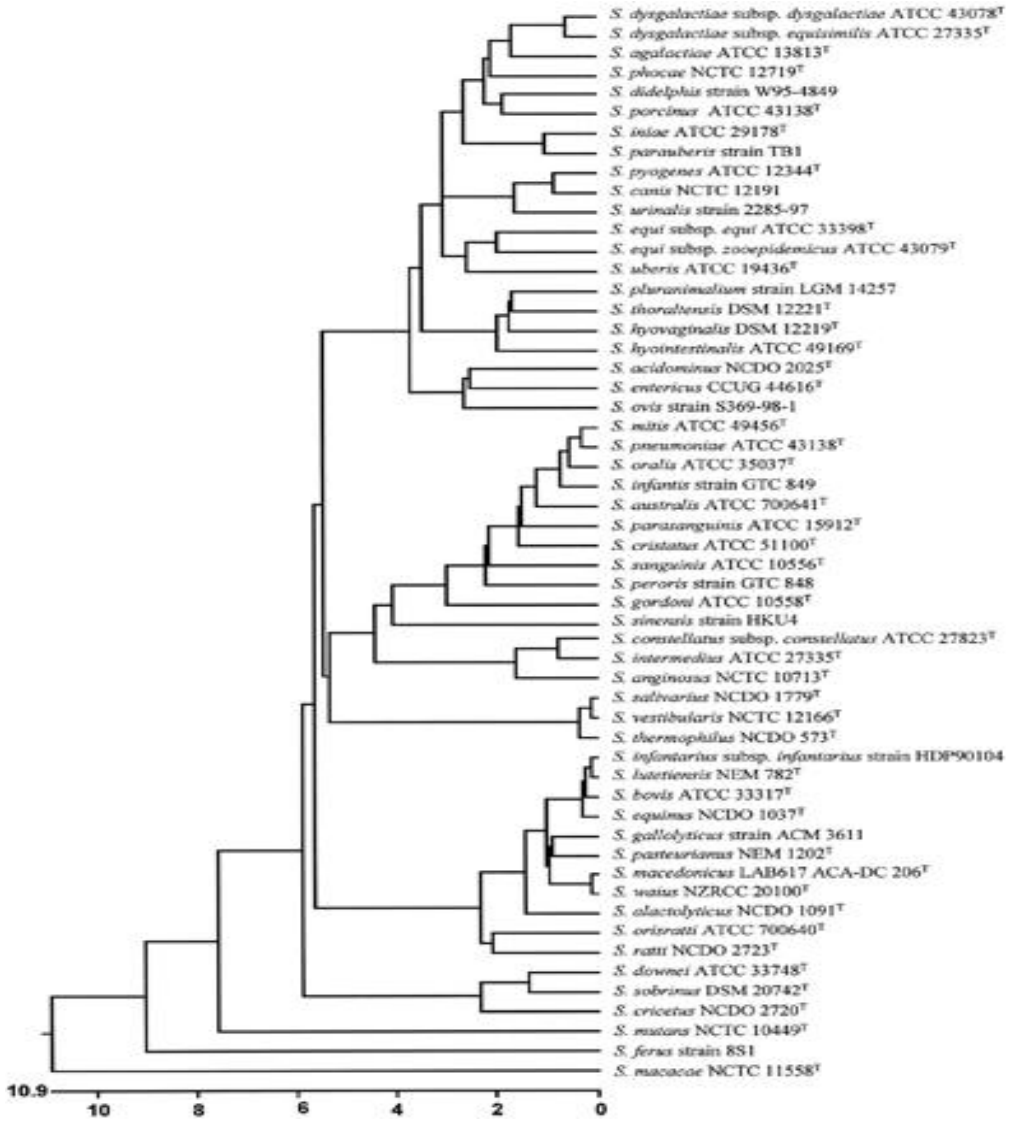

Figure (1): Phylogenetic relationship among 55 *Streptococcus* species based on analysis of 16S rRNA gene sequences *(Facklam, 2002)*.

Streptococcus agalactiae

S. agalactiae is a leading cause of sepsis and meningitis in infants ***(Schuchat, 1998)***. This organism was first described in outbreaks of infections of cattle udders, and because the infection halted milk production, the organism was named *S. agalactiae* ***(Connell, 1982).***

Habitat:

At any given time, 10–40% of healthy adults are commonly colonized by GBS in the gastrointestinal and genital tract but remain asymptomatic. The gastrointestinal tract serves as the primary reservoir for GBS and is the likely source of vaginal colonization ***(Melin and Efstratiou, 2013).***

Morphology:

Gram-positive spherical cells that may be more or less elongated and arranged in pairs or chains of up to 50 cells or more. Chain formation is most pronounced in broth media. Individual cells are typically 0.5-1.0 µm x 1.0-2.0 µm. Growth occurs by elongation on the axis parallel to the chain. After division, the appearance of pairing may remain. It cannot be distinguished on a morphologic basis from other β-hemolytic streptococci ***(Kilian, 1998)***.

Cultural Characteristics:

S. agalactiae is a facultative anaerobe but requires the addition of carbon dioxide for growth. On blood agar, hemolysis is usually of the beta type, though the zones are usually narrow. In some cultures, hemolysis is of the alpha type and in some there is no hemolysis. Colonies are grey, mucoid, convex, regular, usually shiny but sometimes

dry and larger, about 2mm, than those of other streptococci ***(Ross, 1996; Kulkarni et al., 2001).*** When incubated anaerobically in the presence of CO_2 on a starch-containing medium, it gives orange-colored colonies ***(Overman et al., 2002).***

The use of a selective medium supplemented with 15 µg/ml nalidixic acid and 8 µg/ml gentamycin or with 15 µg/ml nalidixic acid and 10 µg/ml colistin is required to detect the presence of even small numbers of *S. agalactiae* in specimens containing many other bacteria, e.g. from throat or vagina ***(Baker et al., 1973; Lim et al., 1986).***

Genetic Characteristics:

S. agalactiae genome reveals substantial similarity with those of the related human pathogens *S. pyogenes* and *S. pneumoniae*. Identified genes unique to *S. agalactiae* that are expected to play a role in colonization or disease include surface proteins genes, capsule synthesis genes, hemolysin gene and several transcriptional regulators. Many of these are associated with mobile elements, including bacteriophages, transposons and insertion sequences, an observation that supports acquisition of virulence traits from other species. The presence of more than 100 genes suggests evolution of additional species-specific functions. Genetic heterogeneity among *S. agalactiae* strains, even of the same serotype, provides evidence that these mechanisms of acquisition, duplication and reassortment have produced the genetic diversity within the species that has permitted *S. agalactiae* to adapt to new environmental niches and to emerge as a major human pathogen ***(Tettelin et al., 2002).***

Virulence Factors:

Like many pathogenic bacteria, GBS encodes many virulence factors that are critical for its ability to cause disease. The most imortant virulence factors are the capsular polysaccharide (CPS) ***(Rajagopal, 2009; Edwards and Nizet, 2011)*** and β-hemolysin which is a pore-forming toxin ***(Whidbey et al., 2013; Rosa-Fraile et al., 2014; Whidbey et al., 2015)***.