

Histopathological Examination of Uterine Tumors and Endometrial Hyperplasias Colonized by *Streptococcus agalactiae* and Antibiotic Susceptibility of the Isolated *Streptococcus agalactiae*

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ABSTRACT

Streptococcus agalactiae was isolated from six leiomyomas of the uterus and two endometrial hyperplasia cases collected from women aged 35 to 80 years, underwent Total Abdominal Hysterectomy (TAH) in Basrah Maternity and Child Hospital due to continuous vaginal bleeding not responding to medical treatment. Sections of leiomyomas stained with haematoxylin and eosin illustrate proliferating smooth muscles fibers with intervening fibrous tissue. Sections from endometrial hyperplasia stained with modified Gram stain demonstrated *S. agalactiae* as diplococci or as aggregates.

Resistance of *S. agalactiae* isolated from the above eight cases, against 14 antibiotics was determined. Results clarified that all isolates (100%) are resistant to 10 antibiotics: tetracyclin, erythromycin, cefotaxine, ampicillin, chloramphenicol, penicillin, cloxacillin, cephalothin, gentamycin and tobramycin. Most isolates demonstrated susceptibility to amoxicillin (62.5 %) which makes it the drug of choice against *S. agalactiae*. Four isolates of *S. agalactiae* (50%) were resistant to vancomycin, which raises a threat of vancomycin resistance genes being transferred to other gram-positive species found in the vaginal and rectal sites.

Streptococcus agalactiae

80-35

*Streptococcus agalactiae**S. agalactiae**S. agalactiae*

Tetracyclin, Erythromycin, Cefotaxine, Ampicillin, : (%100)

.Chloramphenicol, Penicillin, Cloxacillin, Cephalothin, Gentamycin and Tobramycin.

S. (%62)(%50) *.agalactiae***INTRODUCTION**

Group B streptococci (GBS), or *Streptococcus agalactiae*, is a component of the normal flora, colonizing the respiratory, gastrointestinal, and urogenital tracts of humans. Since the 1970s, *S. agalactiae* has emerged as an important human pathogen and an increasingly important cause of invasive infections in immunocompromised adults and the elderly (Baker, 2000; Edwards and Baker, 2005). Vertical transmission of *S. agalactiae* is associated with increased morbidity and mortality in neonates (Gotoff, 2002). Currently, *S. agalactiae* is the leading cause of bacterial sepsis, pneumonia, and meningitis in neonates in the United States, Europe and Asia (Gibbs *et al.*, 2004; Puopolo and Madoff, 2007).

More cases of vaginal GBS colonization were reported in women with gynecological inflammatory conditions, with a significant increase in women with vaginitis and cervicitis (Zhu *et al.*, 1996), cervical cancers (Mikamo *et al.*, 1993) and malodorous and non malodorous gynecologic cancers (VON Gruenigen *et al.*, 2000). Moreover, Genta *et al.*, (2001) reported a case of a diabetic postmenopausal woman with a giant pyomyoma

simulating an ovarian cancer associated with *S. agalactiae* endocarditis and deep venous thrombosis of the right external iliac and femoral veins.

Adherence of GBS and their invasion into variety of tissue-specific epithelial and endothelial cells host tissue is mediated through a complex series of events which has been confirmed in vitro and in vivo (Mikamo *et al.*, 2004; Martins *et al.*, 2007; Al-Hadithi *et al.*, 2009). Followed by colonization leading to the true depiction of pathogenesis of GBS infection (Soriani *et al.*, 2006).

Penicillins including penicillin G are the drugs of choice for intrapartum antibiotic prophylaxis and also for the treatment of GBS infections (CDC, 2002). For severely penicillin-allergic mothers, the alternative treatment is either erythromycin or clindamycin (Apgar *et al.*, 2005). However, increased antimicrobial use against *S. agalactiae* isolates has led to the emergence of antimicrobial resistance (Uh *et al.*, 2001; Culebras *et al.*, 2002). Kimura *et al.*, (2008) characterized and identified the first strains of GBS with reduced penicillin susceptibility (PRGBS) in Japan. Likewise, increased rates of erythromycin and clindamycin resistance among GBS has been reported by De Azavedo *et al.*, (2001); Miller *et al.*, (2004), Desjardins *et al.*, (2004); and Zeng *et al.*, (2006). Additionally, women with a complicated pregnancy were found twice as often colonized with GBS strains resistant to macrolides and clindamycin (Strus *et al.*, 2009).

Hence, the present study has focused on examination of tissues of uterine tumors and endometrial hyperplasia colonized by *Streptococcus agalactiae* and to describe antibiotic susceptibility profile of these isolates.

MATERIALS AND METHODS

Forty two uterine samples were collected from women age ranged (35 to 80 years), underwent Total Abdominal Hysterectomy (TAH) in Basrah Maternity and Child Hospital due to continuous vaginal bleeding not responding to medical and hormonal treatment. These included: 21 uterine tumors and 21 endometrial hyperplasia.

S. agalactiae was identified in six uterine tumors and two cases of endometrial hyperplasia as Gram positive cocci, negative for catalase, positive for CAMP test, capable of growing in 6.5% NaCl but not 40% bile salts, hydrolyzing arginin and gelatin and were resistant to bacitracin (Facklam and Washington, 1991; Collee *et al.*, 1996; Kilian, 1998). Isolates were maintained in Todd Hewitt broth and tryptose blood agar. For testing, the isolates were grown on trypticase soy agar supplemented with 5% sheep blood at 37°C for 18 to 24hrs.

Virulence factors of isolates were confirmed by Al-Hadithi, *et al.*, (2009) including demonstrating existence of the capsule and its intensity using Congo red medium (Freeman, 1979), appearance of turbidity (growth) in Todd Hewitt Glucose Broth (THGB) containing 4 mg/ml of purified Tetracycline (Moffat *et al.*, 1986) which reveals presence of Sialic acid (Nagano *et al.*, 1989) that confers resistance to tetracycline, production of orange to red pigment when colonies were inoculated onto THGB (Gupta and Briski, 2004), blood haemolysis (Collee *et al.*, 1996) and *In Vitro* adherence capability (Biofilm formation) which was detected by the method of Christensen, (1985).

Antibiotic Susceptibility Test

Antibiotic susceptibility of eight *S. agalactiae* isolated from six uterine tumors and two endometrial hyperplasia cases (Al-Hadithi *et al.*, 2009), before processing for histological studies (i.e. before fixing tissues in formalin), was investigated by disc plate method (Mason *et al.*, 1996) towards 14 antibiotics including: penicillin G (10 IU), cloxacillin (5µg), tetracycline (30µg), erythromycin (15µg), ampicillin (10µg), amoxicillin (30µg), trimethoprim (25µg), cefotaxim (30µg), ciprofloxacin (5µg) vancomycin (30µg) and chloramphenicol (30µg) supplied by Oxoid/UK; and gentamycin (10µg) tobramycin (30µg), and cephatollin (30µg) supplied by Al-Razi company/Iraq. Discs were placed on Muller Hinton agar plates supplemented with 5% blood; two plates for each isolate. Replicate plates were seeded with isolates grown overnight in Todd Hewitt broth and were incubated at 37°C for 24hrs. Zones of inhibition were measured by mm and assessed against standard tables (Harley and Prescott, 1996).

Histopathologic Studies

Histopathological examination was performed on formalin-fixed, paraffin-embedded tissues of the uterine tumors and endometrial hyperplasia, processed according to Luna (1960) and stained with haematoxylin-eosin stain.

For the detection of *S. agalactiae* isolates in the tissues; additional sections were prepared and stained by modified Gram's method (McKay, 1970).

RESULTS

Histopathological examination of uterine tumors and endometrial hyperplasia harboring *S. agalactiae*

(Fig. 1) shows section of a leiomyoma of the uterus. The tumor consists of broad bundles of mature smooth muscle cells that run at various angles, with variable amount of fibrous tissues between the bundles. A section from endometrial hyperplasia stained with modified Gram stain (Fig. 2) demonstrates *S. agalactiae* as diplococci or aggregates. No acute or chronic inflammatory cell infiltration or reactions were found in both cases of the leiomyomas and the endometrial hyperplasia from which GBS were isolated.

Fig . 1: Section of leiomyoma of the uterus H.E(X704).

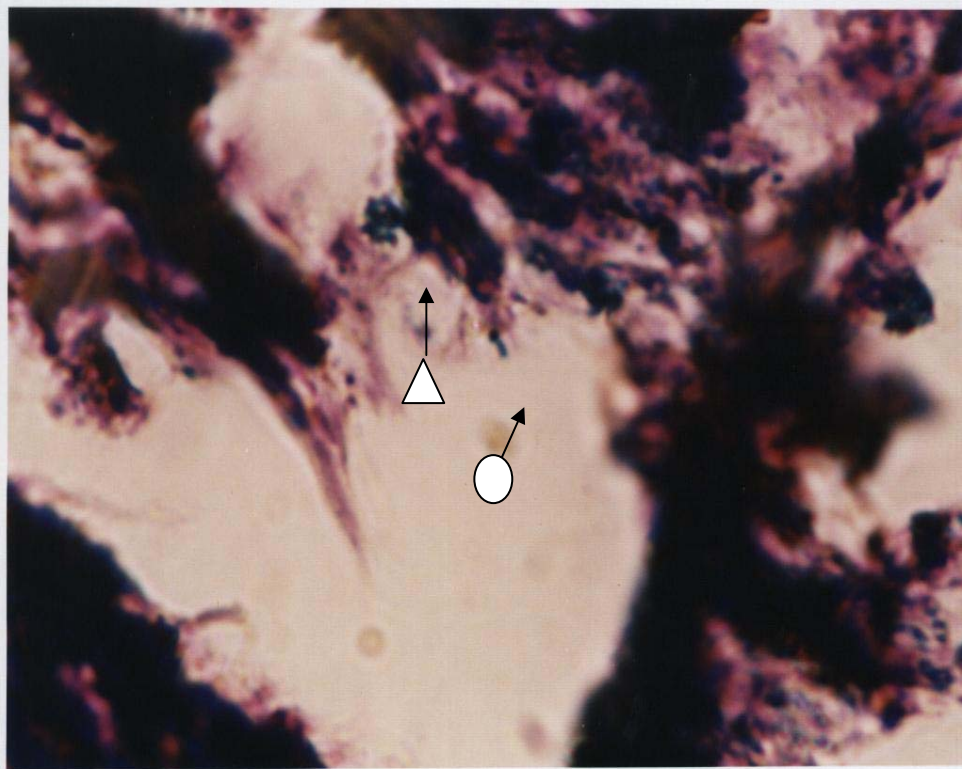
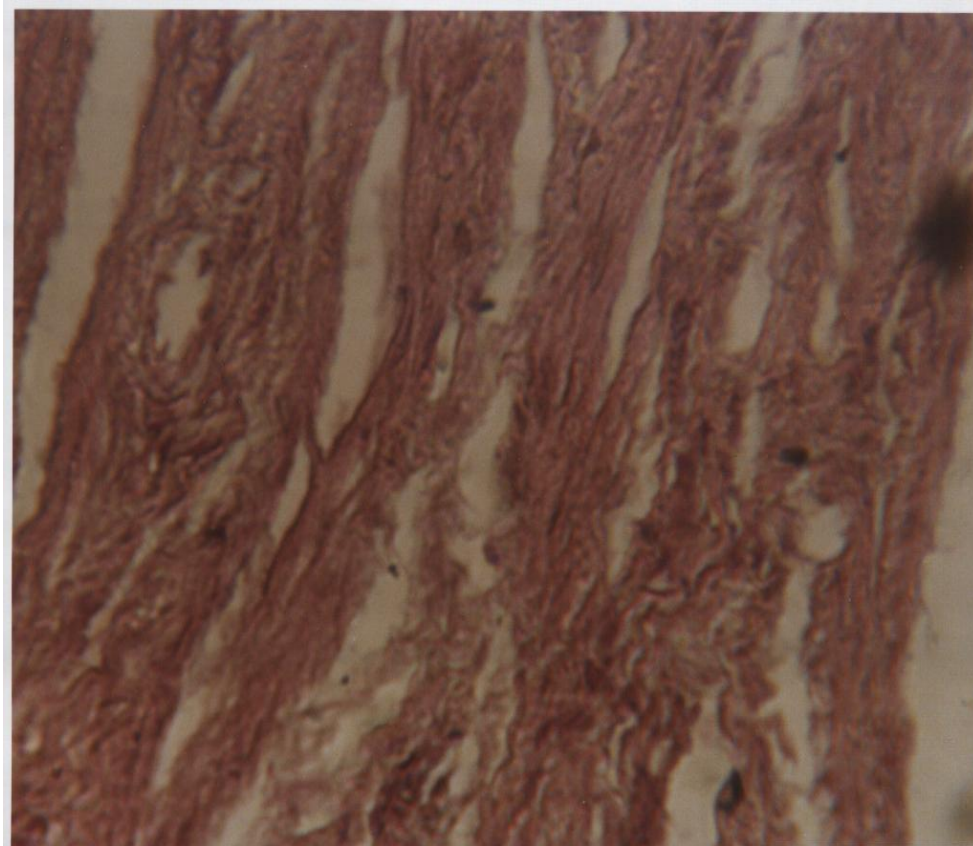


Fig. 2: Modified Gram staining of endometrial hyperplasia (X-1720) showing *Streptococcus agalactiae* cells as aggregates(\blacktriangle) and diplococci(\circ)

Susceptibility of *S. agalactiae* to antibiotics:

Table (1) illustrates that the eight isolates of *S. agalactiae* under study were resistant to 10 out of 14 antibiotics examined (100%); Similar percentage(50%) of vancomycin resistance was detected between isolates from leiomyoma (Ca1 and Ca3) and endometrial hyperplasia (Co1).

Only one GBS isolated from a leiomyoma case was susceptible to ciprofloxacin (12.5 %). Most isolates demonstrated susceptibility to amoxicillin (62.5 %) which makes it the drug of choice against *S. agalactiae*.

DISCUSSION

Group B Streptococcus commonly colonizes healthy adults without symptoms, yet under certain circumstances displays the ability to invade host tissues, evade immune detection and cause serious invasive disease (Maisey *et al.*, 2008).

Uterine leiomyoma is a benign neoplasm of uterine smooth muscle. It is the commonest tumor in females, being found in 30-50% of women during their reproductive life. It is generally arises between 20-40 yrs of age, and tends to stop growing actively after the menopause. Endometrial hyperplasia is an increase in the number of the endometrial glands and their surrounding stromal cells which results eventually in thickening of the endometrium, usually caused by excessive, unopposed action of the estrogens (Colgan *et al.*, 1993; Montag and Kumar, 2007).

Although scarce information is available on bacteriology of pelvic tumors, Mikamo, *et al.*, (2004) indicated that some carcinogens products such as N-nitro compounds, n-butyric acid and n-valeric acid of Enterobacteriaceae, *Streptococcus agalactiae* and anaerobic bacteria, mainly detected in uterine endometrial cancer might contribute to the initiation of endometrial carcinogenesis, during the critical steps leading to GBS dissemination in the host (Pezzicoli *et al.*, 2008) and biofilm formation (Al- Hadithi *et al.*, 2009). Additionally, Soriani *et al.*, (2006) verified the importance of colonization of the colon and vagina in the pathogenesis of GBS infection with a significant increase in women with gynecologic inflammatory conditions (Zhu *et al.*, 1996). Importantly, infection of placental cells can promote ascending in utero infection, whereas invasion of pulmonary epithelium and endothelium promote systemic dissemination (Maisey and Doran 2008). Moreover, Strus *et al.*, (2009) reported that neonates born from colonized mothers with a complicated pregnancy were more often colonized with GBS than those from the mothers with a normal pregnancy (35 % versus 26.7 %).

Even so, microbial etiology of female reproductive tract tumors remains unverified and yet, the question of whether GBS alone causes a cancer is not answered ; however, GBS may be an important cofactor, though not the direct.

A greater concern about antimicrobial resistance associated with GBS is whether increased use of antimicrobial prophylaxis in obstetric care for GBS prevention (CDC, 2002) will lead to the emergence of antimicrobial resistance among other perinatal pathogens.

Although the existence of β -lactam-insusceptible strains of GBS had not been confirmed until the study of Kimura *et al.*, (2008) who identified the first strains of GBS with reduced penicillin susceptibility (PRGBS) in Japan but that study contained clinical isolates stocked from 1995 to 1998. Hence, they consider that PRGBS have indeed existed since the 1990s. Hence, the establishment of "resistance" criteria for GBS and the

development of a feasible and reliable method for screening for reduced penicillin susceptibility in GBS are necessitated (Kimura *et al.*, 2008).

Although the results of phylogenetic comparative analysis carried out by Nagano *et al.*, (2008) implied the absence of epidemic penicillin-insusceptible strains, the present study (despite the use of only few isolates) demonstrates resistance of all GBS isolates (100%) to penicillin (Table 1).

Table 1: Resistance of *S. agalactiae* isolates against 14 antibiotics.

Isolates	Tob	Gen	Ceph	Van	Clox	Pen	Trim	Cipr	Amp	Cephx	Amo	Chl	Ery	Tet
Ca ₁	R	R	R	S	R	R	R	R	R	R	R	R	R	R
Ca ₂	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ca ₃	R	R	R	S	R	R	R	R	R	R	S	R	R	R
Ca ₄	R	R	R	S	R	R	S	R	R	R	S	R	R	R
Ca ₅	R	R	R	R	R	R	S	S	R	R	S	R	R	R
Ca ₆	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Co ₁	R	R	R	S	R	R	R	R	R	R	S	R	R	R
Co ₂	R	R	R	R	R	R	R	R	R	R	S	R	R	R
%	100	100	100	50	100	100	75	87.5	100	100	37.5	100	100	100

Tob: Tobramycin (30µg), Gen: Gentamycin (10 µg), Ceph: Cephalothin (30 µg), Van: Vancomycin (30 µg), Clox: Cloxacillin (5 µg), Pen: Penicillin (10 IU), Trim: Trimethprim (25 µg), Cipr: Ciproflxine (5 µg), Amp: Ampicillin (10 µg), Cephx: Cfotoxin (30 µg), Amo: Amoxicillin (30 µg), Chl: Chloramphenicol (30 µg), Ery: Erythromycin (15 µg), Tet: Tetracycline (30 µg).

In some cases, where *In vitro* susceptibility data may not be available for prenatal isolates or in cases demonstrating severe penicillin allergies and antibiotic-resistant organisms, the alternative treatment is either erythromycin or clindamycin (Apgar *et al.*, 2005). Both antibiotics, and in a similar way chloramphenicol, exert their bacteriostatic effect by interfering with bacterial protein synthesis through binding preferentially to the 50S subunit of the bacterial ribosome (Brooks *et al.*, 2004). Again, the eight isolates examined in the present study were resistant (100%) to erythromycin and chloramphenicol, in addition to another eight antibiotics (Table 1). Increasing resistance of GBS isolates to erythromycin and clindamycin was previously documented by Betriu, (2003) and Manning, *et al.*, (2003). Borchardt *et al.*, (2006) found that clindamycin and erythromycin resistance rates were high among isolates colonizing non pregnant college students and invasive GBS isolates. Zeng *et al.*, (2006) identified resistance to tetracycline, erythromycin, clindamycin and indicated that phenotypic resistance to erythromycin was significantly more common in Asian than in Australasian isolates.

As resistance of *S. agalactiae* isolates against more antibiotics increases, the use of vancomycin will also increase since it has been recommended for prophylaxis of perinatal *S. agalactiae* infection (Miller *et al.*, 2004). On the other hand, higher incidence of *Enterococcus* was reported to be associated with cefazolin (Newton and Wallace, 1998) and cephalosporin (Hillier *et al.*, 1990) prophylaxis, hence empirical therapy with vancomycin will not be effective; as increased use of vancomycin could precipitate an increase in

vancomycin resistant enterococci (VRE) in this population raising the possibility for VRE-associated postpartum infections. Besides, vancomycin resistance gene in this population might be transferred to other gram-positive species found in the vaginal and rectal sites (Chang *et al.*, 2003 ; Miller *et al.*, 2004).

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