AEROBIC AND ANAEROBIC BACTERIAL CAUSES OF PUERPERAL SEPSIS AT MYMENSINGH MEDICAL COLLEGE HOSPITAL

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This thesis entitled **Aerobic and Anaerobic Bacterial causes of puerperal sepsis at Mymensingh Medical College Hospital** is submitted by Dr. Salma Ahmed in partial fulfillment of the requirement for the degree of Master of Philosophy (Medical science) in Microbiology, University of Dhaka. The study was carried out in the Department of Microbiology, Mymensingh Medical College, Mymensingh during the period of July 2006 to June 2007.

Acceptance of the thesis had been approved by the following board of examiners.

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Date of approval

Mymensingh
DECLARATION

I declare that, except otherwise stated, this thesis is entirely my own work and has not been submitted in my form to any other University for any degree.

Dr. Salma Ahmed.
January, 2008
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<tr>
<td>ATCC</td>
<td>American Type Culture collection.</td>
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<td>C.L.E.D</td>
<td>Cystine lactose electrolyte – deficient</td>
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<tr>
<td>DALYS</td>
<td>Disability adjusted life years</td>
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<td>MIU</td>
<td>Motility indole urase.</td>
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<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standard.</td>
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<td>OGSB</td>
<td>Obstetrical and Gynecological Society of Bangladesh</td>
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<td>TSB</td>
<td>Trypticase soya broth.</td>
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<tr>
<td>TGB</td>
<td>Thioglycollate broth.</td>
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AEROBIC AND ANAEROBIC BACTERIAL CAUSES OF PUERPERAL SEPSIS AT MYMENSINGH MEDICAL COLLEGE HOSPITAL
SUMMARY

Puerperal sepsis is an important cause of maternal morbidity and mortality in developing countries. This study was undertaken to isolate and identify the aerobic and anaerobic bacterial agents of puerperal sepsis among the patients admitted in Mymensingh Medical College Hospital during the period from July 2006 to June 2007. Endocervical swab and blood sample were collected from 50 cases of puerperal sepsis and were cultured aerobically and anaerobically. Anaerobiasis was done by using gas pack (BD GAS PAK™ EZ) in Anaerobic jar. The highest incidence of puerperal sepsis was seen in age group 21-30 years comprising of 52% of total cases. Majority of the women (72%) came from lower socio-economic class. Most (74%) of the puerperal sepsis were found in women who had gone normal vaginal delivery at home. As observed complication during delivery, obstructed labour (44%) was at the top of the list. Puerperal sepsis was mostly associated with still birth babies (62%), multiparity (80%) and had no history of antenatal contact (66%). Blood stained and foul smelling discharge was found in 84% women. Out of 50 samples, 42 (84%) yielded positive culture. Among 42 positive cultures 20 (40%) were aerobic and 22 (44%) were mixed growth i.e. aerobic and anaerobic. The isolated anaerobic organisms included *Peptostreptococcus* 14 (63.63%), *Bacteroides fragilis* 3 (13.64%), *Prevotella melaninogenica* 3 (13.64%) and *Clostridium perfringens* 2 (9.09%). The aerobic organisms were *Staphylococcus aureus* 26 (61.90%), *Escherichia. coli* 6 (14.28%) *Staphylococcus epidermidis* 5 (11.90%), *Streptococcus pyogenes* 3 (7.14%) and *Enterococcus faecalis* 2 (4.76%). The organisms were identified by standard biochemical test. Among 50 blood culture specimen 2 (4%) were positive in Trypticase Soya broth with growth of *Staphylococcus epidermidis*. All isolates of
*Staph. aureus* and *Staph. epidermidis* were sensitive to Cephalexin and Vancomycin. Similarly all the strains of *Esch. coli* were sensitive to Gentamicin, Amikacin, Ciprofloxacin and Cephalexin. All the strains of *Strepto. pyogenes* were sensitive to Amoxicillin also. Though all two isolates of *Staph. epidermidis* were sensitive to Ciprofloxacin, but corresponding value for *Staph. aureus* strains were 84.6%. All the isolates of *Enterococcus* were sensitive to Amoxicillin and Cephalexin. As a preliminary study, it was observed that anaerobic culture could be carried out with available logistic arrangement. So it was recommended to design further study on anaerobic bacterial isolation with particular emphasis on their antimicrobial susceptibility.
Chapter – 1

Introduction & Objectives
INTRODUCTION

Puerperal sepsis is an infection of the genital tract at the time interval between rupture of amniotic membrane and the 42\textsuperscript{nd} day following delivery or abortion. Two or more of the following manifestations must be present to define the condition. Those manifestations are- pelvic pain, fever of 38.45\textdegree C or more, abnormal vaginal discharge with foul odor and delayed reduction of uterine size (WHO, 2004). Most of the sudden and unpredictable maternal death occurs due to pregnancy related complications in both developed and developing countries. The most common of those complications is per vaginal bleeding before or after delivery. The second most frequent direct cause of maternal death is puerperal sepsis (Dolea, 2003). During the 19th century, puerperal sepsis took epidemic proportions. Aseptic technique and use of broad spectrum antibiotics have reduced the prevalence of puerperal sepsis to a greater extent. Despite of remarkable advancement in the field of health care pattern and facilities, puerperal sepsis has still been persisting as a significant threat to maternal morbidity and mortality particularly in many developing countries (WHO, 2005). The recorded incidence of maternal sepsis was 1.7, 0.22, 0.07, 0.15 and 4.5 per 100 live births in Nigeria, Niger, South Africa ,USA and Bangladesh respectively (WHO, 2003). One study from Nigeria another from Senegal reported rate of post-partum genital sepsis as 14.8\% & 8.7\% among women who delivered at home compared to 7.9\% & 1.9\% in those who delivered in hospitals with proper health care facilities (Brentlinger, 1998; Jolly, 2000). Maternal mortality was showing an increasing trend in Nigeria as per evident from two reports in the year 2004. One from Abbottbad having mortality of 19.2\% and another from the state of Anambra with mortality rate of 12.1\% (Chukudebelu, 1988). In Norway, 10\%
of maternal death occurred due to puerperal sepsis (Vangen, 2003) and in Poland the rate of sepsis accounted for 27.3%. In both countries, puerperal sepsis was the 2\textsuperscript{nd} leading cause of death (Troszynski, 2003). In West Africa, it was reported that 33.3\% of maternal death occurred due to puerperal sepsis (Prual, 2000). In Industrialized countries such as the United States and Canada showed the incidence of puerperal sepsis as generally not much higher than in the developing countries (Chisembela, 2004). During 20 years period of a study in India, it was found that among the 37155 women delivered, 20.83\% died due to puerperal sepsis or its sequelae (Chhabra \textit{et al}, 2005).

Over 3.5 millions women in Bangladesh become pregnant every year and not less than 20,000 of them die while giving birth to their babies. About 85\% of pregnant women give birth to their babies through normal deliveries without any complications. Where as at least 15\% encounter various complications. Among the complications 26\% are post partum hemorrhage, 16\% Eclampsia, 21\% induced abortion, 11\% puerperal sepsis, 8\% obstructed labor and 18\% comprise the other obstetric complications (The New Nation, 24 July 2003). A study in Bangladesh found that 60\% of women die during the postpartum period compared to 20\% during pregnancy (Safe Mother, 1994). Another study in different rural area of Bangladesh (Gopalpur, Raipur, Singra and Godagari) showed that postpartum infection rate as 14.5\% (Faisel \textit{et al}, 1990). In Mymensingh Medical College Hospital (MMCH) during the year 1984-88, it was found that 17\% maternal death had caused by puerperal sepsis (Begum, 1991). Another study in Dhaka Medical College showed 20.3\% maternal death due to Puerperal sepsis (Bhuiyan \textit{et al}, 1986).
One of the predisposing factor or condition that generally leads to the puerperal sepsis include a home birth in unhygienic conditions should be included at the top of the list. Other factors include- low socioeconomic condition, anaemia, parity as primi-gravida, prolong period after rupture of the amniotic membrane, frequent per vaginal examination and prolonged labor (Chisembele, 2004). Sepsis is an important morbid condition because of its consequences on both fetal and maternal outcome characterized by septicemia, endotoxic shock or the development of peritonitis.

The causative organisms of puerperal sepsis are *Streptococcus pyogenes*, other *Beta-hemolytic streptococcus, Enterococcus faecalis, Anaerobic cocci, Clostridium perfringens, Bacteroids spp, Proteus spp, Esch. coli* other *Coliform* and *Listeria monocytogen* (Cheesbrough,2000). The role of bacteria in peurperal infection was not recognized until the end of the 19th century. In the 1840s, Dr Semmel Weiz observed that “child bed fever” was carried on the hands of birth attendants. Although his theory became hugely controversial, but he insisted for scrubbing hands of birth attendants in chloride solution or in lime before conducting the delivery. Consequently, it was observed that the death rate due to child bed fever plummeted significantly. Thus a relation between contaminated hands and puerperal sepsis was established. Puerperal sepsis has been described for centuries in ancient Indian texts (1500 BC). They recorded that good hygiene reduced perinatal disease. In 1879, Louis Pasteur identified the *Streptococcus* as the causative organism for puerperal sepsis. Since the early 1930s, when Rebecca Lance field reported her grouping system for *Hemolytic Streptococci, Group A streptococcus* was widely acknowledged as the major pathogen associated with puerperal sepsis. *Group B Streptococcus* (GBS) was initially
thought to be a commensal until 1937, while Fry reported seven causes of GBS associated puerperal fever with 3 deaths (Shet et al, 2004). Presently, around 50,000 new mums are affected each year by GBS, which usually lives harmlessly in the vagina or intestine of 10-35% of healthy women. It is one of the commonest bacteria involved in puerperal fever and infection occurs up to two weeks after child birth or abortion. During the 1970s and 1980s, GBS emerged as a significant neonatal and maternal pathogen in the United States and Western Europe with reported mortality rates of 15-50%. In the USA, 10 to 35% of pregnant women are asymptomatic carrier of GBS in the genital and gastrointestinal tract at the time of delivery (Shet et al, 2004). Streptococcus pyogenes is responsible for suppurative infections either local or invasive, associated with or with out any toxic shock syndrome (Loubinoux, 2004). Group G beta hemolytic Streptococcus was isolated from the patient with puerperal sepsis and endometritis (Russle et al, 1989). A study from Finland in 2003 reported that 42 bacterial strains from puerperal sepsis representing 18 different bacterial species were isolated by blood culture; 88% were aerobic and 12% were anaerobic. Among the aerobic bacteria the most common species were Beta Hemolytic Streptococci, Esch. coli and Staphylococcus aureus (KanKuri et al, 2003). Among the anaerobic bacteria most frequent isolates should be anaerobic Streptococci (30%) followed by Bacteroid species (10%), Staphylococci or fecal aerobic Gram negative bacteria (5%) from vaginal swab (Holland and Brews, 1980). As the disease is of polymicrobial in nature, therapy with multiple antimicrobial agents must be the rational choice to cover Gram negative, Gram positive and anaerobic organisms. However, there is no consensus as to the most effective antibiotic therapy for puerperal sepsis. Newer broad spectrum semi
synthetic penicillin as well as cephalosporin or penicillin plus amino-glycoside are found to be sufficient for patients developing endometritis after vaginal delivery. Patients developing endometritis after caesarian section need good antibiotic coverage. Gentamicin along with clindamicin and metronidazole are good options (Basu, 2003).

Though, puerperal sepsis is the common cause of maternal morbidity and mortality, but appears to be largely preventable with good antenatal check-up, aseptic delivery practices and postpartum care. When care is delayed or inadequate, infection can progress quickly to generalized sepsis, which can result in infertility, chronic disability and even death (Dare, 1998). In this local hospital, one study was conducted to see the maternal mortality rate and its causes in the year 1986. But after that study, during the long period, no study particularly on bacterial etiology of puerperal sepsis was done. It is known that bacterial pattern with their antimicrobial susceptibility is a dynamic and changing phenomenon. So time to time surveillance of this event is needed in every health care setting. Moreover, study to isolate anaerobic bacterial pathogens is yet to be performed in the local institute as well as other Microbiology laboratory in our country.

Having the described background, the present study as a preliminary one was carried out to obtain the given objectives.
OBJECTIVES

General Objective:

To find out bacterial causes of puerperal sepsis for better prevention and treatment. So that maternal morbidity and mortality can be reduced.

Specific Objectives:

1. To isolate and identify the aerobic and anaerobic bacterial causes of puerperal sepsis.

2. To observe the antimicrobial susceptibility pattern of the isolated bacterial pathogens.

3. To find out associated risk factors for puerperal sepsis.
REVIEW OF LITERATURE

2.0. OPERATIONAL DEFINITIONS

2.1. PUERPERIUM

According to WHO, the postpartum period or puerperium is the period beginning one hour after the delivery of the placenta and continuing until 6 weeks (42 days) after the birth of the infant (WHO, 1998).

2.2. SEPSIS.

The presence of viable bacteria in the blood or body tissues (Health A to Z, 2006).

2.3. PUERPERAL SEPSIS

Puerperal sepsis is an infection of the genital tract at any time between the onset of rupture of membranes or labor and the 42\textsuperscript{nd} day following delivery or abortion in which two or more of the following are present:

- Pelvic pain
- Fever of 38.45\textdegree C or more measured orally on any occasion.
- Abnormal vaginal discharge
- Abnormal smell, foul odour of the discharge
- Delay in the rate of reduction of the size of the uterus. (WHO, 2004; Carmen and Claudia, 2003; Basu, 2003.)

2.4. ANTENATAL CONTACTS

It was considered regular if at least 4 visits were done (WHO, 1994).

2.5. PROLONGED RUPTURE OF MEMBRANES

More than 24 hours between loss of water and onset of labor pain.
2.6. PROLONGED LABOR

Labor duration greater than 24 hours from the onset of mild pains to the birth of baby.

2.7. RETAINED PLACENTA

Placenta did not come out for more than one hour after baby’s birth.

2.8. DIGITAL EXAMINATION

Up to 6 (satisfactory), between 7 and 13 and more than 13 examination (repeated vaginal examination). (Rani, 2004; Guimaraes, 2007)

2.9. SOCIOECONOMIC CONDITION (Islam, 1992)

Lower income group : Who had monthly income less than Tk. 3000/=  
Middle income group : Monthly income between Tk. 3001 – 20,000/=  
Upper income group : Who had monthly income above Tk 20,000/=  

2.10. CAUSATIVE ORGANISM AND PREDISPOSING FACTORS

Puerperal sepsis is the infection of the genital tract up to 42 days after delivery. It is an important cause of maternal mortality and morbidity.

The predisposing factors or conditions leading to the puerperal sepsis include a home birth in unhygienic conditions, low socioeconomic condition, anemia, primi parity, prolong rupture membrane, multiple vaginal examination, prolong labour (Chisembele, 2004). Sepsis is an important morbid condition because of its consequences on both fetal and maternal outcomes characterized by septicemia, endotoxic shock or the development of peritonitis.

The causative organism of puerperal sepsis are *Streptococcus pyogenes*, other *Betahemolytic streptococcus*, *Enterococcus* species, anaerobic cocci, *Clostridium*
perfringens, Bacteroides, Proteus, E. coli other Coliform and Listeria monocytogen. (Cheesbrough, 2000)

3.11. PHYSICIAL CHANGES IN NORMAL PUERPERIUM (WHO, 2007)

Physical changes those occur during 6 - 8 weeks following delivery can be described as general changes and local changes.

2.11.1. General changes

Temperature normal but may remain a reactionary rise may occur after difficult labour. It does not exceed 38°C and drops within 24 hours. A slight rise may occur at the 3rd day due to engorgement of the breasts. Pulse normal but may rise if there is hemorrhage or infection. After delivery painful uterine contractions occur in early puerperium increasing with suckling due to oxytocin release.

Breasts: Colostrums is secreted in the first 3 days followed by establishment of milk secretion at the 4th day. Than the breasts become engorged, larger, painful, tender while suckling relieves the discomfort. Suckling stimulates prolactin secretion, which causes milk production and oxytocin release, which stimulates milk ejection.

Urine: Diuresis by the 2nd - 4th day, lasting for 3-4 days. Retention of urine may occur due to: atony of the bladder, laxity of the abdomen, recumbancy, reflex inhibition if the perineum is sutured, compression of the urethra by vulval oedema or haematoma.

Bowel: Tendency to constipation due to atony of the intestine, laxity of abdomen and perineum, anorexia and loss of fluids.
**Loss of weight:** due to evacuation of the uterine contents, more fluid loss in urine and sweat.

**Blood:**

Increased coagulability of the blood continues during the first two weeks in spite of significant decrease in a number of coagulation factors. Hemoglobin concentration: tends to fall in the first 2-3 days.

**Menstruation:** is regained by the 6th - 8th weeks after delivery but in lactating women a variable period of amenorrhea may be present.

2.11.2. Local changes

The uterus is involutes as follow: Autolysis of the excess muscle fibres. The blood vessels are obliterated by thrombosis and become degenerated while its remnants are transformed into elastic tissues the deciduals, except the basal layer, is separated. After delivery the uterine weight is 1000 gm. By the end of 6 weeks it is 50 gm. After delivery the length of the uterus is 20 cm and felt at the level of umbilicus. After one week it is midway between umbilicus and symphysis pubis. After 2 weeks it is at the level of symphysis. By the end of the 6th week it is 7.5 cm long uterine ligament are involutes and sub involution predisposes to prolapse and retroversion.

**Lochia:**

It is the genital tract discharge in the first 15 days of puerperium. It is alkaline and composed of blood, decidual fragments, cervical mucus, vaginal transudate and bacteria.
Lochia rubra (red): Consists mainly of blood and decidua. It lasts for 5 days.

Lochia serosa (pale): due to relative decrease in RBCs and predominance of leukocytes. It lasts for 5 days.

Lochia Alba (white): Consists mainly of leukocytes and mucus. It lasts for 5 days. Persistence of red lochia means subinvolution. Offensive lochia means infection. In severe infection with septicaemia, lochia is scanty and not offensive.

Cervix: is closed by the end of the first week.

Vagina: Vaginal rugae appear in the 3rd week.

Vulva: Its gaping disappears by the end of puerperium.

Perineum: regains its tone by the end of puerperium while persistence of its laxity predisposes to prolapse.

2.12. PATHOGENESIS OF PUERPERAL SEPSIS.

In the adult women the genital tract defenses are weakest during and immediately after abortion or labour because of raw placental site, breaks in the epithelial linings of the cervix and vagina, tissues are bruised and devitalized, vulva, vagina and cervix are wide open.

The discharge of liquor and lochia (both alkaline) reduces vaginal acidity. Degenerating blood clots and fragments of deciduas often a nidus for infection. And the patient’s general resistance is lowered by the strain of pregnancy and possibly by anaemia and malnutrition (Jeffcoate’s 1987).

In the majority of instances the organisms giving rise to puerperal infection are present in the vagina before delivery. The normal bacterial flora of the vagina
comprises predominantly the non pathogenic *Lactobacilli* and *Diphtheroids* but other organisms are almost invariably also present and generally cause no symptoms in the non-puerperal woman. Anaerobic *Streptococci* can be isolated from about 30 per cent of vaginal swabs, *Bacterioids* species from 10 per cent, *Staphylococci* or faecal aerobic gram –negative bacilli (*Escherichia coli, Proteus mirabilis*, etc) from almost 5 per cent. *Beta hemolytic streptococci* occur less frequently and are usually of group B. Group A is unusual but very important if they are on occasion present. Other potential pathogens such as *Clostridium perfringenes* are also very often present (Holland and Brews, 1980).

Normal vaginal flora contains both aerobic and anaerobic organisms. An aerobic bacterium tends to occur in the ten fold less concentration than anaerobic bacteria. e.g. $10^8$ aerobic bacteria compared with $10^9$ anaerobic bacteria/ml of vaginal fluid. Component of normal flora may become pathogenic in changed environment (Misheel Dr.1988).

The placental site is covered with adherent fragments of membranes, placenta or blood coat. There may be necrotic deciduals, pus or gas formation which can be caused by a variety of organisms - that is *Streptococci*, gram negative rods in addition to *Cl. Perfringens*. The muscular uterus may also be inflamed and in spreading infection the venous channels in the myometrium may show thrombophlebitis. The peritoneum or the uterus may show roughening from fibrin deposition and purulent exudates. Inflammatory effusions may occur in the broad ligaments following infected lacerations of the cervix and in the absence of treatment may become purulent. Abscesses may also form in association with the tubes ovaries following direct extension from the uterine cavity (Holland and Brews, 1980).
2.13. HISTORICAL BACKGROUND

Historically, puerperal sepsis has been a common pregnancy-related condition, which could eventually lead to obstetric shock or even death. During the 19th century, it took on epidemic proportions, particularly when home delivery practice changed to delivery in lying-in hospitals, as there still was a total ignorance of asepsis. In 1843 Oliver Holmes in Boston, USA, was the first to establish that puerperal fever was contagious and was carried by the unwashed hands of the physician from bed to bed. In 1847 Semmelweis in Vienna, Austria also concluded that examiners might transmit infection from live patients as well as from the dead and ordered his students to scrub with the chlorine solution before every physical examination. This led to a striking decrease of mortality due to puerperal sepsis from 11% in 1846 to 3% in 1847 (Adriaanse, 2000).

2.14. EPIDEMIOLOGY

Maternal sepsis ranked 46th in terms of Disability adjusted life years (DALYs) in Global burden of disease (GBD) 1990 and its burden accounted for 18% of total burden for maternal conditions. Estimated deaths due to puerperal sepsis accounted for 15% of all maternal deaths in GBD 1990 (Dolea, 2003).

Puerperal sepsis often develops after discharge and women are reluctant to go to the hospital, using only hospital data will underestimate the real incidence. The rate of puerperal infections was 2.5% in women who had a vaginal delivery (Yokoe, 2001).

The most conservative estimate of the incidence of postpartum infection was 1.7%. The incidence of serious infection was 0.5% (Nguyen, 1996). Maternal mortality continues to be a major challenge to obstetricians of the developing world. The causes and strategies to prevent it do not differ much in urban and
rural areas. However, conditions in rural areas make the situation difficult. The conditions are the formidable risks that many women may face in their reproductive years. Rural surgeons with their dedication, will power and sustained efforts, can help these women enjoy a safer motherhood (Chisembele, 2004). According to WHO 2003, the incidence of maternal sepsis was 1.7, 0.22, 0.07, 0.15 and 4.5 per 100 live births in Nigeria, Niger, South Africa, USA and Bangladesh. A study from Zaire, Nigeria reported a rate of post-partum genital sepsis of 14.8% among women who delivered at home compared to 7.9% in those who delivered in facility [Brentlinger, 1998]. A study from Senegal demonstrated an incidence of sepsis of 8.7% for home deliveries compared to 1.9% for deliveries in health facilities [Jolly, 2000]. In terms of clean delivery practices, Semmelweis in 1847, documented reductions in mortality due to puerperal sepsis from 11% to 3% as a result of the introduction of scrubbing protocols with chlorine solution before every physical examination [Waterstone, 2001]. Puerperal sepsis is among the leading causes of preventable maternal death. In a study of maternal mortality in a tertiary care hospital in Abbottabad to determine causes and preventable factors, the contribution of sepsis to maternal deaths was 19.2% and it was the third leading cause of death (Begum, 2003). In the state of Anambra of Nigeria, a study showed that sepsis was the fourth leading cause of death and contributed 12.1% to the maternal deaths (Chukudebelu, 1988). In Pakistan, sepsis was among the three leading causes of death in both hospitals and the community (Jafarey, 2002). In Europe and the western countries, sepsis continues to be a major contributor to maternal deaths even though the rate of maternal deaths has drastically gone down. A review covering a period of 20
years, in Norway, on the number and causes of maternal deaths, postpartum sepsis accounted for 4 of the 47 deaths (10%) and was the third leading cause of death (Vangen, 2003). In Poland over a 10-year period, 462 maternal deaths were recorded and sepsis accounted for 27.3% of the direct maternal deaths and was the second leading cause of death (Troszynski, 2003). A study on ‘Maternal Intensive Care and Near-miss Mortality’ in Canada, showed sepsis to be the third main reason for transfer to intensive care unit and accounted for 15% of cases (Baskett, 1998). This was also observed in Brazil where sepsis was among the leading causes of transfer to intensive care unit (Dias, 2002). In South Africa, sepsis is one of the main indications for emergency peripartum hysterectomy (Sebitloane, 2001). Puerperal sepsis is reported to be a major complication of induced abortion in Nigeria (Ikechebelu, 2003). In India, a study showed that 50% of maternal deaths due to sepsis were related to unsafe induced abortion (Prakash, 1991). Sepsis has been shown to have a very high case fatality rate. A study on the ‘Incidence and Case Fatality Rates’ in West Africa looking at severe maternal morbidity from direct obstetric causes, showed sepsis to have a case fatality rate of 33.3% (Prual, 2000). In Malawi, where incidence rate 1.34% have been reported (Kulmala, 2000). A slightly higher incident rate was observed in one study in Sierra Leone of 5.38%, but this could be due to the relatively few numbers of women seen compared to other studies (Leigh, 1997). Interestingly, a higher incidence rate is reported in one study in the United States where the study population may be considered to be of a similar background to those in developing countries. This study looked at women from low socioeconomic backgrounds and reported an incidence rate of 6.18% (Tamura, 2000). A systematic review summarizing the true extent of puerperal sepsis would help
shed more light. Although sepsis is an important public health problem contributing to maternal morbidity and mortality, information on the global magnitude of the problem is limited. Studies reporting incidences of sepsis are widely dispersed in the literature. The provision of the true picture of the problem would better inform decision making in planning of healthcare particularly in developing countries (Chisembele, 2004).

Over 35 lakh reproductive age of women become pregnant in Bangladesh every year and 20000 of them die while giving birth to their babies. About 85 per cent of pregnant women give birth to their babies through normal deliveries while the rest 15 per cent experience various complications. Among the complications 26% post partum hemorrhage. 16% eclampsia, 21% induced abortion, 11% puerperal sepsis, 8%obstructed labor and 18% other obstetric complications (New Nation, 2003). It was observed in one study in Bangladesh that 60% of women die during the postpartum period compared to 20% during pregnancy (Safe Mother,1994). A study in four rural area of Bangladesh Gopalpur, Raipur, Singra and Godagari showed that postpartum infection rate was 14.5% (Faisel et al1990). In Mymensingh medical college hospital during the year 1984-88 it was found that 17% maternal death was caused by puerperal sepsis (Begum, 1991). Another study in Dhaka Medical College showed that 20.3% maternal death due to puerperal sepsis (Bhuiyan et al, 1986).

2.15. BACTERIOLOGY OF PUERPERAL SEPSIS

Puerperal sepsis is a genital tract infection resulted from bacterial invasion during or after labor. The bacteria which are involved in puerperal sepsis can be categorized as follows
2.15.1. Causative Organisms (WHO, 2007)

- Aerobic
  - Gram positive
    - *Haemolytic streptococcus group A* (severe cases).
    - *Non-haemolytic streptococci*.
    - *Staphylococcus aureus*.
    - *Gonococci*.
  - Gram negative
    - *E.coli*.
    - *Proteus*.
    - *Pseudomonas*.
    - *Klebsiella*.

- Anaerobic
  - Gram positive
    - *Anaerobic streptococci (the commonest)*.
  - Gram negative
    - *Cl. perfringens*
    - *Bacteroids*.

2.15.2. General consideration for aerobic bacteria causing puerperal sepsis:

2.15.2.1. *Staphylococcus aureus*

**Taxonomy**

Family: Micrococcaceae

Genus: *Staphylococcus*

Species: *Staphylococcus aureus*

General properties of *S. aureus*
Gram positive cocci those characteristically undergo binary fission through in more than one plane to form irregular clusters. Usually form catalase. Nearly all are facultative anaerobes that grow better under aerobic than anaerobic conditions. The metabolism is respiratory and fermentative and most strains grow in the presence of 10% sodium chloride and between 18 and 40°C. The cell wall contains peptidoglycan, teichoic acid and G + C contained of the DNA 30 – 39 mol % (Topley and Wilsons 8th ed. Vol.2, p. 162).

**Cultural characters**


**Growth characters in common bacteriological media:** After 24 hours incubation in Nutrient agar (NA) Colonies are circular, 2 – 4mm in diameter convex with shining surface and may be pigmented in golden yellow, white or lemon yellow (Cheesebrough, 2000) On blood agar *S. aureus* produces yellow to cream or occasionally white 1-2 mm in diameter colonies, some are beta hemolytic and on Mac Conkey agar most strain is lactose fermenting. Biochemically *S. aureus* is Coagulase, DNA ase and Catalase positive. On Mannitol salt agar which is selective and indicator medium. Most strain of *S. aureus*. Ferments mannitol and form colonies surrounded by yellow zones due to acids production whilst most other *staphylococci* fail to ferments mannitol and form colonies with red or purple zones (Colee *et al*, 1989).
2.15.2.2. *Streptococcus*

**Taxonomy**

Family: Streptococcaceae

Genus: *Streptococcus*, Rosenbach, 1884

**General properties of Streptococcus**

*Streptococcus* is a genus of spherical shaped Gram-positive bacteria, belonging to the phylum Firmicutes. They test positive for oxidase and negative for catalase (Ryan, 2004). Cellular division occurs along a single axis in these bacteria, and thus they grow in chains or pairs, thus the name from Greek *streptos*, meaning easily bent or twisted, like a chain. Contrast this with *staphylococci* which divide along multiple axes and generate grape-like clusters of cells.

**Pathogenesis**

*Streptococcus* species are responsible for many cases of meningitis, bacterial pneumonia, endocarditis, erysipelas and necrotizing fasciitis (the 'flesh-eating' bacterial infections). It should be noted, however, that many *streptococcal* species are non-pathogenic. *Streptococci* are also part of the normal commensal flora of the mouth, skin, intestine and upper respiratory tract of humans. Clinically, individual species of *Streptococcus* are classified primarily based on their hemolytic properties (breakdown of red blood cells in a laboratory). Alpha hemolysis is caused by a reduction of iron in hemoglobin giving it a greenish color on blood agar. Beta only hemolysis is complete rupture of red blood cells giving distinct, wide, clear areas around bacterial colonies on blood agar. Other *streptococci* are labeled as gamma haemolytic, actually a misnomer as no hemolysis takes place (Patterson, 1996).
Beta-hemolytic streptococci are further characterised via the Lancefield serotyping - based on specific carbohydrates in the bacterial cell wall (Faclam, 2002). These are named Lancefield groups A to T, although some species, such as *S. pneumoniae*, do not express Lancefield antigens. Medically the most important groups are the *alpha-hemolytic streptococci*, *S. pneumoniae* and *Streptococcus Viridans*-group and the *beta-hemolytic streptococci* of Lancefield groups A and B (also known as “Group A Strep” and “Group B Strep”)

**Beta-Hemolytic Streptococci**

**Group A**

*S. pyogenes* (also known as GAS) is the causative agent in *Group A streptococcal* infections (GAS) including Strep sore throat, acute rheumatic fever, scarlet fever, Puerperal sepsis, Septicaemia, toxic shock syndrome, acute glomerulonephritis and necrotizing fasciitis. If Strep sore throat is not treated, it can develop into rheumatic fever, a disease that affects the joints and heart valves. Other *Streptococcus* species may also possess the Group A antigen but human infections by non-*S. pyogenes* GAS strains (some *S. dysgalactiae* subsp. *equisimilis* and *S. anginosus* Group strains) appear to be uncommon (Cheesbrough, 2000).

**Group B**

*S. agalactiae*, or GBS causes septic abortion and puerperal sepsis and occasionally urinary tract infection. *S. agalactiae* forms part of the normal microbial flora of the female genital tract. It causes neonatal septicaemia and meningitis (Cheesbrough, 2000). The American College of Obstetricians and
Gynecologists, American Academy of Pediatrics and the Centers for Disease Control recommend all pregnant women, who are between 35-37 weeks gestation, should be tested for GBS. Those who test positive should be given prophylactic antibiotics during labor which can prevent transmission to the infant (Scarag, 2002). In the UK, clinicians have been slow to implement the same standards as the US, Australia and Canada. Only 1% of maternity units test for the presence of Group B Streptococcus.

Cultural characters
After 24 hour incubation on blood agar media *S. Pyogenes* produces beta haemolytic colonies i.e. the colonies are surrounded by a zone of complete haemolysis. Microscopically are gram positive cocci .Occurring characteristicly in short chains.

**Bacitracin sensitivity test:** Identification disc of bacitracin is adding to a blood agar plate. Most strains are sensitive to bacitracin. Biochemically are Catalase negative.

**2.15.2.3. *Streptococcus agalactiae:***
On blood agar media produce grey mucoid colonies surrounded by a small zone of beta haemolysis. Microscopically are Gram positive cocci, occurring characteristicly in short chains but also in pairs and singly .Most strains are capsulated.

**Identification test:** CAMP reaction, Hydrolysis of sodium hippurate, Production of orange pigment.
2.15.2.4. *Enterococcus faecalis*

**Taxonomy**

Family: Enterococcaceae  
Genus: *Enterococcus*  
Species: *E. faecalis*

**Binomial name**

*Enterococcus faecalis* (Schleifer & Kilpper-Bälz 1984)

**General properties:** Prior to 1984, *E. faecalis* was known as *Streptococcus faecalis*. *Enterococcus faecalis* is a Gram-positive commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals (Ryan, 2004). Like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment. The existence of *Enterococci* in such a dual role is facilitated, at least in part, by its intrinsic and acquired resistance to virtually all antibiotics currently in use.

**Pathogenesis.** It is a normal commensal of the vagina and intestinal tract. (Cheesbrough, 2000). *E. faecalis* can cause endocarditis, as well as bladder, prostate and epididymal infections, nervous system infections are less common. *E. faecalis* is resistant to many commonly used antimicrobial agents (aminoglycosides, aztreonam, cephalosporins, clindamycin, the semi-synthetic penicillins nafcillin and oxacillin, and trimethoprim-sulfamethoxazole). Exposure to cephalosporins is a particularly important risk factor for colonization and infection with *Enterococci*. 
Cultural characters:

On blood agar media: Enterococci are mainly non haemolytic but some strains show alpha or beta haemolysis. On MacConkey and CLED agar: E. faecalis ferments lactose producing small dark red magenta colonies on MacConkey agar and small yellow colonies on CLED agar. Enterococcus species are also able to grow in the presence of 6.5% sodium chloride and 40% bile (Cheebrough, 2000)

2.15.2.5. Escherichia coli

Taxonomy

Family: Enterobacteriaceae

Genus: Escherichia

Species: E. coli

Binomial name

Escherichia coli. (Castellani and Chalmers 1919)

Escherichia coli: is a Gram negative usually motile rod. Is one of the main species of bacteria living in the lower intestines of mammals, known as gut flora. When located in the large intestine, it actually assists with waste processing, vitamin K production and food absorption. Discovered in 1885 by Theodor Escherich, a German pediatrician and bacteriologist, E. coli are abundant: the number of individual E. coli bacteria in the feces that a human defecates in one day averages between 100 billion and 10 trillion. However, the bacteria are not confined to this environment and specimens have also been located, for example, on the edge of hot springs. The E. coli strain O157:H7 is one of hundreds of strains of the bacterium that causes illness in humans. As with all Gram-negative organisms, E. coli are unable to sporulate. Thus, treatments which kill all active
bacteria, such as pasteurization or simple boiling are effective for their eradication, without requiring the more rigorous sterilization which also deactivates spores (Feng et al., 2002).

**Culture:** On blood agar E.coli produces 1-4 mm diameter colonies after over night incubation. The colonies may appear mucoid. Some strains are haemolytic. On MacConkey and CLED agar: *E. coli* ferments lactose, producing smooth pink colonies and yellow colonies.

**Biochemically** Indole positive, Reduce nitrate to nitrite, Citrate and H₂S negative (Cheesbrough, 2000).

**Role in microbiology**
Because of its ubiquity, *E. coli* is frequently studied in microbiology and is the current "workhorse" in molecular biology. Its structure is clear and it makes for an excellent target for novice, intermediate, and advanced students of the life sciences. Bacterial conjugation was first discovered in *E. coli* and it remains the primary model to study conjugation (Christie, 2007).

### 2.15.3. General consideration for anaerobic bacteria causing puerperal sepsis:

#### 2.15.3.1. *Peptostreptococcus*

**Taxonomy**

Family: Clostridiaceae

Genus: *Peptostreptococcus* Garrity et al. 2001

**Species:**

*P. anaeobius, P. asaccharolyticus, P. harei, P. hydrogenalis, P. indoliticus*

*P. ivorii, P. lactolyticus, P. magnus, P. prevotii, P. tetradius, P. vaginalis* etc.
**General properties of Peptostreptococcus**

*Peptostreptococcus* is a genus of anaerobic, Gram-positive, non-spore forming bacteria. The cells are small, spherical and can occur in short chains, in pairs or individually. (Ryan *et al.* 2004). *Peptostreptococcus* are slow-growing bacteria with increasing resistance to antimicrobial drugs (Higaki *et al.* 2000). Culture produces a foetid odour Scottmueller (1910) isolated the organisms from puerperal fever and gave the name *peptostreptococcus anaerobius* or *putridus*. This is the commonest anaerobic organism recovered from human infections like pleuropulmonary disease, brain abscess and puerperal infections (Chakraborty, 2003).

**Pathogenesis**

*Peptostreptococcus* species are commensal organisms in humans, living predominantly in the mouth, skin, gastrointestinal and urinary tracts and compose a portion of the bacterial gut flora. Under immunosuppressed or traumatic conditions these organisms can become pathogenic, as well as septicemic, harming their host (Mader and Calhoun, 1996).

**Cultural characters.**

On blood agar after 48 hours anaerobic incubation with 10% Co₂ at 37°C the colonies are 0.5-1.5 mm in diameter round, convex, shiny, opaque, grey and non hemolytic (MacKie and McCartney, 1996). Thioglycollate broth abundant gas produced in broth culture. Biochemically Catalase negative (Cheesbrough, 2000).

### 2.15.3.2. Clostridium perfringens

**Taxonomy**

- Family: Clostridiaceae
- Genus: *Clostridium*
- Species: *C. perfringens*
Binomial name

*Clostridium perfringens* (Hauduroy *et al.* 1937).

General properties of *Clostridium perfringens*

*Clostridium perfringens* (formerly known as *Clostridium welchii*) is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium of the genus *Clostridium* (Ryan, 2004). About 4-6.1um with stubby ends. Occurring singly or in pair often capsulated in tissues (Mackie and McCartney, 1996). *C. perfringens* is ubiquitous in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects and soil. Virtually every soil sample ever examined, with the exception of the sands of the Sahara, has contained *C. perfringens*.

Pathogenesis

*C. perfringens* is commonly encountered in infections as a benign component of the normal flora (Wells and Wilkins, 1996). Infections due to *C. perfringens* show evidence of tissue necrosis, bacteremia, emphysematous cholecystitis and gas gangrene, which is also known as clostridial myonecrosis. The toxin involved in gas gangrene is known as α-toxin, which inserts into the plasma membrane of cells, producing gaps in the membrane which disrupt normal cellular function (Warrell *et al.* 2003). *C. perfringens* type A1: Causes gas gangrene, anaerobic cellulitis, Puerperal infection and Septicaemia (Cheebrough, 2000).

Cultural characters

On Blood agar plates *C. perfringens* grown anaerobically produces double zone of haemolysis, flat, spreading, rough, translucent colonies with irregular margins with. A Nagler agar plate, containing 5-10% egg yolk, is used to presumptively
identify strains which produce α-toxin, a diffusible lecithinase which interacts with the lipids in egg yolk to produce a characteristic precipitate around the colonies. One half of the plate is inoculated with antitoxin to act as a control in the identification. On Robertson,s cooked meat medium: *C. perfringens* is sacchorolytic and slightly proteolytic. Gas is formed. A saccharolytic reaction is shown by Redding of the meat with a rancid smell due to carbohydrate decomposition.A proteolytic reaction is shown by blackening of the meat with a very unpleasant smell due to protein decomposition (Cheesbrough, 2000) Biochemically it ferments common sugars such as glucose, lactose, maltase with production of acid and gas. The organism is indole negative, MR positive and VP negative and form H₄S abundantly. Most strains reduce nitrates. In litmus milk, lactose fermentation causes change in the colour of litmus from blue to red due to production of acid. Casein is coagulated by the acid and the clotted milk is disrupted due to vigorous gas production and this is known as “Stormy clot” (Chakraborty, 2003).

### 2.15.3.3. Bacteroides.

**Taxonomy**

Family: Bacteroidaceae

Genus: *Bacteroides* (Castellani & Chalmers 1919)

**Species**

*B. acidifaciens*, *B. distasonis*, *B. gracilis*, *B. fragilis*, *B. oris*, *B. ovatus*, *B. putredinis*, *B. pyogenes*, *B. stercoris*, *B. suis*, *B. tectus*, *B. thetaiotaomicron* *B. vulgatus*
As normal flora anaerobic Gram-negative bacilli are present in cervix and vaginal fornices, about 80% strains belong to *Prevotella melaninogenicus* group and 15% to Asaccharolytica group (Chakraborty, 2003).

**Epidemiology:** *Bacteroides* and *Prevotella* species are part of the normal flora of the mouth, gastrointestinal tract, or female genital tract. Members of the *Bacteroides fragilis* group predominate in the gastrointestinal tract flora; members of the *Prevotella melaninogenica* (formerly *Bacteroides melaninogenicus*) and *Prevotella oralis* (formerly *Bacteroides oralis*) groups are more common in the oral cavity. These species cause infection as opportunists, usually after an alteration of the body’s physical barrier and in conjunction with other endogenous species. *Prevotella and Porphyromonas* species are associated with infection of the female genital tract (Cheesbrough, 2000). The incubation period is variable and depends on the inoculums and the site of involvement but usually is 1 to 5 days.

**Diagnostic tests:** Anaerobic culture media are necessary for recovery of *Bacteroides* or *Prevotella* species. Because infections usually are polymicrobial, aerobic cultures also should be obtained. A putrid odor suggests anaerobic infection. Use of an anaerobic transport tube or a sealed syringe is recommended for collection of clinical specimens. Rapid diagnostic tests, including polymerase chain reaction and fluorescent in situ hybridization are available in research laboratories.

**Cultural characters** on blood agar. *Prevotella* species grow slower than *Bacteroides*. *P. melaninogenica* produces brown to black colonies after 3 - 7 days incubation (Cheesbrough, 2000).
**Treatment** Some species of *Bacteroides* and almost 50% of *Prevotella* produce beta-lactamase. A beta-lactam penicillin active against *Bacteroides* combined with a beta-lactamase inhibitor can be useful to treat these infections (Ampicillin-sulbactam, Amoxicillin-clavulanate, ticarcillin-clavulanate or piperacillin-tazobactam). *Bacteroides* species of the gastrointestinal tract usually are resistant to penicillin G but are predictably susceptible to metronidazole, chloramphenicol, and sometimes, clindamycin. More than 80% of isolates are susceptible to Cefoxitin, Ceftizoxime linezolid and Imipenem. Cefuroxime, Cefotaxime and Ceftriaxone are not reliably effective.

**2.16. DIAGNOSIS OF PUERPERAL SEPSIS**

**2.16.1. History**

**2.16.2. General examination**

a. Temperature, pulse, blood pressure, level of consciousness.

b. Skin eruption or jaundice (*Cl. perfringens* infection).

c. Tonsils.

d. Breasts, chest and heart.

e. Lower limbs for signs of thrombophlebitis.

**2.16.3. Abdominal examination:**

a. Loin tenderness

b. Abdominal rigidity and tenderness.

c. Uterine size, tenderness and abdominal masses related to the uterus.
2.16.4. **Local examination:**

a) The perineum for infected episiotomy or lacerations.

b) Lochia for amount, colour and odour.

c) Bimanual examination for:
   
i. Uterine size, consistency, tenderness, position and mobility.
   
ii. Cervix: closed or opened, contents felt through it or lacerations.
   
iii. Adnexae: mass.
   

d) Speculum examination: to visualize the cervix and vagina.

2.16.5. **Investigations**

a) Swab and culture: from the cervix and upper vagina for aerobic and anaerobic cultures.

b) Blood culture: taken at peak of temperature in case of septicaemia.

c) Blood picture: haemoglobin and leukocytes.

d) Urine analysis and culture: midstream or catheter specimen.

2.17. **PREVENTION OF PUERPERAL SEPSIS**

a) **Antenatal:**

   i. Proper diet, vitamins and minerals.

   ii. Anemia and diabetes should be treated.

   iii. Local or distant infection should be treated.

   iv. Avoid sexual intercourse late in pregnancy.
b) **Intranatal:**

i. Strict aseptic and antiseptic measures for the patient, attendants and instruments.

ii. Minimize vaginal examinations.

iii. Avoid bleeding and excessive blood loss should be replaced.

iv. Lacerations should be properly sutured immediately.

v. Prophylactic antibiotics in PROM and prolonged or instrumental delivery.

c) **Postnatal:**

i. Maintenance of aseptic precautions.

ii. Care of the perineal or abdominal wounds.

iii. Minimise visitors and keep whom are infected away.

iv. Early isolation of cases of puerperal sepsis.

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**2.18. TREATMENT OF PUERPERAL SEPSIS**

a) **General treatment**

i. Isolation in a separate room or fever hospital.

ii. Diet: light diet rich in vitamins and minerals with plenty of fluids.

iii. Supportive treatment: restoration of fluid and electrolyte balance, correction of anaemia

iv. Symptomatic treatment:

a. Analgesics,

b. Antipyretics and cold fomentations.

v. Observations: pulse, temperature, blood pressure, vaginal bleeding, lochia,

vi. Antibiotic therapy
i. Broad spectrum antibiotic (ampicillin or cephalosporin) + gentamycin + metronidazole or

ii. Clindamycin + gentamycin.

One of these regimens is started till the result of culture and sensitivity.

Antitoxin serum is given in *Cl. perfringens infection*.

b) Promotion of drainage

i. Fowler’s or semisitting position.

ii. Removal of stitches if there is purulent discharge from a wound.

iii. Ergot preparations: help drainage of lochia.

iv. Incision and drainage of the abscess:
   a. In pelvic abscess: posterior colpotomy + drain.
   b. In parametric abscess: incision + drain at the pointing point (usually above the inguinal ligament).


c) Treatment of complicated cases

i. General peritonitis:
   a. No oral feeding.
   b. Ryles tube and suction.
   c. Intravenous fluids.
   d. Parenteral antibiotics.
ii. Thrombophlebitis:
   a. Antibiotics.
   b. Anticoagulant therapy (see DIC).
   c. Immobilisation and elastic stocking.

2.19. ANAEROBIC BACTERIOLOGY

An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen. Obligate anaerobes are bacteria that can live only in the absence of oxygen. Obligate anaerobes are destroyed when exposed to the atmosphere for as briefly as 10 minutes. Some anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those organisms that will grow with or without oxygen. The methods of obtaining specimens for anaerobic culture and the culturing procedure are performed to ensure that the organisms are protected from oxygen.

Microorganisms vary in their need for oxygen and use of it as a means of producing energy. Depending on its atmospheric requirements, an organism can be described as:

- An obligatory (strict) aerobe: Requires free oxygen to survive i.e. *Pseudomonas aeruginosa*.
- A microaerophilic organism: Grows best in the presence of only a trace of free oxygen i.e. *Campylobacter jejuni*.
- An obligatory (strict) anaerobe: Survives only in the absence of oxygen i.e. *Clostridium tetani*.
- A facultative anaerobe: Can live with or without free oxygen i.e. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli*.
- Carboxyphilic organism: Requires an atmosphere which contains carbon dioxide i.e. *Neisseria meningitides*.
2.19.1. Classification of anaerobic bacteria

- Spore-forming:
  Rod, Gram positive - *Clostridium*

- Nonspore-forming:
  Rod, Gram positive  *Propionibacterium*,  *Bifidobacterium*,  *Lactobacillus*,  *Eubacterium*,  *Actinomyces*

  Rod, Gram negative  *Bacteroides*,  *Fusobacterium*,  *Campylobacter*

  Cocci, Gram positive  *Peptococcus*,  *Peptostreptococcus*

  Cocci, Gram negative  *Veillonella*

2.19.2. Pathogenesis of anaerobic infections.

**Virulence Factors**

1. Anti-phagocytic capsule: Also promote abscess formation

2. Tissue destructive enzymes: *B. fragilis* produces variety of enzymes (lipases, proteases, collagenases) that destroy tissue and Abscess Formation

3. Beta-lactamase production: *B. fragilis* – protect themselves and other species in mixed infections

4. Superoxide dismutase production: Protects bacteria from toxic O₂ radicals as they move out of usual niche.
Infection caused by anaerobes commonly is due to combinations of bacteria that function in synergistic pathogenicity. Although studies of the pathogenesis of anaerobic infections have often focused on a single species, it is important to recognize that the anaerobic infections most often are due to several species of anaerobes acting together to cause infection (Brooks et al, 2004). The nonsporing anaerobic organisms are recognized as opportunistic pathogens that may produce disease when the host’s resistance is lowered such as trauma, tissue necrosis, impaired circulation. AIDS, administration of antibiotics, corticosteroids and cytotoxic agents. Diabetes, malnutrition or malignancy act as predisposing factors. The infection due to anaerobes are usually polymicrobial, more than one anaerobe is involve along with aerobic organisms. In most cases the infection remains localized but bacteraemia may occur following dissemination of the organisms. In anaerobic infections the resultant pus is usually putrid and cellulitis is a frequent finding (Chakraborty, 2003).

2.19.3. Culturing of anaerobes bacteria: Methods & Technique:

There are several techniques for obtaining anaerobic conditions:

1. Commercially produced sachets containing oxygen removing chemical i.e. they are non gas generating systems
2. Copper coated steel wool to remove oxygen
3. Reducing agents in culture media

2.19.3.1. Commercially produced sachets containing oxygen removing systems

**Merck anaerocult anaerobic system**

The sachets contained a mixture of iron powder, citric acid, sodium carbonate and kieaclguhr. Addition of a small volume of water activates the chemicals.
Oxygen is rapidly removed leaving anaerobic conditions. Some carbon dioxide is produced.

**Oxoid AneroGen system:**

The active oxygen removing component in AneroGen sachets is ascorbic acid. The sachets are designed for use in 2.5 litre and 3.5 litre capacity anaerobic jars. The paper sachet is placed in the jar immediately before it is closed no water is needed to activate the chemicals within 30 minutes of closing the jars, the oxygen level is reduced to below 1% (carbon dioxide level is 9 – 13%).

**Control of the AneroGen system:**

Use of an anaerobic control indicator in the jar is recommended to ensure anaerobic condition have been produced.

**Coy anaerobic chamber.** The anaerobic chamber used is a flexible glove box (Coy Laboratory Products, Grass Lake, Mich.) kept at 35°C and filled with an atmosphere of 10% carbon dioxide, 10% hydrogen, and 80% nitrogen. The anaerobic conditions in the chamber were monitored with an indicating solution of methylene blue-glucose and a solution of resazurin in cysteine hydrochloride. Both solutions become colorless under anaerobic conditions.

**BBL GasPak system.** The GasPak system included the GasPak jar (2.5 liters) with a catalyst chamber containing new palladium pellets and the GasPak Anaerobe envelope. Before each use the pellets were conditioned in a hot-air oven at 120°C for 2 h. A GasPak Anaerobe envelope was placed in each jar, and 10 ml of water was added to the envelope. Freshly conditioned palladium catalyst was added to the jar, and the atmospheric conditions in the jar system were monitored with a disposable BBL Dry Anaerobic Indicator Strip (Becton Dickinson).
**AnaeroPack system.** The AnaeroPack (Mitsubishi Gas Chemical America, Inc., New York, N.Y.) system included the medium-sized AnaeroPack rectangular container (9.5 by 6.75 by 3.25 in.; 3.2 liters) and two AnaeroPack sachets. The sachets were removed from their foil packs and placed in the container along with a disposable BBL Dry Anaerobic Indicator Strip to monitor the atmospheric conditions. In addition, the AnaeroPack sachets were tested in the GasPak jar (Delaney, 1997)

2.19.3.2. **Copper coated steel wool to remove oxygen:**

This is a single method of obtaining anaerobiasis. It can be adapted for incubating single plate or several plates. The plates can be incubated in a plastic bag providing it is air tight. The system uses steel wool which is activated immediately before use by being dipped in acidified copper sulphate solution. The metallic copper on the surface on the iron rapidly absorbs oxygen. Anaerobic conditions are obtained more rapidly by removing some of the air from the bag before it is sealed. A source of carbon dioxide is added an also an indicator to cheek for anaerobiasis.

2.19.3.3. **Reducing agents in culture media:**

**Thioglycollate broth:**

This is used mainly to culture anaerobes in blood. The medium contains the reducing agents sodium thioglycollate and the indicator methylene blue or resazurin to show that the medium is reduced.

**Cooked meat medium:**

This is used to cultured Clostridium and Bacteroides species. The anaerobes grow at the bottom of the medium among the meat particles which contain effective
reducing substances. The medium shows saccharolytic and proteolytic reactions and also gas production.

2.20. ANAEROBIC JAR (Baird and Tatlock anaerobic jar)

With the availability of oxygen removing systems to produced anaerobic conditions and expensive anaerobic jar with pressure gauge, valves, etc is no longer needed. An acrylic air tight jar such as that supplied by technical service consultants. The complete Gaspak system comprises a transparent polycarbonate anaerobic jar and a plastic lid fitted with a screened catalyst chamber containing palladiumized alumina pellets. There are no taps in the lid to allow evacuation of the jar and no vacuum pumps or cylinders of compressed gas are required. An anaerobic indicator is necessary to check the activity of the palladium catalyst. The Gaskit (Unipath) is a similar development and is said to yield a more assured volume of carbon dioxide (Chesbrough, 2000; Macki and McCartney, 1996).

2.21. SPECIMEN COLLECTION (Linda, 2004)

The keys to effective anaerobic bacteria cultures include collecting a contamination-free specimen and protecting it from oxygen exposure. Anaerobic bacteria cultures should be obtained from an appropriate site without the health care professional contaminating the sample with bacteria from the adjacent skin, mucus membrane or tissue. Swabs should be avoided when collecting specimens for anaerobic culture because cotton fibers may be detrimental to anaerobes. Abscesses or fluids can be aspirated using a sterile syringe that is then tightly capped to prevent entry of air. Tissue samples should be placed into a degassed bag and sealed or into a gassed out screw top vial that may contain oxygen-free pre reduced culture medium and tightly capped. The specimens should be plated as rapidly as possible onto culture media that has been prepared.
2.22. PRECAUTIONS

Anaerobes are commonly found on mucous membranes and other sites such as the vagina and oral cavity. Therefore, specimens likely to be contaminated with these organisms should not be submitted for culture (e.g. a throat or vaginal swab).

2.23. SPECIMENS FROM WHOM ANAEROBES ARE LIKELY TO BE ISOLATED

- Blood
- Bile
- Bone marrow
- Cerebrospinal fluid
- Direct lung aspirate
- Tissue biopsy from a normally sterile site
- Fluid from a normally sterile site (like a joint)
- Dental abscess
- Abdominal or pelvic abscess
- Knife, gunshot, or surgical wound
- Severe burn
- IUD, maxillary sinus, placenta tissues, uterine, endometrial tissue.

2.24. SPECIMENS THAT ARE NOT SUITABLE FOR ANAEROBIC CULTURES:

- Coughed throat discharge (sputum)
- Rectal swab
- Nasal or throat swab
- Voided urine
- Urethral swab

2.25. TURNAROUND TIME: Preliminary report after 2 days; final report after 2-6 days
2.26. SPECIAL INSTRUCTIONS: Use the anaerobic transport systems.

2.27. VOLUME: 2 mL

2.28. MINIMUM VOLUME: 0.5 mL

2.29. CULTURE

Cultures should be placed in an environment that is free of oxygen at 95°F (35°C) for at least 48 hours before the plates are examined for growth. Gram staining is performed on the specimen at the time of culture. While infections can be caused by aerobic or anaerobic bacteria or a mixture of both, some infections have a high probability of being caused by anaerobic bacteria. Anaerobic organisms can often be suspected because many anaerobes have characteristic microscopic morphology (appearance). For example, *Bacteroides* spp. is gram-negative rods that are pleomorphic (variable in size and shape) and exhibit irregular bipolar staining. *Fusobacterium* spp. is often pale gram-negative spindle-shaped rods having pointed ends. *Clostridium* spp. is large gram-positive rods that form spores. The location of the spore (central, subterminal, terminal, or absent) is a useful differential characteristic. The presence of growth, oxygen tolerance and Gram stain results are sufficient to establish a diagnosis of an anaerobic infection and begin antibiotic treatment with a drug appropriate for most anaerobes such as clindamycin, metronidazole or vancomycin.

2.30. IDENTIFICATION (Chakraborty, 2003)

The identification of anaerobes is highly complex and laboratories may use different identification systems. Partial identification is often the goal.

1. Organisms are identified by their colonial and microscopic morphology

2. Growth on selective media
3. Oxygen tolerance

4. Biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography and susceptibility to antibiotics.

5. The antibiotic susceptibility profile is determined by the microtube broth dilution method.

Many species of anaerobes are resistant to penicillin and some are resistant to clindamycin and other commonly used antibiotics.
Chapter – 3

Materials & Methods
MATERIALS AND METHODS

3.1. Place and the period of the study
The study was carried out in the Department of Microbiology, Mymensingh Medical college for a period of one year from July 2006-June 2007.

3.2. Type of study
The study was designed as cross sectional one.

3.3. Population
Clinically diagnosed 50 patients of puerperal sepsis admitted in different wards of Gynecology and Obstetric Department of Mymensingh Medical College Hospital were selected for this study.

3.4. Inclusion criteria for cases
Cases of puerperal sepsis were selected on basis of the following clinical findings (WHO, 2004; Basu, 2003).

1. Fever of $\geq 38.5^0$C within 6 weeks following termination of pregnancy.
2. Pelvic pain with offensive and / or purulent vaginal discharge.
3. Abdominal pain and tender uterus.
4. Sub involution of the uterus.
5. Shock.

Patients having two or more of the above features were considered as puerperal sepsis.
3.5. Exclusion criteria

1. Patients with similar signs and symptoms but diagnosed as having other diseases e.g. carcinoma of the cervix, cervical polyp, ulcerated uterine prolapse and vaginosis.

2. Patients with history of taking prior antibiotic treatment within 7 days.

3.6. Data recording

All relevant history, clinical findings and laboratory records of every case was systematically recorded in a pre designed data sheet (appendix-I) for subsequent analysis by computer programme SPSS version 12.0.

3.7. Specimens

Endocervical swab or secretion and blood from each case were collected following standard procedure for microscopic examination and isolation of bacteria with antimicrobial susceptibility.

3.8 Method of collection (Cheesbrough, 2000)

3.8.1 Cervical swab/secretion

Verbal consent was taken from every patient before sample collection and the purpose of the procedure was explained clearly. Specimens were collected carefully to avoid normal resident flora. For that reason a sterile bivalved Cusco’s vaginal speculum was used to visualize the endocervix under sufficient light. Swab was taken by gentle rubbing of the mucosa of cervix by sterile cotton swab and secretion was taken as aspiration by disposable syringe. One aliquot of collected specimen was immediately inoculated in Blood agar media at
bed side for anaerobic culture. The rest of the specimen was transferred to the Department of Microbiology for further investigations.

3.8.2 Blood

From each patient, 10ml of venous blood from median anticubital vein was collected after disinfection of the skin to be punctured with 2% iodine and absolute alcohol. Then with a 20ml sterile disposable syringe, blood was slowly sucked. After ejecting the needle, one aliquot of 5ml blood was inoculated into blood culture bottle containing 50ml TSB and another aliquot of 5 ml blood into 50ml blood culture bottle containing Thioglycollate broth aseptically. Each bottle was provided with rubber cork and covered with aluminum foil to prevent contamination during inoculation and subsequent processing. (Vandepitte et al, 1991) Then the blood culture bottles containing TSB and Thioglycollate broth were transferred immediately to the Department of Microbiology of Mymensingh Medical College for proper incubation.

3.9 Wet Film and Gram staining (microscopy) of the sample

Wet Film and Smear were prepared from each of the cervical sample. Prepared Smear was stained by Gram staining (Appendix - IV). Wet Film and the stained smears were searched for observing morphology of relevant organisms and number of pus cell (Duguid et al, 1989).

3.10. Culture of cervical specimen: Cervical swab/secretion was inoculated into two plates of Blood agar (Appendix – II), One MacCon Key’s agar (Appendix – II), One Nutrient agar (Appendix – II) and One Mannitol salt agar (Appendix – II) medium. One Blood agar, MacConKey,s agar, Nutrient agar and Mannitol salt agar medium was incubated at 37°C for 24 hours aerobically. Another Blood agar plate was incubated anaerobically at 37°C for 48 hrs. (Cheesbrough, 2000). The
GasPak™ EZ Anaerobe Container system was used for anaerobic culture (Becton, Dickinson and Company. Sparks, USA).

**Test procedure:**

1. The inoculated Blood agar media was placed in an anaerobic gas jar.
2. The activated sachet after removing the foil pack was also placed in the anaerobic jar.
3. ATCC No 27853 type *Pseudomonas aeruginosa* on nutrient agar media was also incubated in the anaerobic jar as a negative biological control (Saini, 2003).
4. The jar was incubated at 37°C for 48 hours.

**3.11. Culture of blood sample by Broth method.**

Blood culture bottles containing TSB and Thioglycollate broth were incubated at 37°C aerobically within an hour after collection of blood at bed side. Blood culture bottles were examined macroscopically for growth in the morning and afternoon on the 1st day of incubation and in the morning of each day there after. Culture that appeared as per hazy appearance of the broth were picked up and Gram stain was done (Blazevic *et al.*, 1974). Subculture were done on Blood agar, Nutrient agar and Mac Conkey agar media and incubated at 37°C for 48 hours aerobically (Coller *et al.*, 1989). Bottle that appeared macroscopically negative were examined with Gram stain on the 1st, 4th and 7th day of incubation and blind subculture were done on the 1st and 4th days to a Blood agar. Sub cultured plates were held for two days before they were discarded as negative. The Blood culture bottles were kept on incubation for 2 weeks and were discarded when there was no growth (Blazevic *et al.*, 1974; Duguid *et al.*, 1989).

(Cheesbrough, 2000; Mackie and MacCartney, 1996)

Isolation of bacteria from the collected specimens were done by inoculating the samples into Nutrient agar, Blood agar, Mac Conkey agar, Trypticase soya broth and Thioglycollate broth media (Appendix - II) within earliest possible time (less then one hour) after collection. Inoculated Petri dishes were then incubated at 37°C for 24 - 48 hours aerobically and anaerobically using gas pack and anaerobic jar. The isolates were identified on the basis of colony morphology, from colony Gram staining and appropriate biochemical tests.

3.12.1. *Escherichia coli*

Isolates of *Escherichia coli* were identified on the basis of rounded colonies of 1 – 4 mm diameter with or without haemolysis in Blood agar. Colonies were pink color in MacConkey’s agar and yellow color in CLED agar media. On Gram stained smear from colonies, those appeared as Gram negative bacilli. Biochemically isolates were Indole positive, motile and non producer of urease in MIU medium. On TSI, both butt and slunt were found as acidic (yellow) with production of gas.

3.12.2. *Staphylococcus aureus*

After 24 hours incubation, colony morphology in different culture media was noted. In Nutrient agar the colonies appeared as circular, having 2- 4 mm diameter, convex with shining surface and showing golden yellow pigment. The appearance of the colonies in MacConkey agar was smaller (0.1- 0.5 mm) with lactose fermenting produce pink colony. In Blood agar the colonies appeared to
be yellow to cream and occasionally white in color, having 1-2 mm in diameter with narrow zone of beta haemolysis and produces pin head colony. On Gram’s stained smear from colonies, bacteria appeared as Gram positive cocci in grape like clusters with some single or paired spherical arrangement. Biochemically isolates were positive to catalase, coagulase production and Mannitol fermentation.

3.12.3. *Streptococcus pyogenes*

Colony in Blood agar media appeared as pin point and beta hemolytic. On Gram’s stained smear from colonies, bacteria appeared as Gram positive cocci characteristically in short chain. Biochemically isolates were Catalase and Oxidase negative. Identification disc of bacitracin was added to a Blood agar plate and all strains became sensitive.

3.12.4. *Enterococcus faecalis*

After 24 hours incubation, colony in Blood agar were mainly non haemolytic but some strains show alpha or beta haemolysis. Colonies were small dark-red magenta colour in MacConkey agar and those on CLED agar media were yellow. Gram’s stained smear from colonies revealed Gram positive cocci, occurring in pairs or short chains. Biochemically isolates were Catalase negative.

3.12.5. *Peptostreptococcus*

After anaerobic incubation with 10% CO$_2$ at 37$^\circ$ C the colonies were round, convex, shiny, opaque, grey and non hemolytic in Blood agar. On Gram’s staining isolated colony revealed Gram positive cocci, arranged in short chains or single and paired cocci. Biochemically isolates were catalase negative.

3.12.6. *Clostridium Perfringens*
Isolates of *Clostridium Perfringens* were identified on the basis of double zone of hemolysis in Blood agar media. On Gram’s stained smear from colonies, revealed short, plump, Gram positive rod with stubby end, arranged singly or in pairs. Biochemically isolates were catalase and oxidase negative.

### 3.12.7. *Bacteroid fragilis.*

After 48 hours of incubation with 10% CO$_2$ at 37°C in Blood agar *B. fragilis* produced grey, glistening, non haemolytic, 1-2mm diameter colonies. On Gram’s staining, isolated colony revealed as Gram negative rods.

### 3.12.8. *Prevotella melaninogenica*

After 48 hours of incubation in Blood agar media, pigmented or brown colonies appeared. On Gram’s staining the *Prevotella* species were seen as Gram negative bacilli and appeared as slender rods or coccobacilli.

### 3.13. Antimicrobial susceptibility testing by disc diffusion method

All the aerobic isolates were put into antibiotic susceptibility test by Kirby-Bauer disc diffusion technique (Bauer *et al*., 1966) as per recommendation of NCCLS, 1997. Panel of antibiotics were used. All tests were performed on Muller-Hinton agar (appendix-II). The surface was lightly and uniformly inoculated by cotton swab. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards (Appendix-II). The swab stick was then took out and squeezed on the wall of the test tube to discard extra suspension. Inoculated plates were incubated at 37°C for 24 hours. On the next day, plates were read by taking measurement of zone of inhibition. Results were recorded and graded as Resistant (R) and Sensitive (S) according to the reference zone of inhibition of particular antibiotic (NCCL, 1998). Known control strain ATCC, No. 25922 and ATCC No. 25923 were used for quality control.
3.13.1. Antimicrobial agents used

A total of 10 antimicrobial agents were used for determining antibiogram of isolated organisms according to Gram positive and Gram negative panel recommended by NCCLS, 1997. Antibiotics Amoxycillin, Oxacillin, Cephalexin, Cephradine, Cefuroxime, Ciprofloxacin, Gentamicin, Amikacin, Rifampin and Vancomycin.

3.13.2. Standardization of the disc

In order to standardize the disc potency, a representative disc was tested against the reference strains of Esch. Coli, American type culture collection (ATCC), No. 25922 and Staph. aureus, ATCC No. 25923. Zone of inhibition were compared with standard values as recommended by NCCLS (1998).
Chapter – 4

Results
Results

This study included clinically diagnosed 50 cases of puerperal sepsis. All those patients were diagnosed on the basis of set inclusion criteria such as fever of $>38.5^\circ$C, foul smelling per-vaginal discharge, lower abdominal pain and sub-involution of uterus. Maternal age, level of education and socio-economic condition are shown in Table 1 (Figure 1.1 – 1.3). Majority (52%) women came from 21 to 30 years age group followed by age below 20 years (30%). Minimum cases (18%) were from the age group of 31-40 years. Most of the women (66%) were illiterate, 18% had education at secondary level and 16% at primary level. Again majority of the women (72%) came from lower socio-economic class and rest 28% were from middle class.

Table 2 (Figure 2.1 – 2.5) shows different risk factors associated with puerperal sepsis. Most (74%) of the puerperal sepsis cases were found in women who underwent normal vaginal delivery at home and 18% had history of Caesarean section. As observed complication during delivery, obstructed labor (44%) was at the top of the list and hemorrhage (04%) was the minimum incidence. In 48% cases, preterm rupture of membrane were noted and in 46% cases, membrane was ruptured in due time. Vaginal examination of 6-10 times before delivery were found in 48% cases, no examination in 26% cases and more than 10 times were found in 08% cases. Puerperal sepsis was mostly associated with still birth babies (62%) and multiparity (80%).Women those had no history of antenatal contact mostly (66%) developed puerperal sepsis.
Table 1: Age distribution, level of education and Socio-economic conditions of study population (n = 50)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 20</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>21 – 30</td>
<td>26</td>
<td>52</td>
</tr>
<tr>
<td>31 – 40</td>
<td>09</td>
<td>18</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>Primary level</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Secondary level or higher</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Socio-economic class as per monthly income.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower (Up to Tk. 3000/=).</td>
<td>36</td>
<td>72</td>
</tr>
<tr>
<td>Middle (Tk &gt;3000 – 20,000/=)</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Upper(TK &gt; 20,000/=)</td>
<td>00</td>
<td>00</td>
</tr>
</tbody>
</table>
Figure-1.1: Distribution of patients by age group
Figure-1. 2: Distribution of patients by educational status
Figure-1.3: Distribution of patients by monthly family income

Table 2: Different risk factors in relation to puerperal sepsis
<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Frequency of puerperal sepsis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Place and mode of delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal vaginal delivery at home.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LUCS</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Normal vaginal delivery at hospital</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Complication during delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstructed labour</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Preeclampsia &amp; Eclampsia</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td><strong>Rupture of membrane</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm rupture membrane</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Rupture in due time</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>Artificial rupture</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><strong>Number of vaginal examination before delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 5</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>6 – 10</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>&gt;10</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>None</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td><strong>Outcome of baby</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still birth</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Live birth</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td><strong>Parity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of multi para</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Number of primi para</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><strong>Antenatal contacts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Irregular</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>None</td>
<td>33</td>
<td>66</td>
</tr>
</tbody>
</table>
Figure-2.1: Distribution of patients place & mode of delivery

Chi-square value 43.386, p value <0.001. So it is highly significant.
Figure-2. 2: Distribution of patients by type of complications

- 44% Obstructed labour
- 26% Retained placenta
- 16% Eclampsia
- 10% Haemorrhage
- 4% None
- 4% None
Figure-2.3: Distribution of patients by type of rupture of membrane

Chi-square value 20.885, p value <0.001. So it is highly significant.
Figure-2.4.: Distribution of patients by birth outcome

- **Live Birth**: 62%
- **Still Birth**: 38%
Figure-2.5: Distribution of patients by receiving Antenatal care

Antenatal care taken

- 66% none
- 24% regular
- 10% irregular
Table 3 (Figure-3) indicates nature of vaginal discharge in women with puerperal sepsis. Blood stained and foul smelling discharge were found in 84% women, 14% had purulent and odorous discharge and only 2% has non-odorous watery discharge.

Table 4 demonstrates that out of 50 cases, 42 (84%) yielded positive culture from endocervical swab and 8 (16%) were culture negative. Among 50 blood culture specimen, 2 (4%) were positive in Trypticase soya broth with growth of *Staphylococcus epidermidis*.

Table 5 Shows that out of 50 endocervical specimen, 20 (40%) yielded growth aerobic, 22 (44%) yielded mixed growth i.e. both aerobic and anaerobic and 08 yielded no growth in culture.

Table 6 (Figure 4) shows distribution of different aerobic bacterial isolates. *Staphylococcus aureus* was at the top of the list 26 (61.90%). Isolation rate of *Escherichia coli, Staphylococcus epidermidis, Streptococcus pyogenes* and *Enterococcus faecalis* were 6 (14.28%), 5 (11.90%), 3(7.14%) and 2( 4.76%) respectively.

Table 7 (Figure 5) demonstrates the distribution of anaerobic bacteria. Out of 50 cases, 22 anaerobic bacteria were isolated from cervical swab, Among those, *Peptostreptococcus* were 14 (63.63%), *Bacteroides fragilis* were 3 (13.64%) *Prevotella melaninogenica* were 3 (13.64%) and *Clostridium perfringens* were 2 (9.09%).
Table 3: Nature of vaginal discharge

<table>
<thead>
<tr>
<th>Discharge</th>
<th>No. of case</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood stained and odorous</td>
<td>42</td>
<td>84</td>
</tr>
<tr>
<td>Purulent and odorous</td>
<td>07</td>
<td>14</td>
</tr>
<tr>
<td>Watery, non odorous</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure-3:  Distribution of patients by criteria of discharge

Criteria of Discharge

<table>
<thead>
<tr>
<th>Criteria of Discharge</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood stained odorous</td>
<td>54%</td>
</tr>
<tr>
<td>Purulent odorous</td>
<td>14%</td>
</tr>
<tr>
<td>Watery</td>
<td>2%</td>
</tr>
</tbody>
</table>
Table 4: Specimen wise result of culture.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Culture positive</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocervical swab/Secretion n = 50</td>
<td>42 (84%)</td>
<td>08 (16%)</td>
</tr>
<tr>
<td>Blood n = 50</td>
<td>2 (4%)*</td>
<td>48 (96%)</td>
</tr>
</tbody>
</table>

*Staphylococcus epidermidis*
Table 5: Pattern of culture in 50 endocervical specimens

<table>
<thead>
<tr>
<th>Pattern of growth</th>
<th>No</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only aerobic growth, no growth in anaerobic culture.</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Growth in both aerobic and anaerobic culture</td>
<td>22</td>
<td>44</td>
</tr>
<tr>
<td>No growth in aerobic and anaerobic culture.</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 6: Distribution of Aerobic bacteria from Endocervical specimen (n = 42).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Pure aerobic</th>
<th>Aerobic from mixed growth</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>14</td>
<td>12</td>
<td>26 (61.90%)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2</td>
<td>4</td>
<td>6 (14.28%)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>2</td>
<td>3</td>
<td>5 (11.90%)</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>2</td>
<td>1</td>
<td>3 (7.14%)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td></td>
<td>2</td>
<td>2 (4.76%)</td>
</tr>
<tr>
<td>Total</td>
<td>20 (47.61%)</td>
<td>22 (52.38%)</td>
<td>42 (100%)</td>
</tr>
</tbody>
</table>
Figure - 4: Distribution of patients by presence of aerobic organism in culture of swab

Detection of aerobic organism

- S.aureas: 62%
- E. Coli: 14%
- S. Epidermidis: 12%
- S. Pyogenes: 7%
- Enterococcus: 5%
Table 7: Distribution of Anaerobic bacteria of Endocervical swab (n = 22)

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>No. of isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peptostreptococcus</em></td>
<td>14</td>
<td>63.63</td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>3</td>
<td>13.64</td>
</tr>
<tr>
<td><em>Prevotella melaninogenica</em></td>
<td>3</td>
<td>13.64</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>2</td>
<td>9.09</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>22</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Figure-5: Distribution of patients by presence of anaerobic organism in culture of swab

![Detection of Anaerobic organism](image)

<table>
<thead>
<tr>
<th>Detection of Anaerobic organism</th>
<th>Pepto. ST</th>
<th>Bacteroides</th>
<th>Prevotella</th>
<th>CL. Perfringens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence (%)</td>
<td>63%</td>
<td>14%</td>
<td>14%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Table 8: shows Gram’s staining and culture in 50 endocervical specimens.
Gram positive cocci with plenty of pus cell were found in 25 specimens, of which 20 (80%) yielded growth of *Staphylococci*. Gram positive cocci in chain with plenty of pus cells were found in 2 specimens, those showed growth of *Streptococci* (100%). Mixed Gram positive cocci and Gram negative bacilli with plenty of pus cell were found in 23 specimens, of those 20 (87%) yielded mixed growth.

Percentage of antibiotic sensitivity (S) of different aerobic bacteria is shown in the Table 9. All (100%) 26 isolates of *Staphylococcus aureus* and 5 isolates of *Staph. epidermidis* were sensitive to Cephalexin, Cephradine, Cefuroxime and Vancomycin. Corresponding values towards Amoxycillin, Oxacillin, Ciprofloxacin and Rifampicin were recorded as 30.77% & 40%, 53.85% & 40%, 84.61% & 100%, 92.31% & 100% respectively. All 03 isolates (100%) of *Streptococcus. pyogenes* were sensitive to Amoxycillin, Cephalexin, Cephradine, Cefuroxime and Ciprofloxacin. Numbers of isolates of *Enterococcus spp.* were 02 and all (100%) were sensitive to Amoxycillin, Oxacillin, Cephalexin, Cephradine and Gentamicin. All 06 (100%) isolates of *Esch. coli* were sensitive to Cephalexin, Cephradine, Cefuroxime, Gentamicin, Amikacin and Ciprofloxacin.
<table>
<thead>
<tr>
<th>Morphology and pus cell</th>
<th>Number of specimen</th>
<th>Growth with number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive cocci in clusters with plenty of pus cell.</td>
<td>25</td>
<td><em>Staphylococci</em> 20</td>
</tr>
<tr>
<td>Mixed gram positive cocci and gram negative bacilli with plenty pus cell</td>
<td>23</td>
<td>Mixed growth 20</td>
</tr>
<tr>
<td>Gram positive cocci in chain with plenty of pus cell.</td>
<td>2</td>
<td><em>Streptococci</em> 02</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>42</td>
</tr>
</tbody>
</table>
Chapter – 5

Discussion
**Discussion**

Puerperal sepsis is an important cause of hospitalization due to its clinical morbidity as high fever with chill, tachycardia, sub involution of uterus, lower abdominal pain, profuse and foul smelling lochial discharge. Usually, the infection is of poly-microbial in origin with a mixture of both aerobic and anaerobic organisms (Dennis, 2006). Incidence of aerobic bacteria tends to occur in one-tenth proportion to that by anaerobic bacteria. Component of normal flora may become pathogenic in changed environment (Misheel, 1988). This study was conducted in one of the largest tertiary hospital where a large number of patients particularly from lower socio-economic condition seek admission for management of puerperal sepsis. The study aimed to determine causative organisms with special emphasis on anaerobic bacteria, because it was the preliminary but pioneer study in this institute regarding isolation of anaerobic bacteria. The criteria for diagnosing puerperal sepsis in the present study were supported by other investigator, such as the study done by Good Burn *et al* (2000) in rural area of Bangladesh found that the key features for diagnosis of maternal post partum genital tract infection by a symptoms complex of any two out of three symptoms, e.g. Foul smelling discharge, lower abdominal pain and fever. Different predisposing factors in relation to puerperal sepsis were also observed during the study period.

In the present study, most of the patients (52%) were in the age group of 21 -30 years. This finding was similar to that of the study done in Comilla Medical College Hospital where they found 59% cases in the same age group (Begum, 2005). Another study from India also reported highest incidence (59.4%) of puerperal sepsis in the age group between 21-30 years (Modi, 1986). The age
distribution observed in the present study was also consistent with previous study done in the same department of Mymensingh Medical College Hospital and in Dhaka Medical College Hospital (Rawsan, 2001; Shahanaz, 2003). The highest incidence of puerperal sepsis in younger age group of female might be due to the reason that majority of women become pregnant in this age group (Pokharel, 2004). Regarding educational level and socio-economic class of women those suffered from puerperal sepsis, this study showed that most of the cases (66%) were illiterate and came from low socioeconomic class (72%). Keeping agreement with the said findings, other study also recorded higher incidence of puerperal sepsis in people of low socio-economic class having low level of education from New Zealand (Maharaj, 2007). Other studies from Bangladesh recorded 99-100% cases of puerperal sepsis were from low socio-economic status (Baker, 2005; Begum, 2005). Relatively higher incidence of puerperal sepsis in illiterate women of low socio-economic class is well explained by the facts that they are lacking of the medical knowledge regarding health care, personal and environmental hygiene either in antenatal or in post natal period. Their false beliefs and social stigma regarding pregnancy, delivery and peri-natal care keep them away for seeking necessary medical advices and aids. In many instances, though medical care is available but they can not afford it due to monetary problems (Dolea, 2003). In addition, socio-economic status and level of education in people of Mymensingh district is relatively lower than those of other districts (Jahan, 2004).

The most prevalent risk factors (Table 2) in relation to puerperal sepsis in the present study were recorded as follows: multiparity 80%, normal vaginal delivery at home 74%, no history of antenatal check-up 66%, history of still birth babies
62%, preterm rupture and multiple vaginal examination (48%), history of obstructed labour 44%, retained bit of placenta (16%) and toxaemia of pregnancy (eclampsia/pre-eclampsia) 10% cases. In accordance with those recorded risk factors obtained in the present study, Moharaj (2007) found that home birth in unhygienic condition, low socioeconomic status, prolong rupture membrane, multiple vaginal examinations and obstructed labour as the mostly responsible risk factors for puerperal sepsis. Another study done in Pakistan showed home delivery in 53.12% and multiparty in 72% cases where repeated vaginal examination, instrumentation and manual removal of placenta caused puerperal sepsis (Naheed, 2002). Mitra et al (1997) found that 75% of rural women in India received no antenatal care in their study almost simulating with the findings of the present study where it was recorded that 66% of the patients had no antenatal check-up. One Nigerian study (Dare, 1998) reported the incidence of puerperal sepsis as 1.7%, but it was higher (66%) among the patients those did not register in antenatal clinics. Prolong labour (65.7%), frequent vaginal examination during labour (50.7%) and premature ruptures of membrane (31.5 %) were observed as the closely related predisposing factors. Similar findings were also seen in India and Brazil (Basavaraj, 2006; Chin, 2000). Not only the puerperal sepsis, but the maternal mortality in a rural referral hospital in Nigeria found 10 times more mortality (p<0.001) among unbooked patients for antenatal care than that for booked patients (Igberase, 2007). Risk factor of puerperal sepsis in Alexandria Egypt found as very low socioeconomic score, no antenatal care and frequent vaginal examination as per calculating the odd ratios (OR) by logistic regression analysis (Mahally, 2004). Similar to the present study, other studies from Sir Salimullah Medical College and Mitford Hospital also reported multiple vaginal
examination during home delivery, prolong labour, premature rupture membrane and Caesarean Section 20%, 10%, 8% and 5% cases respectively (Baker, 2005). In Bangladesh, 90% delivery took place at home and about 20,000 women die every year due to pregnancy related complications such as post partum hemorrhage, septic abortion, eclampsia, puerperal sepsis and obstructed labor (OGSB, 2002). One study done at Comilla Medical College hospital and Comilla Sadar hospital recorded home delivery and eclampsia in 87% and 8% respectively in cases of puerperal sepsis (Begum, 2005). Home delivery (74%) in relation to puerperal sepsis was also observed by Khatun (2003). Another study done in Mymensingh Medical College Hospital showed that obstructed labour, preterm rupture membrane and pre eclampsia as important risk factors in 50% 32% and 12% cases of puerperal sepsis respectively which was consistent with our study (Jahan, 2004).

Study done in Germany on 51 women of labour found that 27 and 24 developed endometritis following vaginal delivery and Caesarean section respectively (Gerstner, 1981). Other study from Finland observed different risk factors for postpartum sepsis as 3.2 times more following Caesarian section than after vaginal delivery (Kankuri, 2003). But in our study, puerperal sepsis was mostly associated with vaginal delivery at home that did not correlate with the studies mentioned above. The reason behind the fact should be the most possible chance of transmission of infecting organisms from exogenous source due to mal-handling by traditional birth attendants or untrained ladies leading to injuries and local wounds. Besides the mentioned factor, another aspect should be the chance of more activation of endogenous flora due to breach in normal environmental conditions (Maharaj, 2007).
The nature of vaginal discharges (Table 3) observed in the present study was as follows: odorous with blood stained, purulent and watery 84%, 14% and 2% respectively. Observation by Gibbs (1980) found 58 cases of endometritis as complication of vaginal delivery where the diagnostic criteria included fever over 100°F, uterine tenderness and foul smelling lochial discharge. In an observation conducted in Bangladesh, it was noted that occurrence of foul smelling vaginal discharge remained high (31% up to 2 weeks and 7.1% up to 6 weeks of delivery) in groups whose delivery was conducted by untrained personnel at home (Goodburn, 2000). Persistent discharge might be due to prevailing infection either in mild or in severe form. The infectious agents of course are being transmitted from the hands of non-trained personnel including Dais, neighbours or in-laws. Certain practices, such as not cleaning the birth canal with water and antiseptic, frequent digital examination with unwashed hands and intra-vaginal application of ghee or other herbal products by untrained health personnel were found to enhance the risk of vaginal infections (Tazeen, 2006).

In the present study, out of 50 samples (Table 4), 84% yielded growth in culture of endocervical swab/secretion and 4% in blood culture. Bacteria isolated from blood culture were identified as *Staphylococcus epidermidis*. Notably, as same organisms were also isolated in aerobic culture of endocervical swab of the same patients, so these two were considered as pathogenic. One study done in Siriraj Hospital, Thailand found that 50% of all cases yielded positive culture from cervical and intrauterine swab (Chaisil, 1991). Another study done by Perine (1980) found 67% culture positive from endocervical secretion. In both studies isolation rate of bacteria were lower than that obtained in the present study. The reasons behind this outcome might be due to the magnitude of the infection,
culture properties of the causative bacteria with pattern of antimicrobial resistance and of course the time of specimen collection. Similarly, positive rate of blood culture of the present study (4%) did not match with the findings of following studies: Basavaraj (2006) showed 20% positive blood culture comprising Klebsiella species as the most common bacteria out of 102 cases. Coagulase negative Staphylococci found in only one case. Callen, et al (1980) found 100% culture negative results in 127 blood culture specimens taken from patients of puerperal sepsis. The various isolation rate of blood culture from cases of puerperal sepsis might be due to techniques adopted for the procedure and also the history of irregular drug treatment. More over, puerperal sepsis affects the mucosa and other anatomic parts of the genital tract. The condition remains associated with bacteraemia or septicaemia when virulent bacteria from the affected parts invade blood stream. In the present study, minimum isolation of bacteria from blood culture might be due to not having associated bacteraemia or septicaemia.

In the present study, out of 50 endocervical specimens, aerobic and mixed growth (both aerobic and anaerobic) was observed in 40% and 44% samples respectively (Table 5). This finding was compared with other study where trans-cervical endometrial swabs were taken from 51 women having clinical signs of endometritis, fever over 38°C, uterine tenderness and purulent lochial discharge. On culture, aerobic bacteria were isolated from 85.2% cases following vaginal delivery and 75.1% following CS which was not consistent with our findings (40%). Mixed growth (aerobic and anaerobic) was observed in 48.1% cases that showed very good correlation (44%) with our findings (Gerstner, 1981). The lower rate of isolation of aerobic bacteria in our study might be due to the reason
that probability of taking antibiotics before collection of endocervical swab was not completely ruled out. Because, administering prophylactic antibiotic injection or capsule following delivery is almost routinely practiced by rural practitioners or even in hospitals.

Distribution of aerobic bacteria (Table 6) as the cause of puerperal sepsis observed in the present study was as follows: *Staph. aureus* 61.90 %, *Staph. epidermidis* 11.90 %, *Streptococcus pyogenes* 7.14 %, *Enterococcus faecalis* 4.74% (aerobic Gram positive bacteria 85.72%) and *E. coli* 14.28 % (Gram negative bacteria). Keeping agreement with our study, Chaisil Watana (1991) showed predominance of (78%) of Gram positive cocci in one study where 8% isolates were Gram negative bacteria. Other study was done in UK on 286 women where vaginal swabs from all of them were cultured and heavy growth of *Staph. aureus*, *Beta Haemolytic Streptococcus (Gr A,C or G)*, *Strepto. pneumoniae* or *Haemophilus influenzae* were observed (Dykhuizen, 1995). Pokharel (2004) reported *Staph. aureus* (50%) as the commonest organisms causing postpartum genital infections in women who delivered their babies with premature rupture of membrane, whereas women who delivered their babies with rupture of membrane in due time developed postpartum genital infections by *Esch. coli* (28.8%). The difference in the pattern of bacteria appeared as statistically significant (p<0.05). Other studies done in abroad or in home observed variations in the distribution of aerobic bacteria those caused puerperal sepsis. In this context, one study (Baker, 2005) done in SSMC and Mitford Hospital found *Esch. coli* as the predominant bacteria (50%) followed by *Staphylococcus. spp* (30%) and *Streptococcus. spp* (20%). Distribution of same bacteria in other study done in USA was reported to be 36%, 28% and 21% respectively (Decherny, 2003). Other study done in
Pakistan (Mayo hospital, Lahore) reported isolation rate of Gram positive cocci as 40% and Gram negative bacilli as 60% from cases of puerperal sepsis. The most common Gram positive bacteria were *Staph. aureus, Staph. epidermidis* and *Enterococcus*. The most common Gram negative bacteria were *Esch. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Anaerobes were equally important (Naheed, 2002). Other studies from India reported *Esch. coli* as the most common organism isolated from maternal genital tract followed by *Staph. aureus* and *Klebsiella pneumoniae* (Roa, 1992; Basavaraj, 2006). In western country, *Group B streptococcus* was found to be the most common organism implicated in maternal genital tract colonization resulting puerperal infection of mother and also to babies (Beargie, 1975). Another study (Gerstner, 1981) observed that in cases of puerperal sepsis the most common bacteria were *Staph epidermidis, Esch. coli, Enterococci* and *Streptococci*. The variation of bacteria isolated from genital tract those cause puerperal infection as endogenous source may represent regional variation of genital flora (Basavaraj, 2006). In addition, the source of infection might be exogenous where pathogens from nearby skin flora or from contact with contaminated instruments, dressings or pads are implanted in the mucosa of genital tract (WHO, 1994).

In the present study, the rate of isolation of anaerobic bacteria was 44% with following distribution: *Peptostreptococcus* 63.64%, *Bacteroides fragilis* 13.64%, *Prevotella melaninogenica* 13.64% and *Clostridium perfringens* 9.09%. Having remarkable similarity with the findings of the present study, one study done in the department of Obstetrics and Gynecology at Cook Country hospital isolated anaerobic bacteria from all 33 patients of post-partum genital infections. The commonest isolates were species of *Peptostreptococcus, Bacteroides and*
Veilonella (Thadepalli, 1973). Predominance of *Peptostreptococcus* was also observed in other study. As for example, study done in Bethesda Navy hospital and Walter Reed Army hospital found *Peptostreptococcus* as the predominant anaerobic bacteria in obstetrical and gynaecological cases. The anaerobic Gram positive cocci were generally found mixed with *Prevotella* (Brook, 2006). One study (Lang, 1980) in West Germany, isolated anaerobic bacteria is more than 33% of women with post-partum endometritis, abscess formation and similar pathologic conditions. The majority isolates comprised of *Bacteroides fragilis* followed by *Peptostreptococcus*. Another study from Harare hospital Ggynae and Obstetrics unit also found pigment producing *Bacteroides* as predominant species causing anaerobic infection in puerperal sepsis cases (Mason, 1989). Findings of those two studies were not in good agreement with the present study. Because, majority anaerobes isolated in the present study were identified as *Peptostreptococcus*. It is to be mentioned that anaerobic bacteria have been found in the vagina of 70 to 86% of healthy women as commensal (Rudoip, 1976), but their ability to colonize the vagina during pregnancy should be impaired due to increased vascularity that leads to increased oxygen supply. Still anaerobic infection affecting genital tract during puerperal period is not usually uncommon. The reasons behind this might be improper manipulation at the time of parturition causing local tissue damage, resulting hypoxic state and necrosis. In addition, facultative bacteria from endogenous or exogenous source consume the tissue level oxygen aggravating the anaerobic state. Modern techniques for anaerobic culture have further improved the isolation rate of anaerobic bacteria (Gall, 1983; Rudoip, 1976). Those factors mentioned above can well explain the considerable incidence of anaerobes as causative agents for post-partum genital infections.
In the present study, antimicrobial susceptibility (Table 9) of different aerobic bacterial isolates was seen. All the strains of \textit{Staph. aureus} and \textit{Staph. epidermidis} were sensitive to Cephalexin and Vancomycin. Similarly all the strains of \textit{Esch. coli} were sensitive to Gentamicin, Amikacin, Ciprofloxacin and Cephalexin. All the strains of \textit{Strepto. pyogenes} were sensitive to Amoxycillin also. Though all five isolates of \textit{Staph. epidermidis} were sensitive to Ciprofloxacin, but corresponding value for \textit{Staph. aureus} strains were 84.6%. All the isolates of \\textit{Enterococus} were sensitive to Amoxycillin and Cephalexin. Although number of isolates was not that high, still we compared our data with other studies. Kankuri (2003) reported different aerobic bacteria as showing 81% sensitive results against first and second generation Cephalosporins. Another study from Nepal (Pokharel, 2004) also observed 100% strains of different aerobic bacteria as sensitive to Cephalexin. Findings of two studies mentioned above were well consistent with our results. \textit{Esch. coli} and other Gram negative bacteria showed 100% susceptibility towards Gentamicin in a study by Modi (1986) in India that also stood highly comparable to our results. Isolates of the genus \textit{Staphylococcus} in our study were 100% susceptible to Vancomycin that was in close accordance with a report from USA (Rotus, 2007). Regarding susceptibility to Ciprofloxacin (quionolone) among different aerobic bacteria as found in the present study, well compared with that of the study by Martens \textit{et al} (1991) conducted on 231 patients of postpartum endometritis. In this regard, susceptibility towards second generation Cephalosporins was also consistent with our results. Isolates of \textit{Enterococcus} of that mentioned study showed >85% susceptible results towards Ampicillin and Gentamicin. In our study, only 02 strains were identified as \textit{Enterococcus faecalis} and those were tested against
Amoxycillin instead of Ampicillin and found sensitive. Those strains were also sensitive to Ciprofloxacin and Gentamicin. Another study reported following pattern regarding antimicrobial susceptibility of 55 strains of *Enterococcus faecalis* from the lower genital tract. Sensitivity towards Carbenicillin, Azlocillin and Ampicillin were 92.7%, 91.2% and 83.9% respectively. The value for resistance towards Gentamicin and Amikacin were 49.5% and 46.5% respectively. With the exception of limited low number of strains in our study, we also observed similar pattern of antimicrobial susceptibility of *Enterococcus* strains (Shopova, 1995). What ever might be the pattern of susceptibility obtained in our study, property of antibiotic resistance among bacterial population is not a static phenomenon. It is related with magnitude of clinical use, proper dose, adequate duration and close monitoring of bacterial eradication from the site of infection. Despite all those factors having at substandard level in our clinical practice, we can comment that the susceptibility pattern of aerobic bacterial strains of the present study was not alarming.

On analyzing the findings of the present study, it can be concluded that puerperal sepsis is the common life threatening condition in the postnatal period particularly following vaginal delivery at home. Multiparous, illiterate women from low socio-economic class not undergoing antenatal checkup bear significant risks for developing puerperal sepsis. Prevalence of anaerobic bacteria as the cause of puerperal sepsis is significantly observed in the present study. Aerobic bacteria those colonize the gravid birth canal of mothers appeared to be the common agent of post partum sepsis. Since, we could not carry out antimicrobial susceptibility of anaerobic bacteria due to unavoidable limitations, so we can
strongly recommend further research in this field along with regular logistic arrangement of anaerobic culture in this institute.

Table 9: Percentage (%) of antibiotic sensitivity (S) of different aerobic bacteria.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
<th>S. pyogenes</th>
<th>Enterococcus spp.</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>30.77</td>
<td>40</td>
<td>100</td>
<td>100</td>
<td>66.66</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>53.85</td>
<td>40</td>
<td>100</td>
<td>100</td>
<td>33.33</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cefradine</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Not seen</td>
<td>Not seen</td>
<td>Not seen</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>84.61</td>
<td>100</td>
<td>Not seen</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>84.61</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>92.31</td>
<td>100</td>
<td>Not seen</td>
<td>Not seen</td>
<td>Not seen</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100</td>
<td>100</td>
<td>Not seen</td>
<td>100</td>
<td>Not seen</td>
</tr>
</tbody>
</table>
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Appendices
APPENDICES

Appendix – I

DATA SHEET

Title: Study on Aerobic and Anaerobic bacterial causes of puerperal sepsis at Mymensingh Medical College Hospital.

Comments: I am a student of MMC in the course of M. Phil Microbiology. I would like to collect some information about you. The data collected from you will be used for research work only. I expect that you will kindly cooperate with me. Thank you.

Identification number:

Date:

1 Name of the patient:

2 Age:

3 Name of the husband:

4 Date of admission:

5 Address

Vill:

P.O:

Upazilla:

Dist:

6. Educational level of the mother

   i) Illiterate

   ii) 1-V (primary)

   iii) VI-SSC (Secondary)

   iv) Above SSC
7. Monthly income of family
   i) Up to 3000
   ii) 3001-6000
   iii) 6001-10,000
   iv) More than 10,000.

8. Parity
   i) 0
   ii) 1-2
   iii) 3-4
   iv) 5 and above.

9. Antenatal care
   i) Regular
   ii) Irregular
   iii) None

10. Duration of pregnancy
   i) 1-3 month
   ii) 4-6 month
   iii) 7-9 month

11. If abortion, Mode of abortion
    i) Spontaneous
    ii) Induced

12. Place of abortion or delivery
    i) Home
    ii) Hospital
    iii) Others
13. If pregnancy continue up to term, mode of delivery
   i) LUCS
   ii) Vaginal
   iii) Others

14. Duration of delivery
   i) 8-12 hours
   ii) 12-16 hours
   iii) Above 16 hours
   iv) Others

15. Outcome of delivery
   i) Live birth
   ii) Still birth

16. Rupture of membrane before delivery
   ii) Premature rupture membrane
   iii) Rupture in due time
   iv) Artificial rupture membrane
   v) Others

17. Number of vaginal examination
   i) up to 5
   ii) 6-10
   iii) More than 10

18. History of intrauterine manipulation for delivery of fetus and placenta
   i) Yes
   ii) No
19. Presence of laceration or incision  
   i) Yes  
   ii) No  

20. Any infection of the wound.  
   i) Yes  
   ii) No  

21. Fever  
   i) 99°F—100°F  
   ii) 101°F-104°F  
   iii) More 104°F  

22. Duration of fever  
   i) 1-5 days  
   ii) 6-10 days  
   iii) More than 10 days  

23. Lower abdominal pain  
   i) Yes  
   ii) No  

24. Is there any vaginal discharge  
   i. Yes  
   ii. No  

25. If yes, criteria of the discharge.  
   i. Blood stain & foul smelling  
   ii. Purulent & odorous  
   iii. Watery  
   iv. Others
26. Other medical disorder of the patient
   i. UTI
   ii. Cardiovascular
   iii. Breast
   iv. Others

27. Is there any complication during delivery
   i. Hemorrhage
   ii. Obstructed labor
   iii. Eclampsia and pre eclampsia
   iv. Others

LABORATORY FINDINGS

Specimen: Blood, Cervical swab/secretion

Cervical Swab

<table>
<thead>
<tr>
<th>Gram staining done</th>
<th>Blood agar media</th>
<th>Mac Con keys agar media</th>
<th>Nutrient agar media</th>
<th>Mannitol salt agar media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive</td>
<td>Cocci/ bacilli.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram Negative</td>
<td>Cocci/bacilli.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For Aerobic Organism

<table>
<thead>
<tr>
<th>Organism isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes \ No.</td>
</tr>
</tbody>
</table>

A. If yes, Colony character in:

Blood agar media:

Mac Con keys agar media:

Nutrient agar media.

Mannitol salt agar media.

B. Gram staining

<table>
<thead>
<tr>
<th>Gram Positive</th>
<th>Cocci/ bacilli.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Negative</td>
<td>Cocci / bacilli.</td>
</tr>
</tbody>
</table>

C. Biochemical test:  
  i) Catalase test  Positive\Negative  
  ii) Coagulase test  Positive\Negative  
  iii) Oxidase test  Positive\Negative  
  iv) CAMP reaction  Positive\Negative  
  v) Nagler reaction  Positive\Negative  
  VI) MIU  
  vii) TSI  

D. Serological test  

E. Name of the organism:  

F. Antibiotic sensitivity  

**Antibiogram of isolates (NCCLS, 1998):**  

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Zone of diameter (mm)</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>18 – 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>13 – 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>14 – 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephradine</td>
<td>13 – 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15 – 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>12 – 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12 – 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>13 – 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>12 – 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamycine</td>
<td>12 – 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>13 – 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>15 – 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>10 – 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>27 – 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>16 – 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>13 – 15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S= sensitive, R= Resistant, I= Intermediate sensitive

For Anaerobic Organism

Organism isolated
Yes/No.

A. If yes, Colony character in: Blood agar media:
   Robertsons cooked meat media.
   Thioglycolate broth.

B. Gram staining: Gram Positive Cocci/bacilli.
   Gram Negative Cocci/ bacilli.

C. Biochemical test:
   Catalase test Positive/ Negative
   Oxidase test Positive / Negative
   Nagler reaction Positive/Negative

D. Serological test

E. Name of the organism:

F. Antibiotic sensitivity.

**Blood Culture.**

**Growth present in:**
1. Trypticase soya broth.
2. Thioglycolate broth.
3. No Growth.

1. For Trypticase soya broth:
   a. Sub Culture and see colony morphology: Blood agar media:
      Mac Con keys agar media:
      Nutrient agar media.
      Mannitol salt agar media.
      Gram Negative Cocci/ bacilli.
   c. Biochemical test:
      i) Catalase test Positive/ Negative
      ii) Coagulase test Positive/ Negative
      iii) Oxidase test Positive /Negative
      iv) MIU
      v) TSI
2. For Thioglycolate broth.
   Gram staining: Gram Positive Cocci / bacilli.
   Gram Negative Cocci / bacilli.

Appendix – II

The composition and methods of preparation of different media, chemicals and reagents used in this study are given below –
Nutrient agar medium

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of Animal Tissue</td>
<td>5.00</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>1.50</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>1.50</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Twenty eight grams of dehydrated nutrient agar medium was added to 1000 ml of cold distilled water in a flask and heated to boiling to dissolve the medium completely. The medium was then sterilized in an autoclave at 121°C and 15 lbs pressure for 15 minutes. The sterile media were stored in a refrigerator at 4°C for future use.

Blood agar medium

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart infusion</td>
<td>500.00</td>
</tr>
<tr>
<td>Tryptose</td>
<td>10.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Forty grams of the dehydrated blood agar medium was suspended in 1000 ml cold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45°C in a water bath. Defibrinated 5-10% sheep blood was then added to the medium.
aseptically and distributed to sterile petridishes. Sterile media was stored in refrigerator at 4°C for future use.

**Mannitol salt agar**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.00</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>75.00</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10.00</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

One hundred and eleven grams of dehydrated mannitol salt agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45°C in a waterbath and distributed to sterile petridish. The sterile media was stored in refrigerator at 4°C until use.

**Muller Hinton agar medium**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef dehydrated infusion</td>
<td>300</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>17.50</td>
</tr>
<tr>
<td>Ingredients</td>
<td>gram/liter</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Peptone</td>
<td>19.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0</td>
</tr>
<tr>
<td>Na- Deoxycholat</td>
<td>1.0</td>
</tr>
<tr>
<td>Neutral Red</td>
<td>0.03</td>
</tr>
<tr>
<td>Crystal Violet</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Fifty two grams of dehydrated MacConkeys agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes.

**Triple sugar iron agar:**

Composition:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.00</td>
</tr>
<tr>
<td>Ingredients</td>
<td>gram/liter</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.00</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.00</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Sixty five grams of dehydrated triple sugar iron agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes.

**C.L.E.D Agar:**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone bacteriological</td>
<td>7.00</td>
</tr>
<tr>
<td>Peptone</td>
<td>4.00</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.00</td>
</tr>
<tr>
<td>L. Cystine</td>
<td>0.128</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.02</td>
</tr>
<tr>
<td>Acid fuchsin</td>
<td>0.10</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Thirty six grams of dehydrated triple sugar iron agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes.
**MIU Medium Base:**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymic hydrolysate</td>
<td>10.00</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.01</td>
</tr>
<tr>
<td>Agar</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Eighteen grams of dehydrated triple sugar iron agar medium was suspended in 950 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. Cool to about 50 – 52°C and aseptically add 5ml sterile 40% Urea Solution per 95ml basal medium. Mix well and dispense into sterile test tubes. Allow to cool in an upright position.

**Trypticase soya broth (for blood culture)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypticase soya broth</td>
<td>30gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>PABA</td>
<td>0.05gm</td>
</tr>
<tr>
<td>Sodium polyenthol sulphonate</td>
<td>0.5gm</td>
</tr>
</tbody>
</table>

Thirty grams of dehydrated Sayabean casein digest Medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15
minutes and dispensed 50ml into sterile blood culture bottle and cap was replaced tightly and wrapped with aluminum foil. Stored at + 4°C.

**Thioglycollate broth media**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gram/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic digest of casein</td>
<td>15.00</td>
</tr>
<tr>
<td>Dextrone</td>
<td>5.50</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.50</td>
</tr>
<tr>
<td>L – cystine</td>
<td>0.50</td>
</tr>
<tr>
<td>Sodium thioglycolate</td>
<td>0.50</td>
</tr>
<tr>
<td>Resazurin sodium</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Thirty grams of dehydrated Fluid thioglycollate Medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes and dispensed 50ml into sterile blood culture bottle and cap was replaced tightly and wrapped with aluminum foil. Stored at + 4°C.

Note: If upper one third appeared pink coloured, the medium may be restored by heating on a water bath or in free flowing steam until the pink colour disappears.

PH after sterilization 7.1 ± .2

Use: For sterility testing of biological and for cultivation of anaerobes aerobes and micro aerophiles as per USP.

**MacFarland Standard 0.5**
Composition and preparation 1% (V/V) solution of chemically pure 0.36 (N) sulphuric acid and 1.175% (W/V) solution of chemically pure (0.048M) barium chloride was prepared in two separate sterile flasks. Than 9.9ml of sulphuric acid and 0.1ml of barium chloride were added to the clean screw capped test tube and sealed. The barium sulphate suspension corresponds approximately to Mcfarland standard tube number 1 with corresponding cell density of $3 \times 10^8$ organism/ ml to made the turbidity standard of cell density to one half of the Mcfarland standard tube number 1 which correspond to cell density of $1.5 \times 10^8$ organism/ml for determination of antibiotic sensitivity by Kirby-Bauer inoculated technique. 0.5ml of 1.75% (W/V) barium chloride (Bacl$_2$ 2H$_2$) was added to 99.5ml of 1% (V/V) sulphuric acid (0.36N), mixed well and 5 – 10 ml was distributed in sterile capped test tube and sealed.

APPENDIX-III

The composition and methods of preparation of different stains, diluents and chemicals used in this study are given below:

Crystal violet Gram stain:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal violet</td>
<td>20g</td>
</tr>
<tr>
<td>Ammonium oxalate</td>
<td>9g</td>
</tr>
<tr>
<td>Ethanol or methanol, absolute</td>
<td>95ml</td>
</tr>
<tr>
<td>Distilled water to</td>
<td>1 liter</td>
</tr>
<tr>
<td>Lugol’s iodine solution</td>
<td></td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>20g</td>
</tr>
<tr>
<td>Iodine</td>
<td>10g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 liter</td>
</tr>
</tbody>
</table>

Alcohol fixative solution

To make 200ml:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (ethyl alcohol), absolute</td>
<td>180ml</td>
</tr>
</tbody>
</table>
Acetic acid, glacial 10ml
Distilled water 10ml

Immerse the fixative for 20 minutes. Rinse with 95% ethanol and allow the smear to dry.

Carbol fuchin

Basic fuchin 10g
Ethanol or methanol 100ml
Phenol 50g
Distilled water 1 liter

Oxides reagent

To make 10ml
Tetramethyl-p-phenylenedimine(dihydrochloride) 0.1g
Distilled water 10 ml

Appendix – IV

Different tests:

Catalase test: (Cruick shank et al, 1975)

Procedure:

A small amount of the culture to be tested was picked up from an agar media with cleaned sterile platinum loop. This was inserted into H₂O₂ (3%) solution held in a small, clean tube. The production of gas bubbles from the surface of solid culture materials indicates a positive reaction. *Staph aureus* was used as positive control and *Streptococcus* as negative control.

Oxidase test: (Cruick shank et al, 1975)

Procedure:
A piece of filter paper was placed in a clean petridish and 2 or 3 drops of freshly prepared oxidase reagent (tetramethyl-p-phenylene diamine dihydrochloride 10gm/l) was added. With a sterile glass rod 1 or 2 colonies of the test organisms was taken and rubbed on the filter paper. Development of blue purple colours within 10seconds indicates positive test. *Pseudomonas* was used as positive control and *Salmonella* as negative control.

**Bacitracin sensitivity test:** (Rose, 1989)

Discs containing 0.04 unit of bacitracin was place on subculture Blood agar plate. *Streptococci* of lancefield group A were sensitive (zone of inhibition >15mm diameter). Other *Streptococci* (i.e *Str. viridans*) were resistant i.e. gave no inhibition or only a small zone of inhibition.

**Coagulase test:** (Duguid, 1989)

Human plasma was collected from discarded blood bag for transfusion and preserved in 15ml screw cap test tube at -20\(^{\circ}\)C for months and one test tube for an use at 4\(^{\circ}\)C for one week.

**Slide coagulase test**

One drop of normal saline (0.85% sodium chloride) was placed on a clean microscopic slide. With minimum of spreading one or two colonies of test organisms were taken and emulsified in the drop of saline to form a smooth milky suspension. Undiluted human plasma was added to the bacterial suspension with the help of one inoculating were loop. Appearance of coarse clumping visible to the naked eye within 5 – 10 sec indicative positive reaction. A positive control test was done using coagualase positive culture of staph aureus.
Gram's staining method (Cheesebrough, 2000)

Gram's staining was done for morphological identification of bacteria. A drop of distilled water was taken on the middle of a clear glass slide. Then bacterial colony (fresh culture) was transferred with a sterilized inoculating loop on the drop of water and a very thin smear was prepared on the slide by spreading the colony uniformly. The smear was fixed by passing it over the flame for two or three times. The smear was flooded with crystal violet (Appendix-III) solution and allowed to stand for two minutes and then washed thoroughly with gentle stream of tap water. The slide was then immersed with Gram's iodine (Appendix-III) solution for one minute and then washed with tap water. Then the smear was decolorized with acetone alcohol (Appendix-III) for 5-10 seconds.

Acetone alcohol was drained off and washed thoroughly with gentle stream of tap water. The slide was then counterstained with diluted carbol fuchsin(1:10) for one minute. After washing with tap water, slide was dried and examined under microscope with high power objective (X100) using immersion oil.
Appendix – V

BD GasPak™ EZ Gas Generating Container systems

Intended use

The GasPak™ EZ Gas Generating Container systems are multiuse systems that produced atmospheres suitable to support the primary isolation and cultivation of anaerobic, microaerophilic or capnophilic bacteria by use of gas generating sachets inside multi use incubation containers.

Principle of the procedure:

The gas pack EZ gas generating sachet consist of a reagents sachet containing inorganic carbonate, activated carbon, ascorbic acid and water. When the sachet is removed from the outer wrapper, the sachet becomes activated by exposure to air. The activated reagents sachet and specimen are placed in the gas pack EZ incubation container and the container in sealed the sachet rapidly reduces the oxygen concentration within the container. At the same time inorganic carbonate produces carbon dioxide. For the cultivation of anaerobic bacteria the gas pack EZ anaerobe container system sachet produced an anaerobic atmosphere within 2.5 hour with grater than 15% carbon dioxide within 24 hours.

Warning and precaution:

Discard sachet after reaction is complete:

Do not use sachet if outer foil is damaged or open in any manner.

Avoid direct sunlight and excessive temperature.

Do not stack sachet when using more than 1.

Do not open container until incubation is complete.
Storage instruction:
On receipt store sachet at 2 – 25°C in a dry environment

Product deterioration
Do not use reagent sachets if outer package has been damage or open
Do not use container that show cracks, chips damage gaskets or other irregularities

Active ingredients: Ascorbic acid, activated carbon and water

Warning: Activated carbon is irritating to eyes, respiratory system and skin

Test procedure:

(1) Place the desired plates in the rack inside the gas pack EZ incubation container.

(2) Remove the gaspack EZ container system sachet from the carton . Remove the outer foil packaging.

(3) Place the activated sachet in the gaspack EZ incubation container with the plates.

(4) The sachet should be placed along side the plates between the plate rack and the outside of the container. (Add an anaerobic indicator to the container or jar at same time).

(5) Closed the gaspack EZ container by seating the lid on the container bottom. Snap all four latches completely closed.

(6) The jar is then incubated at 35°C temperature for 48 hours.

(7) After incubation, open the container removed plate and disposed sachet in the appropriate manner.
Maintenance recommendation:

Proper care of the gaspack EZ incubation container consist of the following:

1. Avoid contact with abrasives.
2. Avoid contact with solvents and detergents.
3. Rinse and dry thoroughly after cleaning with the mild detergents.
4. Never autoclave container or lid.

User quality controlled:

Each gaspack EZ gas generating container system should be tested periodically for its ability to provide adequate conditions for the growth of appropriate bacteria.

<table>
<thead>
<tr>
<th>System</th>
<th>Quality control organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaspack EZ anaerobic container system</td>
<td>Bacteroides fragilis ATCC\textsuperscript{TM} 25 – 85</td>
</tr>
</tbody>
</table>

Appendix – VI

PHOTOGRAPH

3. Swab culture Cross tabulation

<table>
<thead>
<tr>
<th></th>
<th>Swab culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>Artificial</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Due time</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>Premature rupture membran</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>42</td>
</tr>
</tbody>
</table>

**Chi-Square Tests**

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>20.885(a)</td>
<td>2</td>
<td>.000</td>
</tr>
</tbody>
</table>

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .48.