"BACTERIOLOGICAL STUDY OF CHRONIC SINUSITIS WITH SPECIAL REFERENCE TO ANAEROBES"

By

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Under the Guidance of

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LIST OF ABBREVIATIONS

BA	Blood agar
BAP	Blood agar plate
BBE	Bacteroides Bile Esculin agar
CA	Chocolate agar
DNase	Deoxyribonuclease
FESS	Functional Endoscopic Sinus Surgery
KVLB	Kanamycin Vancomycin Laked Blood agar
SPS	Sodium Polyanethol Sulphonate
TSI	Triple Sugar Iron

ABSTRACT

Background and objective :-

Chronic sinusitis afflicts significant percentage of the population and causes considerable long term morbidity. Objective is to isolate and speciate aerobic and anaerobic bacteria from clinically diagnosed cases of chronic sinusitis and to determine the antibiotic susceptibility pattern of aerobic organisms.

Material and methods :-

The study included 50 patients with signs and symptoms of chronic sinusitis over a period of one year (Jan 2007-Jan 2008). Material was collected from chronically inflamed sinuses during FESS. Clinical samples were transported to the laboratory in fluid thioglycollate media. The macroscopic and microscopic findings were noted. Aerobic and anaerobic cultures were put up. For aerobic, 5% blood agar, mac conkey agar and chocolate agar (5-10% co2) media were used and for anaerobic, blood agar with haemin and vitamin K, Kanamycin, Vancomycin laked blood agar (KVLB) and Bacteroides Bile Esculin agar (BBE). The media were placed in McIntosh Fildes jar with Iternal Gas Generating System and incubated at 37^0C for minimum of 3-5 days. Anaerobic growth was identified using standard techniques. Antibiotic sensitivity testing of aerobic organisms were carried out by the Kirby Bauer's disk diffusion technique.

Results :-

Bacterial growth was present in 33 of the 50 samples. Only aerobes were obtained in 17 (34%) samples, pure anaerobes in 9 (18%) cases. Anaerobic growth was totally seen in 16 (32%) of the samples, no growth was seen in 17 (32%) of the

samples. Of the aerobes Staplylococus aureus was the commonest isolate and in anaerobes Prevotella species were the commonest isolate followed by by Bacteroides species.

Conclusion:-

Although aerobes are commonly isolated in chronic sinusitis, anaerobes also play a important role in chronically inflamed sinuses and therefore appropriate therapy should be directed at these anaerobes as well, as early as possible to decrease the long term morbidity of this disease.

Key words :bacteriology, anaerobes, chronic sinusitis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PHOTOGRAPHS

LIST OF GRAPHS

INTRODUCTION

In ancient times the paranasal sinuses, without any anatomical differentiation were thought to be a system of hollow spaces through which mucus produced by the brain was drained. Leanardo da Vinci in Milano in 1489 was the first to prepare and draw anatomical specimens of the paranasal sinuses. Many diseases were attributed to such structures, from halitosis to acne[1].

Sinusitis is an inflammation of the paranasal sinuses whose etiology includes both infectious agents and allergic mechanisms. The causative organisms can be bacteria, fungi or viruses. Sinusitis results from any condition causing ostial obstruction or from pathophysiological changes in the mucociliary transport mechanisms[2].

0.5% to 2.5% of adult patients with viral upper respiratory tract infection will develop acute bacterial sinusitis[3]. Unresolving acute sinusitis that fails to respond to antimicrobial therapy can lead to chronic infection. Because of its persistent nature chronic sinusitis can become a significant cause of morbidity. Untreated it can reduce the quality of life and the productivity of the affected person. Chronic sinusitis is associated with exacerbation of asthma and serious complications such as brain abscess and meningitis which can produce significant morbidity and mortality. Sinusitis is usually diagnosed on clinical and radiological grounds[4].

The microbiology of acute sinusitis is mainly made up of aerobic and facultative bacteria whereas in chronic sinusitis it has been shown to be polymicrobial consisting of both aerobic and anaerobic bacteria. The recovery rate of anaerobic bacteria varies from 25-65%[5, 6, 7].

There has been a great deal of variation in the reporting of the bacteriology of chronic sinusitis, differences can be attributed to the different countries in which the studies were performed, characteristics of study populations and differences in sampling methods and the types of laboratory facilities.

The bacteria usually recovered from chronic sinusitis are aerobic bacteria like, Staphylococcus aureus, Streptococcus pneumoniae, H. influenzae and also gram negative organisms. The frequently isolated anaerobic bacteria are, Bacteriodes spp, Prevotella spp, anaerobic cocci and Fusobacterium spp[6, 7, 8, 9, 10].

Microenvironmental conditions are a key factor in the development and maintenance of chronic anaerobic sinusitis. Aerobic and air tolerant bacteria colonize the sinus initially, consume oxygen and alter gas tension, resulting in an environment that favours anaerobes. Once a chronic infection is established, the sinus provides a good anaerobic environment because of the inflammation and reduced drainage[11].

The successful treatment of chronic sinusitis necessitates a thorough knowledge of the prevailing bacteriology. In most cases of chronic sinusitis it is seen that an attempt to culture the anaerobes is not made as it is less rewarding due to inherent technical complexities and treatment is usually directed against only the aerobic bacteria isolated which does not help in resolving the problem of chronic sinusitis where anaerobic bacteria are known to play an important role. Keeping this in mind, in our study we have given special importance to isolation of anaerobic bacteria in patients presenting with chronic sinusitis at the ENT OPD of KLE's Prabhakar Kore Hospital, Belgaum.

AIMS AND OBJECTIVES OF THE STUDY

1. To isolate and speciate the aerobic and anaerobic bacteria isolated form clinically diagnosed cases of chronic sinusitis.

2. To carry out antibiotic sensitivity testing for aerobic isolates.

REVIEW OF LITERATURE

Historical Review

Nasal region studies, the olfactory function and the knowledge about the paranasal sinuses date back from the most remote times, as well as the attempts to treat disorders of this area.

Egyptian physicians were the precursors of nasal surgeries. They used instruments to remove the brain through the nose, as part of the mummification process.

Hippocrates, in V century B.C. had already described parts of the nasal anatomy. Leonardo da Vinci drew the nasal conchae and the paranasal sinuses.

Wright in his "A history of laryngology and Rhinology" notes that Vesalius (1542) attributed the earliest knowledge on the existence of sinuses to Galen (130 – 201 A.D.)

Nathaviel Highmore in England in 1651 presented the first detailed description and drawing of the maxillary sinus and hence it is named Highmore's antrum.

During the XVII and XVIII centuries, the major scientific discussion about the nasal region regarded the function and objective of paranasal sinuses.

In England – 1707, Drake and Cowper, reported some cases of halitosis caused by maxillary sinus suppuration. They treated the patient by pulling out teeth, thus opening the maxillary sinus through the alveolus.

In France – 1765, Jourdain, tried to cure maxillary sinuses suppuration by irrigating the structures through their natural ostium, present in the middle meatus, but

he was not very successful. In 1743, Lamorier was already opening the maxillary sinus through the tooth socket which remained the standard procedure for a long time.

J.F.L. Deschamps of Paris, in 1804 wrote the first paper dedicated entirely to the nose and nasal sinuses. He gave lengthy attention to the sense of smell and stated that sinuses had nothing to do with olfaction.

L.Grunwald in his textbook on "Nasal suppuration" in 1893 firmly established the subject of sinus disease. Prior to this point, most felt that nasal pus of the maxillary antrum was associated with the teeth rather than the nose.

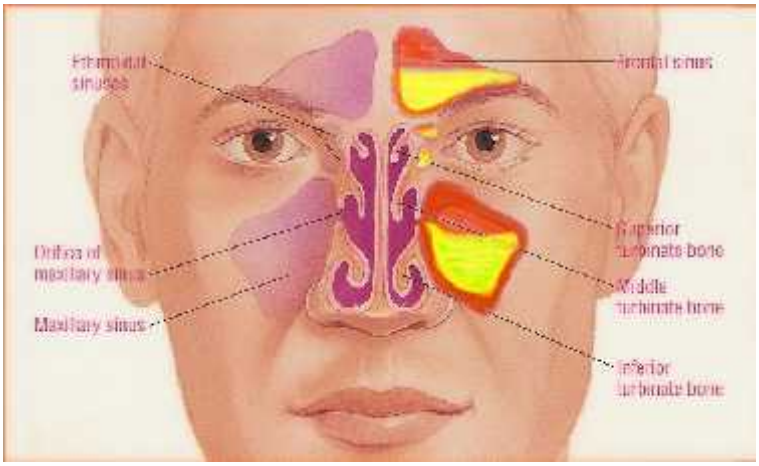
G. Caldwell of America (1893) and Luc of France (1894) independently suggested making a wide opening in the canine fossa and establishing a permanent counter – opening in the nasal cavity for operating on the maxillary antrum. This procedure is known today as Caldwell Luc method.

However current knowledge regarding the anatomy of these structures is greatly due to the works of Emil Zuckerkandl, from Austria, who in 1870 described details of the nose and paranasal sinuses in anatomical studies, thus opening a new field for scientific and surgical knowledge in this area.

Scholars such as Grunwald, Onodi, Hajek witnessed the birth of rhinology as a speciality, making up the basis of our current concepts of diagnosis and treatment of the nasal cavities and paranasal sinuses[1,12].

Anatomy of Paranasal Sinuses

Paranasal sinuses are air – containing cavity in certain bones of skull. They are four on each side.



Clinically paranasal sinuses have been divided into two groups.

Anterior Group:

This includes maxillary, frontal and anterior ethmoidal. They all open in the middle meatus.

Posterior Group:

This includes posterior ethmoidal sinuses which opens in the superior meatus, and the sphenoid sinus which open in the sphenoethmoidal recess[13].

Flora of the Normal Sinuses

The bacteriology of the normal sinuses has been studied by few authors. As most of these studies are on maxillary sinus, it is being discussed here.

The bacteriology of the maxillary sinus has been found to be quite diverse. Several variables for this diversity include the type and methods of specimen collection transportation and cultivation.

Bjorkwell in 1950 first studied the bacterial findings in the normal maxillary antrum. In his work, clinically and radiographically healthy sinus was defined as a normal sinus[14]. Brook (1981) was the first to culture bacteria from normal maxillary sinuses. He defined normal sinuses as 'none (of patients) suffered from sinusitis,

allergic rhinitis in the past'. He reported the presence of aerobic and anaerobic bacteria in normal maxillary sinuses of 12 patients. Anaerobes were isolated is all 12 specimens, in 5 (42 %) they were the only organisms isolated, and in 7 (58%), they were recovered mixed with aerobes. There were 33 anaerobic isolates and the predominant ones were Bacteroides spp. Prevotella spp, anaerobic gram positive cocci and Fusobacterium spp. There were 16 aerobic isolates and the predominant ones were beta haemolytic Streptococci, alpha haemolytic Streptococci and Streptococcus pneumoniae. He concluded that the endoscopically normal maxillary sinus is not sterile and the organisms isolated are the same as seen in chronic maxillary sinusitis[15].

Rong-San Jiang in their study, cultured bacteria from 47 swab specimen and 15 mucosal specimens. The culture rate was 56.6% from swab specimens and 48.4% from mucosal specimens. Sixty four bacterial isolates were identified from 47 swab specimens. The common aerobic bacteria were Streptococcus viridans, Staphylococcus epidermidis and Streptococcus pneumoniae and anaerobes were Peptostreptococcus spp, Propionibacterium spp, Bacteriodes spp[16].

Mucous membrane of Paranasal Sinuses:

Paranasal sinuses are lined by mucous membrane which is continuous with that of the nasal cavity through the ostia of sinuses. It is thinner and less vascular compared to that of the nasal cavity. Histologically, it is ciliated columnar epithelium with goblet cells which secrete mucus. Cilia are more marked near the ostia of sinuses and help in drainage of mucus into the nasal cavity.

Development of Paranasal Sinuses:

Paranasal sinuses develop as outpouchings from the mucous membrane of lateral wall of nose. At birth, only the maxillary and ethmoidal sinuses are present and are large enough to be clinically significant.

Growth of sinuses continues during childhood and early adult life. Radiologically, maxillary sinuses can be identified at 4-5 months, ethmoids at 1 year, frontals at 6 years and sphenoids at 4.

Lymphatic Drainage:

The lymphatics of maxillary, ethmoid, frontal and sphenoid sinuses form a capillary network in their lining mucosa and collect with lymphatics of nasal cavity. Then they drain into lateral retropharyngeal and/or jugulo digastric nodes.

Physiology of Paranasal Sinuses:

Ventilation of sinuses

Ventilation of paranasal sinuses takes place through their ostia. During inspiration air current causes negative pressure in the nose. This varies from – 6mm to 200mm of H_2O depending on the force of inspiration. During expiration, positive pressure is created in the nose and this sets up eddies which ventilate the sinuses. Thus ventilation of sinuses is paradoxical, they are emptied of air during inspiration and filled with air during expiration.

Mucus drainage of sinuses

Mucus secreted in the paranasal sinuses travels to the ostium in a spiral manner. Here the cilia are very active and propel mucus into the meatuses from where it is carried to the pharynx. The mucus from anterior groups of sinuses travels along

the respective lateral pharyngeal gutter situated behind the posterior pillar and that from posterior group is spread over the posterior pharyngeal wall to be finally swallowed.

Functions of Paranasal Sinuses:

It is not clear why nature provided paranasal sinuses. Probable functions are:

1) Air conditioning of the inspired air by providing large surface area over which air is humidified and warmed.
2) To provide resonance to voice.
3) To act as thermal insulators to protect the delicate structures in the orbit and the cranium from variations of intranasal temperature.
4) To lighten the skull bones.
5) Vehicle for trapping viruses, bacteria, foreign material for removal.

Sinusitis

Sinusitis in the simplest form is an inflammation of the mucous membrane of one or more of the paranasal sinuses. The disease may involve the bone of sinus or surrounding structures before patient seeks help.

Classification: By considering the duration of the disease and the pathological changes that occur in the mucosa of the sinuses it has been classified by Litton as:

1. **Acute sinusitis**: Acute inflammation of the sinuses, which resolves within 3 weeks.
2. **Subacute sinusitis**: Where the inflammation of the sinuses persist for more than 3 weeks and resolves within 3 months.

3. **Chronic sinusitis**: Where inflammation of the sinuses persists even after 3 months.

Sinusitis may occur in a single or in several sinuses at a time. Sinusitis may be of open type or closed type depending on whether the ostium is open or blocked. Acute sinusitis is a common disorder in both children and adults. About 0.5% to 2.5% of adult patients with viral URI's will develop acute bacterial sinusitis. The persistence of URI for more than 7 to 10 days usually indicates the development of sinusitis. Maxillary and ethmoid sinuses are most frequently involved.

Chronic sinusitis is a common disease worldwide. It affects 32 million people each year. It is highly prevalent in the United States affecting an estimated 16% of the population with an economic burden of 6 billion. In India the prevalence rate is 12%, more worrisome is the fact that prevalence has doubled since the 90's.

Chronic Sinusitis

Chronic inflammation of the sinuses persisting even after 3 months is considered as chronic sinusitis.

Since the maxillary antrum is the sinus most frequently infected in adults and the sequelae of these infections can be protracted and dangerous, the proper identification of the infecting organism, along with the appropriate correction of the underlying anatomical abnormality, must be accomplished early if the recurrent cycles of infection are to be prevented[13,17.]

Etiology:

The etiologic factors, which favor the development of chronic sinusitis as, described by Koltai et al[18] in their study are varied and include anatomic changes that prevent adequate drainage of the sinus through its natural ostia. Physiologic

abnormalities of the mucous membrane lining with altered ciliary activity, and host immune incompetence predisposes to recurrent infections. Obstruction of the nose and sinuses may be due to naturally occurring septal deviations, the sequelae of trauma, or the result of inadequately controlled nasal allergies. Friedman R et al[19], describes allergy especially asthma, as an important predisposing factor in sinusitis. Itzhak Brook et al[20], in their study incriminates dental infections also as a source of sinusitis. When sinusitis occurs, oxygen gets absorbed mostly by the sinus mucosa. The possible implication of the oxygen consumption in the diseased sinus is the formation of a bacteria host relationship in favor of certain bacteria[21]. According to Aust R, it is therefore plausible that the reduced oxygen tension in the sinus during the serous phase better meets the requirements for the growth of those bacteria isolated in acute sinusitis, Streptococcus pneumoniae and Haemophilus Influenza. In contrast, the complete lack of oxygen in the purulent secretion supports the growth of the anaerobic organisms recovered in chronic sinusitis[22].

Frederick J et al[6] describes unresolving acute sinusitis that fails to respond to antimicrobial therapy can lead to chronic infection, with potentially serious local and systemic complications. Carenfelt C et al[23] enumerated factors that may allow organisms to survive antimicrobial therapy within the inflamed sinuses. These include inadequate penetration of antimicrobial agents into the sinus cavity, a high protein concentration (that can bind antimicrobial agents), decreased multiplication rate of organisms in the sinus cavity that can interfere with the activity of bacteriostatic agents and increased acidity within the sinus cavity that reduces the efficacy of some antimicrobial agents (i.e. aminoglycosides and quinolones).

Aust R[22] and Carenfelt et al[23] have documented that as chronicity develops, the aerobic and facultative species are gradually replaced by strictly anaerobic bacteria.

These changes may be due to the selective pressure of antimicrobial agents that enable resistant organisms to survive. More over, the effects of chronic infection may lead to the development of conditions appropriate for the growth of anaerobic bacteria. These changed conditions include the reduction in oxygen tension and an increase in acidity within the sinus cavity which reduces blood supply and therefore, the consumption of oxygen by the aerobic component of the sinus cavity bacterial flora. Other factors as described by Brook I, are the emergence over time or selection of anaerobic bacterial strains that possess essential virulence factors such as a capsule[21].

Predisposing factors for chronic sinusitis are :

1. Dental Infection: Dental infections are an important source of sinusitis. Odontogenic maxillary sinusitis may occur by draining of an apical dental root abscess into the maxillary sinus. It has been reported that the disease is usually frequent in cases of aged of the second and third decades. In most of them, either the first or second molar tooth is assumed to be the origin of the disease[24].

   Itzhak Brook has studied the incidence of dental caries in relation to maxillary sinusitis. He found that in 18% of cases the sinusitis could be directly traced to apical infection, in 30% of cases dental abscess was present which was thought probably to be the etiological cause and in 41% of patients a dead tooth was present in relation to the floor of the sinus, which probably might have been the origin of the infection and in only 11% of the cases all the teeth were normal[20].

2. Injuries to the facial bones may result in collection of blood in the sinus cavity and it may become secondarily infected.
3. Mechanical obstruction to the normal aeration of or drainage of the sinus.

   This can occur because of,

   a) Deviated nasal septum
   b) Unilateral choanal atresia
   c) Foreign bodies placed in the nose
   d) Fractures of the nose following trauma.

4. Swimming: When water enters the nose forcibly particularly while diving, infection may spread to the sinuses.
5. Barotrauma: Here as a result of rapid pressure changes, volume and pressure of air decreases in the sinus, leading to the ostial block. If the person is suffering from acute rhinitis at the same time chances of getting sinus infection are quite high.
6. Abnormalities of local defence mechanisms: In cystic fibrosis, Young's syndrome, Kartagener's syndrome etc, the local defence mechanisms that is the mucociliary blanket cannot function in a normal way leading to stasis of secretions in the sinuses and predispose to infection.
7. Systemic Infections: Leads to lowered resistance to infections and may result in spread of infection to the sinus easily.
8. Blood borne infection is a rare possibility.

Anthony W Chow in their "Infections of the sinuses and parameningeal structures" summarizes the factors that predispose to sinusitis.

1. Impaired mucocililary function

- Viral upper respiratory tract infection
- Cold or dry air
- Chemicals, drugs
- Cystic fibrosis
- Ciliary dysmotility syndrome.

2. Obstruction of sinus ostia

- Viral URI
- Allergic rhinitis
- Rhinitis medicamentosa
- Anatomic abnormalities (Eg: nasal polyps, deviated nasal septum, foreign body, tumors)

3. Immune defects

- Immunoglobulin A deficiency
- Immunoglobulin G_2 and G_4 subclass deficiency
- Acquired immunodeficiency syndrome
- Wegener's granulomatosis
- Diabetes malletis

4. Increased risk of microbial invasion of the sinuses

- Odontogenic infections
- Nasotracheal intubation

- Head trauma
- Swimming or diving
- Cocaine sniffing[13, 17]

Microbiology:

The microbiology of chronic sinusitis has been an interesting topic in the literature. A wide variety of aerobic, anaerobic bacteria and fungi are responsible for the causation of chronic sinusitis.

Bacteriology:

The studies dealing with bacterial findings of chronic maxillary sinusitis have been widely investigated in attempting to define the correct pathogens implicated. However, the results from different reports are extremely diverse as evidenced by Karma et al[25] and Su et al[26]. Besides the real differences in bacterial flora, this has been blamed on differences in sample sources and sampling techniques, contamination by normal flora, the uncontrolled precultural use of antibiotics, differences in the population studied etc. Jiang et al[10], Su et al[26] have documented that the mucosal specimens reflect the real bacteriology of sinusitis more accurately than swab specimens.

Studies have demonstrated the usefulness and general accuracy of endoscopic samples in chronically infected sinuses[27].

The maxillary sinuses have usually been considered as the primary focus of the disease and therefore the majority of microbiological studies on chronic sinusitis have concentrated on the maxillary sinuses.

Bacteriological studies of chronic maxillary sinusitis by different people shows the culture positivity rates ranging from 54% to 92% consisting of aerobic bacteria, anaerobic bacteria and in some cases a mixed population of both aerobic and anaerobic bacteria accounting for a significantly high percentage of cases[23, 25, 26, 28, 29]. Karma et al[25] documents only 54% of positive cultures in their study. The culture positivity rate was 92% by Itzhak Brook[2] followed by 90% by Erkan et al[30]. A 87% culture positivity by Archana Thakur et al[31], 81% by Hartog et al[32] and 75% by Fredrick et al[6].

The occurrence rate of aerobic bacteria as the causal organisms in chronic maxillary sinusitis ranges from 12% by Erkan et al[30] and Itzhak Brook[21] to 52% by Hartog et al[32].

During the past decade medical literature has emphasized the role of anaerobes in chronic sinusitis. The report by Fredrick and Braude[6], established the importance of anaerobes in chronic sinusitis. They isolated anaerobes from 31% of cases with chronic sinusitis. However Itzhak Brook[21] documents an incidence as high as 56% of anaerobes as causative organisms of chronic maxillary sinusitis. Archana Thakur et al[31] in their study describes an isolation rate of 28%, where as Hartog et al[32] documents anaerobic etiology in only 5% of cases. Studies of Doyle, Woodham and Goldenhersh et al[33] identified few or no anaerobes or alpha haemolytic streptococci predominated over other bacteria. Very often more than one bacterial species can be found, in contrast to what is seen in acute sinusitis.

Many studies confirm the polymicrobial etiology of chronic maxillary sinusitis consisting of aerobic, anaerobic and both aerobic and anaerobic bacteria[7, 23, 25, 31, 32]. Erkan et al[30] describes more than one bacterium were involved in causation of chronic maxillary sinusitis in 36% of cases. The presence of mixed flora was also reported

from 32% of cases by Itzhak Brook[21], 25% of cases by Archana Thakur et al[31] and from 20% of cases by Frederick et al[6]. Isolation rates of mixed flora as low as 13% by Karma et al[25] and 6% by Jiang et al[10] has also been reported.

The maxillary sinus is the most frequently studied in the bacteriology of chronic sinusitis because it is the most accessible, and was once considered the focus of primary disease. In contrast, frontal, ethmoid and sphenoidal sinusitis have been studied only occasionally or rarely.

Frontal sinusitis is a potentially devastating infection with a high frequency of intracranial complication. In contrast to maxillary sinusitis, the microbiologic features of frontal sinusitis are not well established and only a few reports document the organisms isolated. The role of anaerobic bacteria in this infection is also not well studied, although their role was recorded in a few cases. Itzhak Brook in his study has recorded anaerobic bacteria as the main isolates[9].

The role of the bacteriology of the ethmoid sinus in chronic sinusitis has not been clarified. However, because of the popularization of FESS, it has become much easier to collect the specimens directly from the ethmoid sinus than before. Therefore more research has been devoted recently to the study of the bacteriology of the ethmoid sinus. In a study conducted by Jiang et al[10], aerobic and facultative were the predominant bacteria and anaerobic bacteria was seen in only one specimen.

Sphenoid sinusitis is rare but potentially devastating infection. In contrast to maxillary sinusitis, the microbiology is also not well established and role of anaerobic bacteria is not well studied either. Itzhak Brook in his study has documented anaerobic bacteria as the most common isolates in chronic sphenoid sinusitis[34].

Anaerobic Bacteriology:

Anaerobic organisms most commonly implicated in the causation of chronic sinusitis are Peptostreptococcus spp. Bacteroides spp. Prevotella spp. Fusobacterium spp. Porphyromonas spp. and Propionibacterium spp. Most studies document Peptostreptococcus spp, as the dominant anaerobe isolated from patients with chronic sinusitis. The isolation rates in different studies vary from 2% to as high as 60%[16, 21, 23, 33]. A 60% and 54% isolation rate was described by Itzhak Brook et al[7, 21] in two separate studies, followed by 57% by Ziuzio et al[35] and 40% isolation rate by Erkan et al[30], 54% by Frederick J et al[6].

Bacteriodes spp was the prominent isolate (21%) followed by Peptostreptococcus in a study by Itzhak Brook[21]. However in most other studies Bacteroides spp was the next common anaerobe to Peptostreptococcus spp as evidenced by Archana Thakur et al[31] (23%), Frederick et al (23%)[6] and Itzhak Brook et al[7] (15%).

Prevotella spp was a predominant isolate (48%) in the study of Rajiv Arora et al[36]. It was the next common organism isolated from patients with chronic sinusitis (27%) by Erkan et al[30]. Itzhak Brook et al[7] describes an isolation rate of 21% of Prevotella spp in his study.

The other anaerobes that are not uncommonly isolated from patients with chronic sinusitis include Fusobacterium spp, 17% and 15% by Archana et al[31] and Itzhak Brook et al respectively[7]. Propionibacterium spp was isolated in 22% of cases by Erkan et al[30], followed by Hartog et a[32] and 13% by Archana Thakur[31]. An isolation rate of 13% of Clostridium spp was given by Erkan et al[30] and 45% by Rajiv Arora et al[36] and Itzhak Brook et al[7].

Review of anaerobic bacteriology

Infections due to anaerobes are being recognized more frequently during the last decade. The reluctance to identify all anaerobic isolates in specimens from mixed infections is due to the time consuming nature of the work involved, the preconception that anaerobes from such specimens are difficult to purify and the lack of knowledge as to the precise pathogenic role of each organism in a mixed infection.

It was Leuwenhook in 1686 who first reported that animalcules could exist in the absence of oxygen. Pasteur (1861) observed that occurrence of butyric fermentation in the absence of oxygen and concluded that it was due to a bacillus which he called "vibrion butyrique". He introduced the terms "aerobies" and "anaerobies" to designate microorganisms that live in the presence and absence of oxygen respectively[37].

Association of anaerobes with human infection was recognized as early as 1890, when Clostridium tetani was isolated in pure form and role of toxin in tetanus was established. Viellon and Zuber (1898) isolated Bacteroides species from the intestinal tract. Rist (1898) isolated anaerobes from an infected bone. Within the next 30 years many diverse types of anaerobic bacteria were isolated and the significant role of anaerobes in a variety of human diseases was established[38].

Metrinadazole was first used against anaerobic infections in 1962 by Shin in the treatment of acute ulcerative gingivitis. It is still an effective, safe and the drug of choice for anaerobic infections.[39]

A brief survey of the evolution of anaerobic culture technique is as follows:

1. Until 1916: The methods used were mainly fluid cultures and deep agar shake cultures.

2. In 1916: McIntosh and Fieldes introduced an anaerobic jar based on the combustion principle for removal of oxygen, allowing the use of agar plates and surface cultivation. It required an electronically heated platinum catalyst and 100% hydrogen.
3. In 1939: An improved version, Brewer jar was introduced in the United states.
4. In 1950: Roll tube technique of Hungate was described.
5. In 1958: Stokes isolated a large number of anaerobes with the aid of a jar employing a catalyst active at room temperature and Baird – Tatlock jar was introduced.
6. In 1966: Moore's modifications known as Virginia Polytechnique Institute (VPI) method became available.
7. 1966 – 1968: A number of major developments occurred in anaerobic methodology. Gaspak self contained combustion jar system with its own gas generating package was introduced. Anaerobic chambers, anaerobic cabinet, glove box and selective media were also introduced[40].

Pathogenicity of anaerobes in sinusitis

Pathogens were thought to owe their disease producing ability to unique characteristics, but it is now recognized that most micro-organisms that colonize the body have the capacity to cause disease under predisposing circumstances. Many of the anaerobes involved in the upper respiratory tract infections possess a number of virulence factors that confer pathogenicity. Encapsulation has been shown to confer virulence to Bacteroides species and capsule production is increased in a number of infections. Increased encapsulation of Bacteroides and Prevotella species has been observed in both acute and chronic episodes when compared with healthy sinuses.

Fusobacterium species, have been shown to possess the capability of producing lipopolysaccharides associated with virulence as well as neutrophil cytotoxic substances and DNAase. Other virulence factors associated with anaerobes include the ability to adhere to mucosal epithelial cells and coagulation promotion and spreading factors.

Further evidence of the pathogenic role of anaerobes in chronic upper respiratory tract infections has been obtained with use of an experimental animal model of sinusitis. It was found that infection with Bacteroides fragilis alone can cause prolonged and severe inflammation of the sinus mucosa of greater severity than that observed in experimental pneumococcal sinusitis.

The majority of chronic upper respiratory tract infections are polymicrobial, both aerobic and anaerobic flora are isolated. Mixed infections enhance the overall virulence of the microbial community because of the synergistic potential of mixtures of aerobic and anaerobic organisms. One mechanism of synergy is the protection of susceptible pathogens by β-lactamase producing organisms[11].

Aerobic Bacteriology:

Aerobic organisms most commonly isolated in chronic maxillary sinusitis are Staplylococci aureus, alpha haemolytic Streptococci, H. influenzae and Coagulase negative Staphylococci. Staphylococcus aureus was the predominant aerobe isolated by Hartog et al[32] in 42% of cases, followed by 21% by Ziuzio et al[35], 17% by Fredrick et al[6] and 15% by Itzhak Brook[21].

Alpha haemolytic Streptococci were the predominant aerobes in studies by Sener et al[41] who isolated the organism in 69% of cases followed by Su et al[26] who

isolated in 54% of cases. An isolation rate of 38% was documented by Karma et al[25], 16% by Itzhak Brook[21], 6% by Erkan et al [30].

The isolation rates of enteric gram negative bacilli were from 4% to as high as 52% in different studies[7,28,29,30,31]. Enteric gram negative bacilli were isolated in 52% of cases by Archana Thakur et al[31], followed by an isolation rate of 22% by Erkan et al[30]. Itzhak Brook[21] documents 10% isolation followed by 5% by Jiang et al[16]. Coagulase negative Staphylococci was incriminated for its causative role in chronic maxillary sinusitis in 39% of cases by Sener et al[41]. They were the most common isolates in 36% of cases as described by Su et al[26], Jiang et al[16] isolated from 12% of cases.

Mycology

Chronic fungal sinusitis is a disease of immunocompetent hosts and is usually noninvasive, although slowly progressive invasive disease is sometimes seen.

Non invasive disease, which is typically associated with hyaline molds such as Aspergillus species, dematiaceous molds such as Curvularia or Bipolaries species, can present as a number of different scenarios. In mild indolent disease, which usually occurs in the setting of repeated failures of antibacterial therapy, only nonspecific mucosal changes may be seen on sinus CT. Endoscopic surgery is usually curative in these patients, with no need for fungal therapy. Another form of disease presents with long standing, often unilateral symptoms and opacification of a single sinus on imaging studies as a result of a mycetoma (fungus ball) within the sinus. Treatment for this condition is also surgical, although systemic antifungal therapy may be warranted in the rare case where bony erosion occurs.

A third form of the disease known as allergic fungal sinusitis, is seen in patients with a history of nasal polyposis and asthma, who often have had multiple sinus surgeries. Patients with this condition produce a thick, eosinophilic mucus with a consistency of peanut butter that contains sparse fungal hyphae on histologic examination. Patients often present with pansinusitis[42].

Pathogenesis

Chronic sinusitis results from previous episodes of acute inflammation which has resolved incompletely or persistent obstruction of the sinus from oro-antral fistula and persistent tooth infection. These conditions cause irreversible damage to the sinus mucosa leading to impairment of drainage of secretion which leads to accumulation of secretion and infection which further causes damage to the sinus mucosa thus causing a viscious cycle of events.

The frequent involvement of anaerobes in chronic sinusitis may be related to the poor drainage and increased intranasal pressure that occurs during inflammation. This can reduce the oxygen tension in the inflamed sinus by decreasing the mucosal blood supply and depressing the ciliary action. The lowering of the oxygen content and P^H of the sinus cavity supports the growth of anaerobic organisms by providing an optimal oxidation reduction potential[43].

Pathology

Pathological changes present in chronic sinusitis have been recognized over the years and are as follows:

1) The most common type is hypertrophic changes. The depth of the mucosa is greatly increased and phlebitis and perilymphangitis can occur leading to

oedema and polyp formation called hypertrophic or polypoidal sinusitis. There may be associated fibrosis in lamina propria.

2) The mucuos membrane may be destroyed in spots by ulceration and bare bone may be exposed which may be soft in consistency due to caries and in some cases bone may be absorbed or sequestra may be formed.

3) In some cases chronic inflammatory process may induce atrophic changes in sinus mucosa with increase in submucosal fibrous tissue known as atrophic sinusitis[17].

Clinical Presentation

Symptoms

The symptoms are variable they may be severe enough to prevent the patient from going to work or they may be mild. For convenience they are considered under separate headings, but they are often vague, overlapping and changeable.

1) Pain: over the cheek on the affected sides referred to the floor of the frontal sinus, preauricular and temporal areas and the upper teeth and gums. Headache may be the presenting complaint in sphenoid or frontal sinusitis. The pain is usually mild and dull aching type.

2) Swelling: of the cheek on the affected side may be present. In ethmoid sinusitis there may be excessive tearing and oedema of eyelids.

3) Nasal obstruction: can occur on one or both sides or intermittently on either side if both sides are involved, this may be due to mucosa swelling or anatomical obstruction.

4) Nasal discharge: mucopurulent or purulent discharge, sometimes foul smelling is present if it is open type and if it is closed type, discharge may not be present.

5) Sense of smell: is reduced or there may be sensation of unpleasant smell.

6) Sensation of irritation in the throat: may be present due to postnasal drip or inflammation of the pharyngeal mucosa.

7) Hawking and dry cough: may be present due to postnasal drip.

Signs:

1. Anterior Rhinoscopy: There may be erythema of the nasal mucosa. The turbinate may be hypertrophied with thick mucopurulent secretions in the middle meatus. Deviation of the septum, polyps and foreign bodies may be seen.
2. Posterior Rhinoscopy: Mucopurulent or purulent discharge may be seen on the superior surface of the soft palate or on the roof of the nasopharynx. Choanal atresia or polyps may be detected.
3. Tenderness over the affected sinus can be elicited by applying pressure on the canine fossa or anterior face of the maxilla and on palpitation oedema of the cheek may be detected.
4. Examination of the oral cavity and throat in maxillary sinusitis of dental origin, gum disease, halitosis and dental caries may be detected.

5. Ear examination may show retraction of the tympanic membrane on the affected side, which may look dull and tuning fork tests may or may not indicate conductive deafness on the affected side[13].

Complications of Chronic Sinusitis

Extension of infection from sinuses to other parts may occur by

1) Thrombophlebitis of perforating veins
2) By direct extension through an ulcerating or necrotic portion of sinus wall.
3) By way of preformed dehiscence
4) Through vascular channels in the form of bacteraemia.
5) It is questionable whether infection from sinuses can spread via lymphatics.

Complications that occur include,

a. Pan sinusitis: Infection may spread to other paranasal sinuses.

b. Middle ear infection may occur

c .Pharyngitis, tonsillitis, laryngitis and tracheobronchitis may occur due to spread of infection.

6) Osteomyelitis: of the maxilla is a rare complication and occur in children usually in the first year of life as the maxilla contains bone marrow at that time (cancellous bone) which later becomes membranous bone. The condition presents as a painful swelling of cheek and lower eyelid with fever. The infection usually subsides with medical treatment.
7) Bacteremia and septecemia are reported even in cases where the sinus infection was present without producing any local symptoms.

8) Meningitis, Cavernous sinus thrombosis, Brain abscess and Subdural empyema can also occur but are rare complications[44]

Nosocomial Sinusitis

The discussion of sinusitis would not be complete without a mention of nosocomial sinusitis as nosocomial infections are gaining much importance in the study of infections.

Paranasal sinusitis has been increasingly recognized as an important nosocomial infection in the intensive care unit (ICU) patient. In contrast to out patient sinusitis, which commonly presents with overt symptoms, suppurative sinusitis in the critically ill is often clinically silent. The sequelae of this infection can be catastrophic and justify early aggressive diagnostic enquiry. Intracranial extension and fulminant sepsis are well documented and occur more commonly than with outpatient sinus infections.

Nasal intubation of the air way, especially in the unfavourable circumstances that prevail during exigent treatment of the critically injured, likely traumatizes the nasal mucosa. The resulting oedema may obstruct the sinus ostia. Nasogastric tubes have been implicated similarly. Coexisting facial trauma and resulting sinus hematomas amplify this process by providing a culture medium for bacterial growth[45].

MATERIAL AND METHODS

Source of data

Material was collected from the sinuses in patients with chronic sinusitis, undergoing FESS (Functional Endoscopic Sinus Surgery) at ENT of KLE and MRC Belgaum over period of one year from Jan 2007 to Jan 2008. The study comprised of 50 cases of clinically suspected cases of chronic sinusitis.

Detailed clinical history regarding age, sex, chief complaints, past history of sinusitis, seasonal variation, and presence of any other associated illness was obtained from each patient.

Pus samples collected from the sinuses were studied in the department of Microbiology, J.N. Medical College Belgaum.

Method of Collection of data

Inclusion Criteria:-Patients with clinical signs and symptoms of chronic sinusitis with no H/o recent antibiotic intake.

Exclusion Criteria :- Patients with acute sinusitis.

Samples were collected from each patient with help of sterile swabs during sinus surgery. Three swabs were collected, one each for Gram's stain, aerobic culture and anaerobic culture and brought to the microbiology laboratory in fluid thioglycollate medium.

Laboratory Methods:-

Macroscopic examination: - In the laboratory the sample was inspected for colour and purulence and the findings recorded.

Microscopic Examination:-

Direct smear was made by using one swab. The smear was stained by Gram's method [Hucker's modification of Gram's stain was followed where in counterstain safranin was used and kept for 5 mins[46] (details given below)] for an immediate presumptive diagnosis of the number and type of microorganisms present in the sample. Morphology of the organism and other observations in the gram stained smear were recorded.

Gram stain Procedure

1) A thin smear of the material was made on a clean glass slide and allowed to air dry.
2) The material was fixed by passing the slide three to four times through the flame of a bunsen burner so that the material does not wash off during staining procedure.
3) Slide was placed on staining rack and the smear overlayed with crystal violet solution.
4) After 1 minute the slide was washed thoroughly with distilled water.
5) Then the smear was overlayed with Gram's iodine solution for 1 minute and washed again with water.
6) The smear was held between the thumb and fore finger and the surface flooded with a few drops of acetone- alcohol decolorizer, until no color washes off.
7) The smear was washed with running water and placed on staining rack. The surface was overlayed with safranin counter stain for 5 minutes and washed with running water.
8) The smear was placed in a upright position in a staining rack, allowing excess water to drain off and smear to dry.

9) The stained smear was examined under 100 X (oil) immersion objective of the lens[47].

Bacteriological Methods

Aerobic Culture Methods

The culture swab was inoculated onto.

1) 5% Sheep blood agar.
2) Chocolate agar
3) Mac Conkey agar plate.

The plates were incubated at 37^0C for 24 hrs aerobically (Mac Conkey agar) and under 5% Co_2 (Blood agar and Chocolate agar).

All the isolates were identified and characterized biochemically by standard procedures as described by Mackie & Mc Cartney Practical Medical Microbiology.

Antibiotic Sensitivity Testing

The antimicrobial susceptibility testing was done for aerobic and facultative isolates by disc diffusion method as described by Kirby and Bauer, on Mueller Hinton Agar.

Different antibiotics and concentration of discs used were as follows:

Antibiotics	**Concentration per disc**
1) Amikacin(Ak)	30mcg
2) Ciprofloxacin(Cf)	5mcg
3) Amoxycillin / Clavulanic acid (Ac)	30mcg
4) Cefotaxime(Ce)	30mcg

5) Erythromycin(E) 15mcg

6) Oxacillin(Ox) (1mcg)

7) Co-Trimoxazole(Co) (1.25/23.75mcg)

For antimicrobial sensitivity testing a single colony was inoculated in peptone water and incubated at 37^0C for 4 to 6hrs and turbidity adjusted to Mac Farland's 0.5. Muellor Hinton Agar plate was inoculated with culture by means of cotton swab and antibiotic discs were applied and incubated over night at 37^0C. Zone of inhibition was measured. Interpretation was made according to the Kirby Bauer chart[46].

To detect MRSA Muellor Hinton agar with 5% NaCl was used and incubated at 35^0C for 24 hrs[48].

Control strain used was Staphylococcus aureus ATCC 25923.

Culture for anaerobic organisms

Each sample was inoculated onto

1) Blood agar supplemented with Haemin and Vit K.
2) Kanamycin Vancomycin laked blood agar (KVLB)
3) Bacteroides bile esculin agar (BBE)

↓

Single colony of each distinct type

↓

Aerotolerance testing

↓ -ve → Aerobic (CA) incubation 5-10% in candle jar

↓

Purity blood agar plate

↓

Add antibiotic Identification discs	**Perform**	**Record**
	1. Gram stain	1. Colony Morphology
	2. Spot indole test	2. Pigment
- Sodium polyanethol Sulphonate (SPS) disc	3. Catalase test	3. Haemolysis
		4. Fluorescence
- Nitrate disc		5. Pitting
- Susceptibility to Kanamycin (1mg) Vancomycin (5μg), Colistin (10μg)		

1) Blood agar was supplemented with Haemin (5mcg/ml) and Vit K (10mcg/ml). Blood agar plates used for anaerobic isolation were prepared with Brucella agar base. It is a non selective media.

2) Kanamycin (75mcg/ml) Vancomycin (7.5mcg/ml) laked blood agar (KVLB).

KVLB agar inhibits the growth of most facultative bacteria and allows for earlier pigmentation of Prevotella melaninogenicus. Most Bacteroides spp. grow well on it, while Fusobacterium spp. and most gram positive anaerobes are inhibited.

Preparation:

First Brucella agar base was prepared to which 75μg/ml Kanamycin base was added and autoclaved. Vancomycin 7.5μg/ml and laked blood (5%) was aseptically added after antoclaving. Laked blood was prepared by freezing whole blood overnight and then thawing.

3) Bacteroides bile esculin agar (BBE) containing Gentamycin 100μg/ml, 20% bile, 0.1% esculin, 0.05% ferric ammonium citrate (HI MEDIA) was used.

BBE agar is useful for rapid detection and isolation of members of the B. fragilis group. Members of the B. fragilis group grow well, producing dark colonies with brown to black halos[49].

The method used for obtaining anaerobiosis in the jar was "internal gas generating system" described by lakshminarayana and Vaidhyalingam.

Procedure in brief is as follows,

Catalyst:

Palladium pallets reactivated every time before use by drying at 150^0C - 160^0 C for 1-2 hrs.

Indicator

Methylene blue prepared by mixing equal volumes of,

a. 6% glucose containing 1mg/ml thymol as preservative

b. Sodium hydroxide solution prepared by diluting 6ml of 0.1 N NaOH to 100ml of distilled water.

c. Methylene blue prepared by adding 3ml of 0.5% w/v solution of methylene blue to 97ml of distilled water. Mixture is placed in anaerobic jar after it is made colourless by heating in a boiling water bath.

Principle

In this system the hydrogen and CO_2 gas mixture required for creating anaerobiosis is obtained from the following reactions.

(1) Citric acid + Sodium bicarbonate $\rightarrow$

$C_6H_8O_7 + 3NaHCO_3$

Sodium citrate + Carbon dioxide

$Na_3(C_6H_5O_7) + 3H_2O + 3\ CO_2$

(2) Sodium borohydride + water $\rightarrow$

$NaBH_4 + 2H_2O$

Sodium metaborate + Hydrogen

$NaBO_2 + 4H_2$

Operation of the gas generator

a) 1g sodium borohydride was taken in 30 ml test tube

b) 1g sodium bicarbonate and 1g citric acid were taken in the 5 ml test tube, which was placed inside the 30 ml test tube.

c) The stem of 20 ml funnel was plugged lightly with cotton to control the flow of water. The funnel was placed in 30 ml test tube in such a way that the stem of the funnel dips into 5 ml test tube. Entire unit was kept inside the jar with the

indicator. 20ml of distilled water was poured in the funnel just before closing the lid of the jar.

The water poured into the funnel drips into the 5 ml test tube librating CO_2. CO_2 being heavier stays within displacing the air. Once the 5 ml test tube is filled with water, it overflows into the 30 ml test tube liberating hydrogen, which being lighter gas, rushes out with CO_2. The palladium catalytically reduces the oxygen present within the jar to form water. Catalyst is exothermic, so warming of the lid of the jar can be felt[50].

After 72 hours of incubation at 37^0C anaerobic jar was opened. The plates were examined for the presence of colonies. When the colonies appeared on the anaerobic plates each predominant distinct colony was subcultured to purity blood agar plate (BAP). From a pure culture on a BAP, following was recorded,

- Colony morphology, including size of colony, shape, color, internal appearance (such as speckling) and general appearance (eg: mucoid transparent, opaque)
- Pigment
- Haemolysis
- Fluorescence
- Pitting

Single colony of each distinct type was plated on to blood agar plates with antibiotic identification discs.

- Sodium polyanethol sulphonate (SPS) disc for rapid presumptive identification of Peptostreptococcus anaerobius.

- The 3 antibiotic discs Kanamycin 1 mg, Colistin 10μg and Vancomycin 5μg were placed on the first quadrant of the purity BAP, which aid in preliminary grouping of anaerobes and serve to verify the Gram's stain.
- A nitrate disc was placed on the 2nd quadrant for subsequent determination of nitrate reduction.

Chocolate agar plate was inoculated for incubation in candle jar at 37^0C to test for aerotolerance.

If there was no growth on plates after 72 hours of anaerobic incubation, the plates were reincubated for an additional period of 48 hours and for a maximum period of 1 week.

The following tests were done from the purity plate,

Catalase test : Growth was removed from blood agar plate to a drop of 15 % hydrogen peroxide on a glass slide and observed for evolution of bubbles.

Spot Indole test : A loopful of growth from a pure culture on a blood agar plate was removed and this growth was smeared on filter paper that has been saturated with 1 % paradimethylaminocinnamaldehyde in 10 % (V/V) concentrated hydrochloric acid. A positive reaction was indicated by the rapid development of blue colour around the growth. Negative reaction gave no color change or a pinkish color.

Nitrate test : This test was done using nitrate discs. The disc was removed from surface of plate and placed in a clean petridish. One drop each of reagents A and B were added. Development of pink to red color indicated nitrate had been reduced to nitrite. If no colour developed in a few minutes, a small amount of zinc dust was added and waited for 5 minutes. Development of red colour indicated that nitrate was not reduced. If no colour developed it was taken as positive test[49].

Nitrate reagents

Solution A

Sulfanilic acid	0.5g
Glacial acetic acid	30.0ml
Distilled water	120.0ml

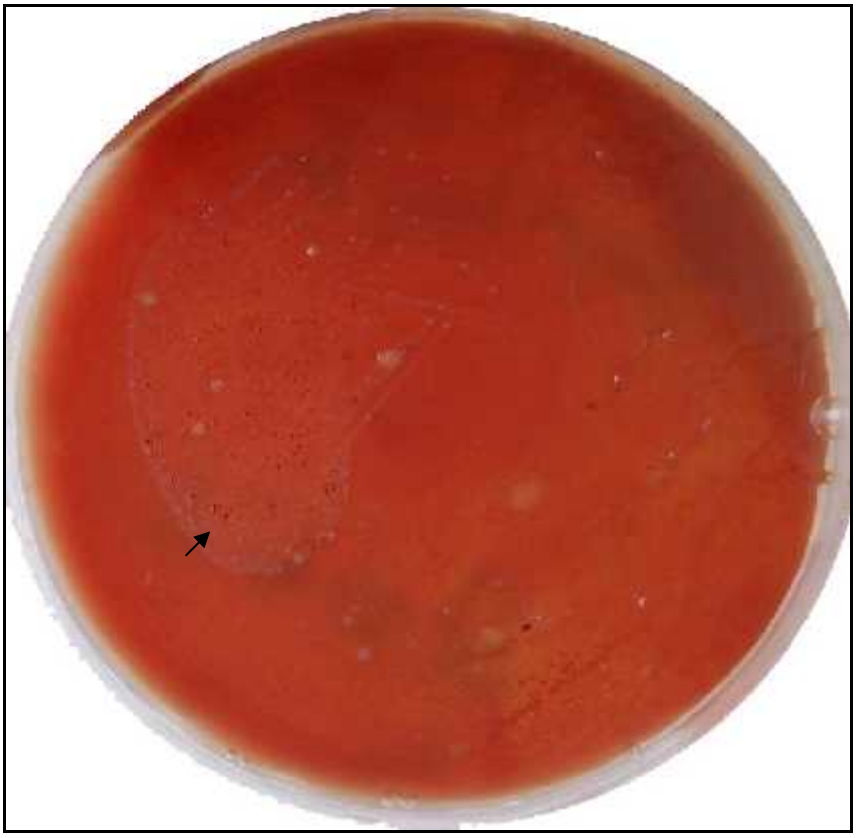
Solution B

1,6-Cleve's acid	0.2g
(5-amino-2-naphthalenesulfonic acid)	
Glacial acetic acid	30.0ml
Distilled water	120.0ml

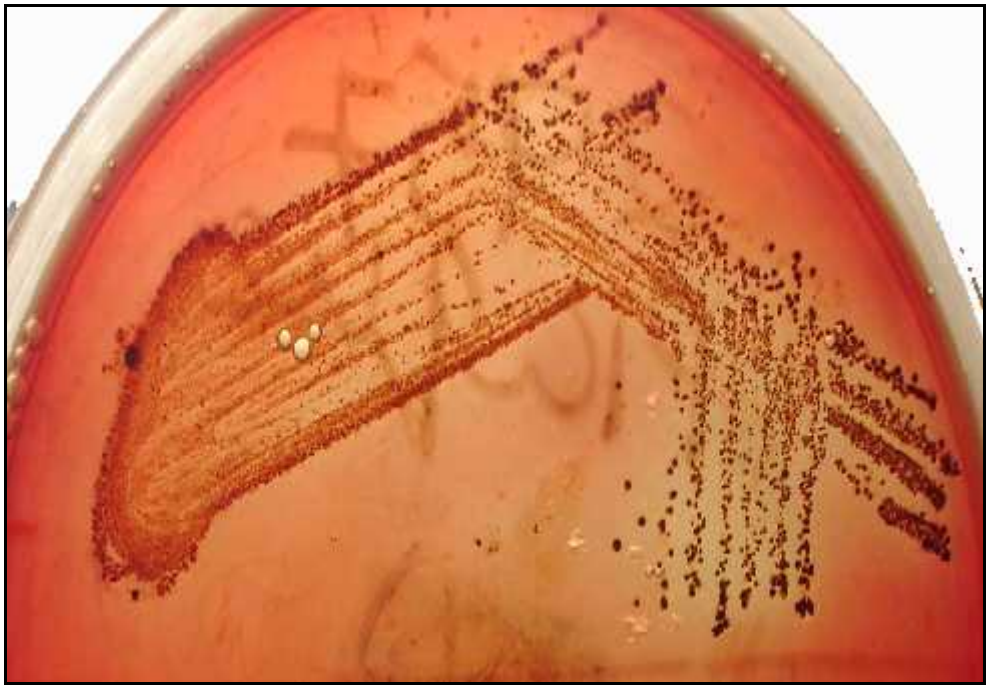
Photograph No. 1: McIntosh Fildes Jar

Photograph No. 2: Growth on primary blood agar plate showing Prevotella spp.

Photograph No. 3: Growth on primary blood agar plate showing Porphyromonas spp.



Photograph No. 4: Growth of Prevotella spp. on blood agar

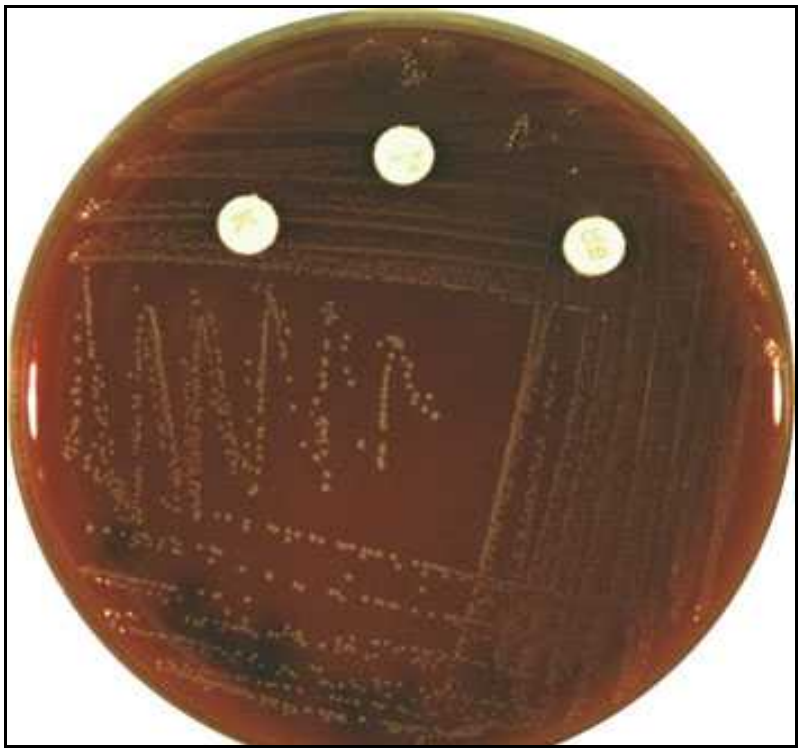


Photograph No. 5: Prevotella spp. showing sensitivity to Colistin disc

Photograph No. 6: Bacteroides fragilis on blood agar

Photograph No. 7: Bacteroides fragilis on BBE agar

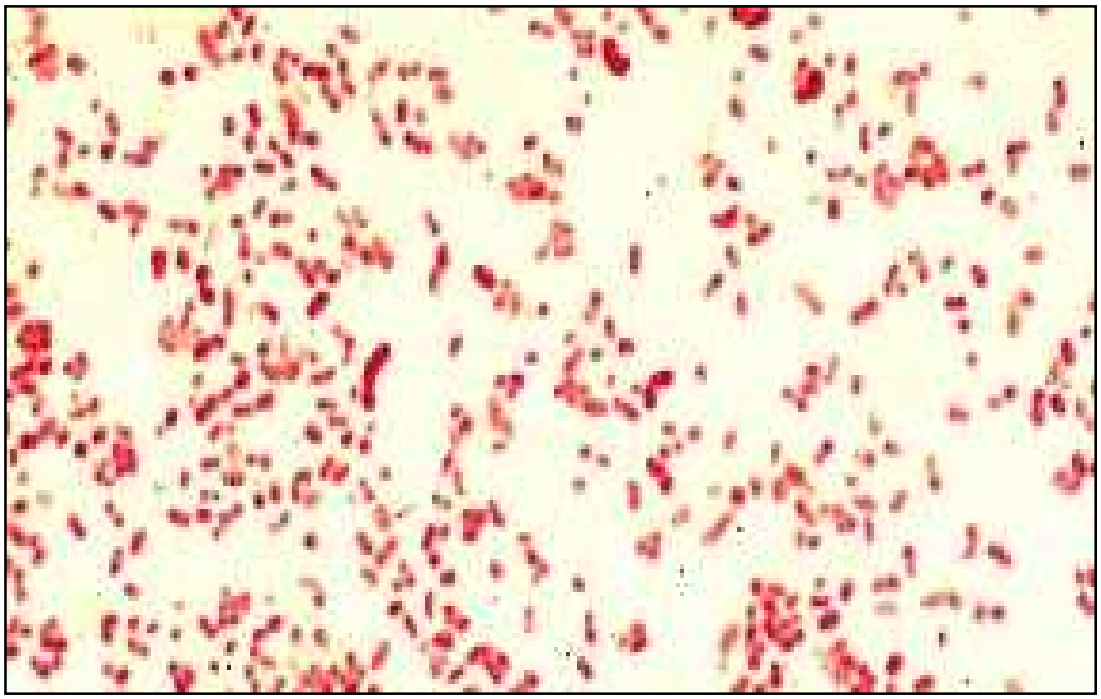
Photograph No. 8: Bacteroides fragilis showing resistance to Kanamycin, Vancomycin & Colistin discs



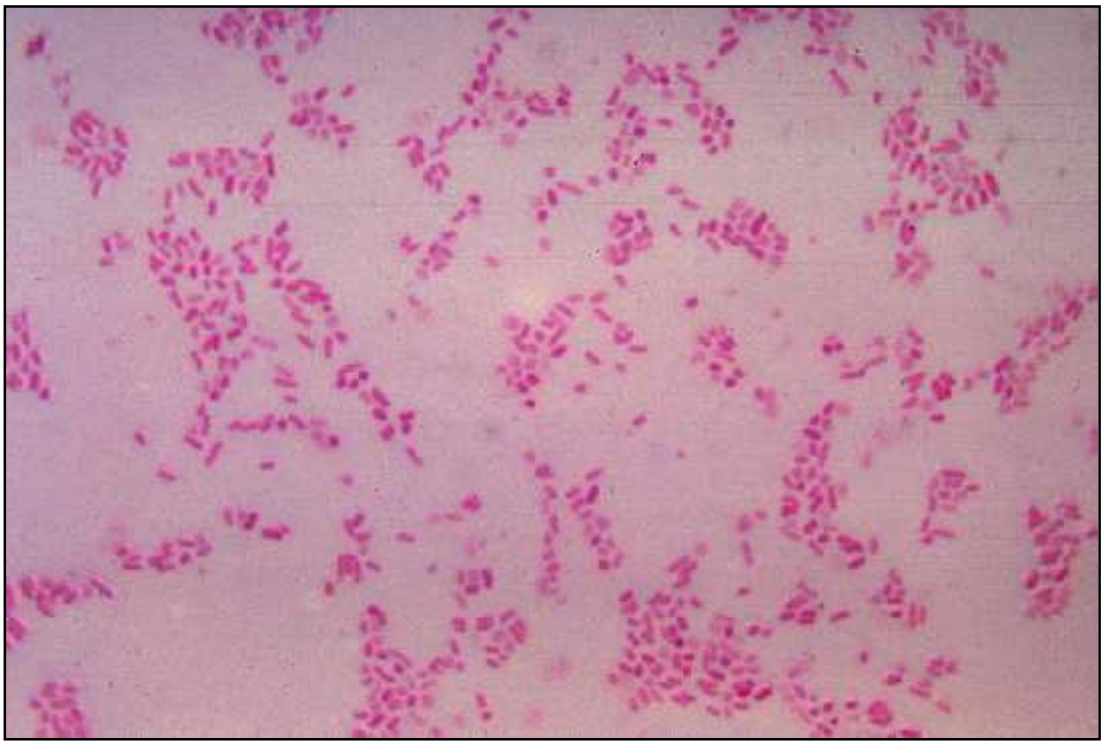
Photograph No. 9: Bacteroides urealyticus on KVLB agar

Photograph No. 10: Peptostreptococcus anaerobius showing sensitivity to SPS disc on blood agar

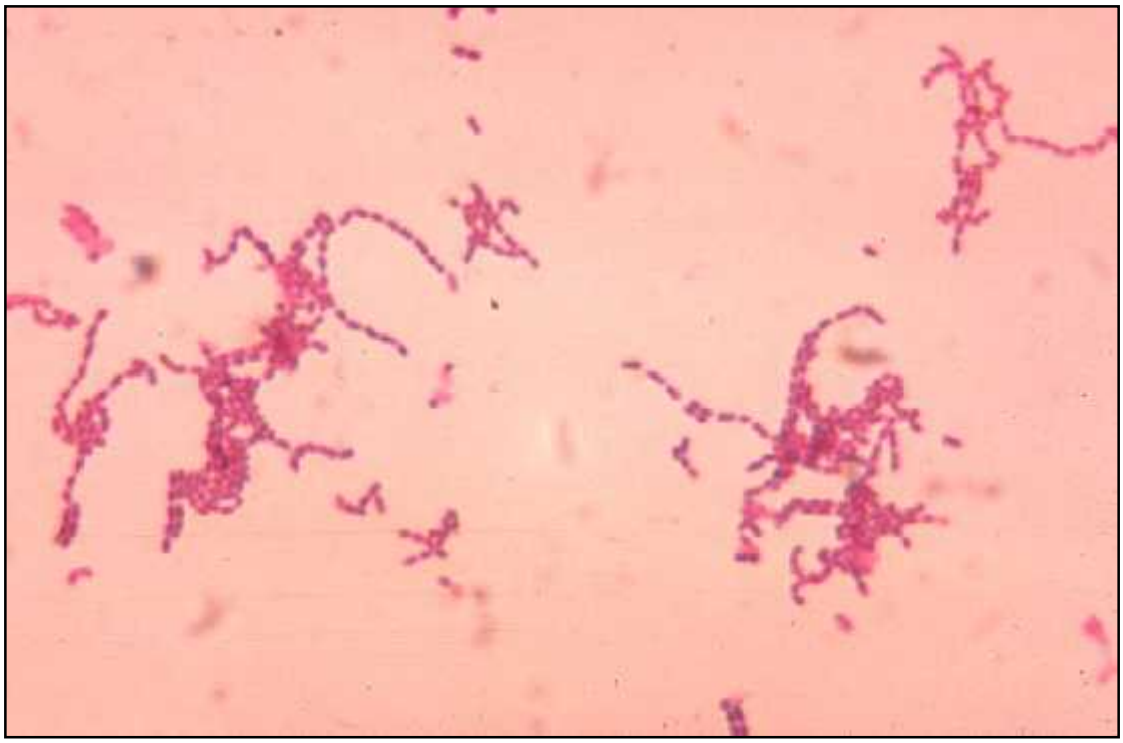
Photograph No. 11: Gram's stain showing pleomorhic GNB of Prevotella melaninogenica



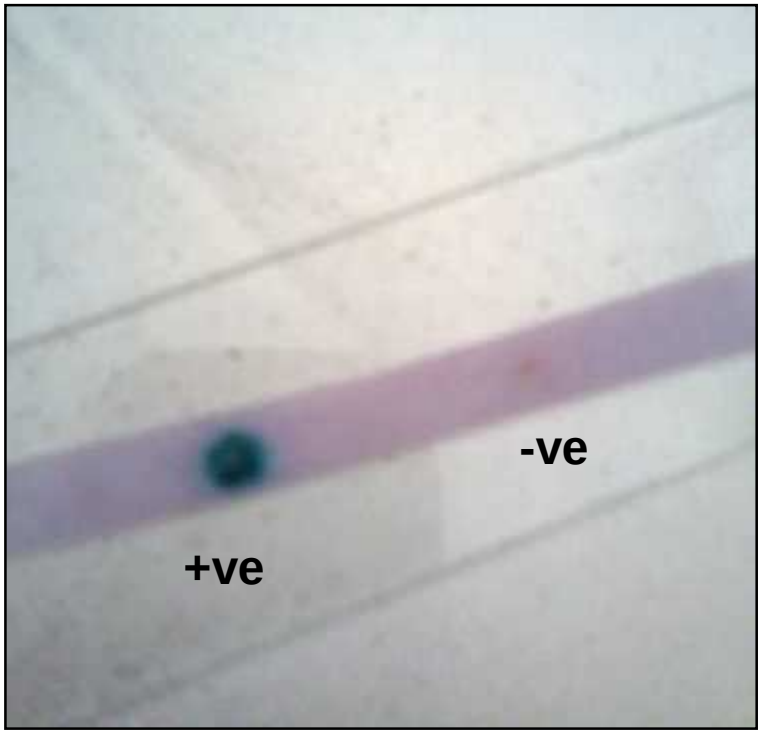
Photograph No. 12: Gram's stain showing GNB of Bacteroides fragilis



Photograph No. 13: Gram's stain showing GPC of Peptostreptococcus anaerobius



Photograph No. 14: Spot indole test



RESULTS

Table 1: Age and Sex distribution of Chronic Sinusitis (n=50)

Age (years)	Male		Female	
	Number	Percentage	Number	Percentage
20-30	11	22	6	12
31-40	13	26	7	14
41-50	7	14	4	8
51-60	2	4	-	-
Total	**33**	**66**	**17**	**34**

The table shows that the disease is more common in males between the age groups 31-40.

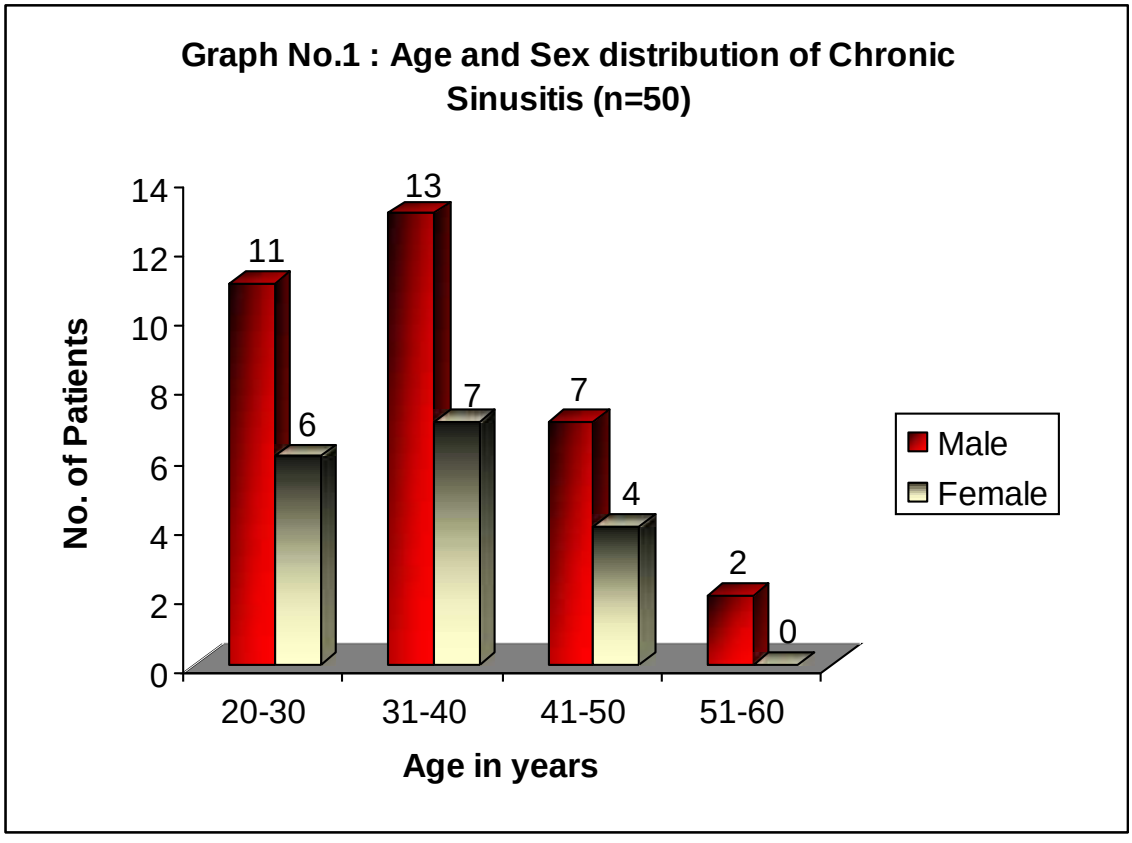


Table 2 :Predisposing factors existing in chronic sinusitis cases.

	Predisposing conditions	Number	Percentage
1.	Dental disease	8	16
2.	Obstructive conditions (eg: Deviated nasal septum, nasal polyps)	5	10
3.	Allergic rhinitis	2	4
4.	Upper respiratory tract infection	4	8
5.	Other allergic states	2	4
6.	No predisposing conditions	29	58

The table shows that dental disease and obstructive conditions (DNS, nasal polyps) are common predisposing conditions for chronic sinusitis.

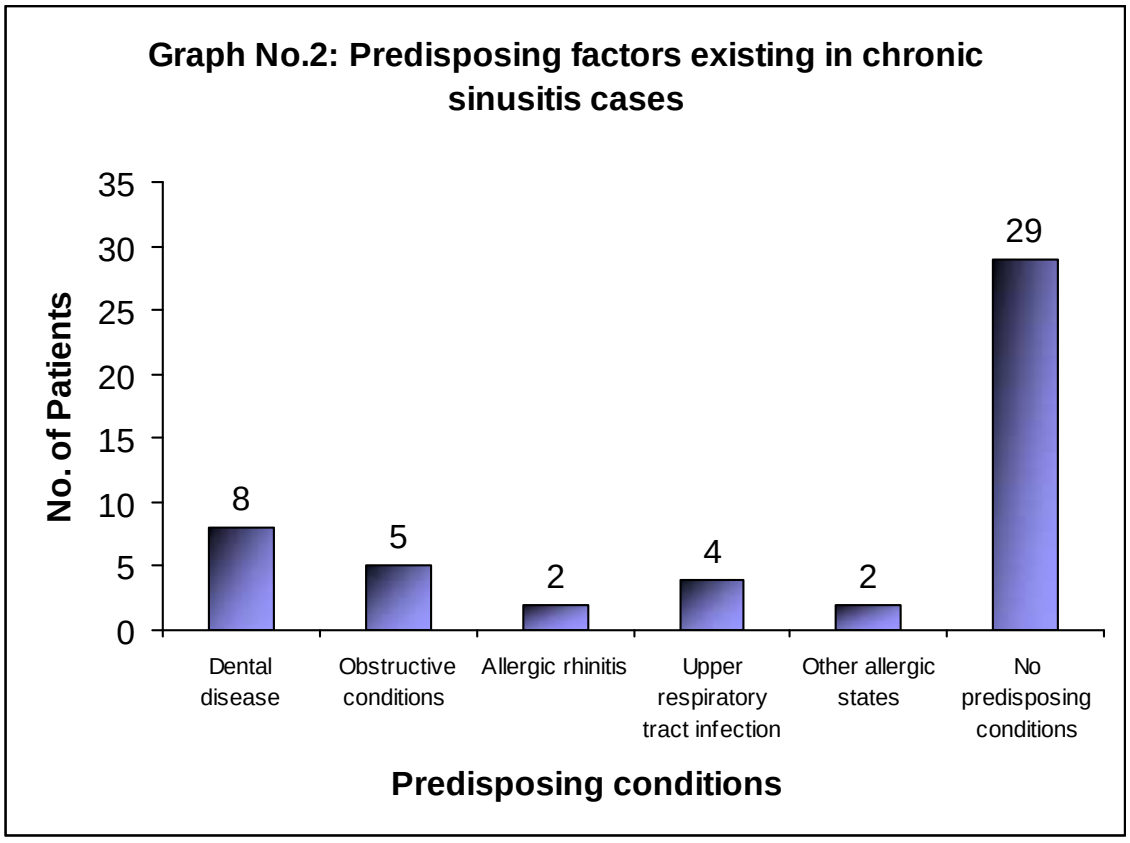


Table 3 : Common clinical symptoms in study subjects (n=50)

	Symptoms	Number	Percentage
1.	Facial pain (pain in the region of cheeks)	30	60
2.	Headache/Heaviness of head	10	20
3.	Nasal obstruction	27	54
4.	Nasal discharge	16	32
5.	Fever	3	6
6.	Other constitutional symptoms	10	20

Table shows that the commonest symptom is facial pain followed by nasal obstruction and nasal discharge.

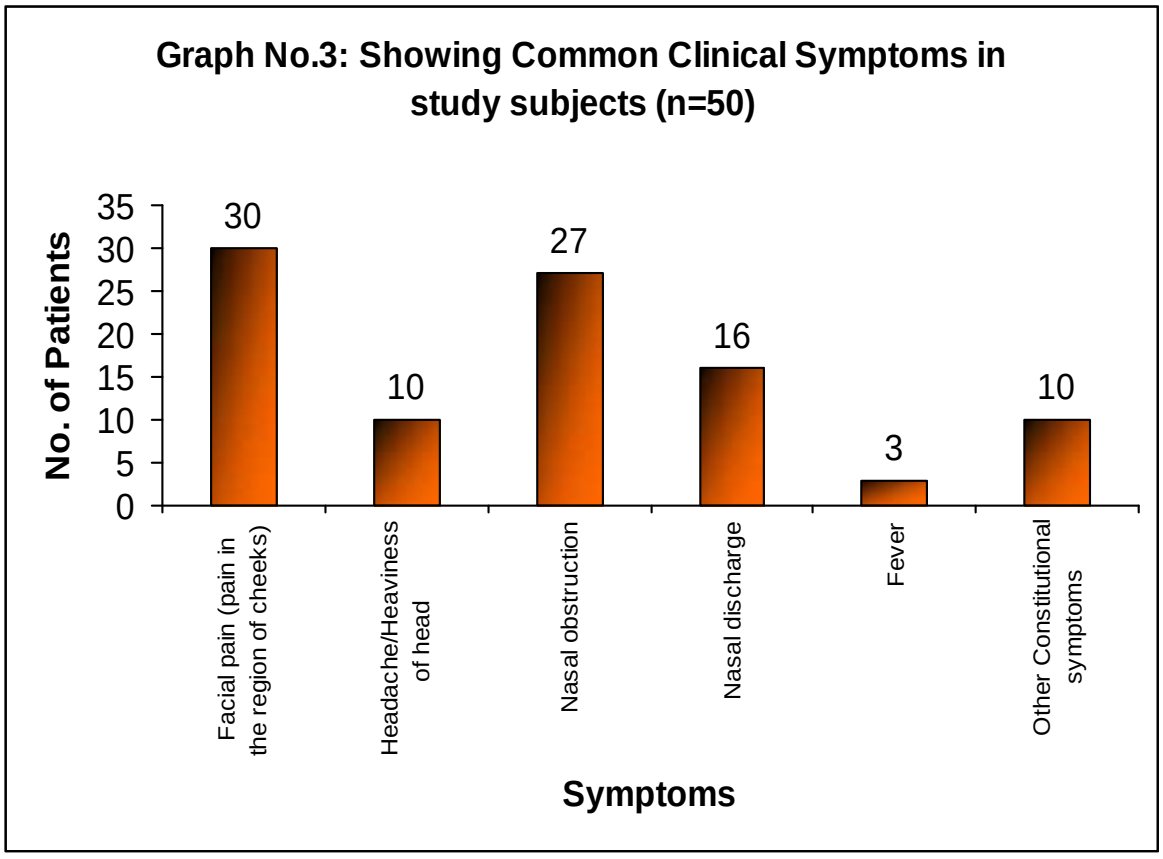


Table 4 : Distribution of types of sinusitis in study subjects (n=50)

	Sinusitis	Number	Percentage
1.	Chronic maxillary sinusitis	46	92
2.	Chronic frontal sinusitis	4	8

Graph No.4: Distribution of types of sinusitis in study subjects (n=50)

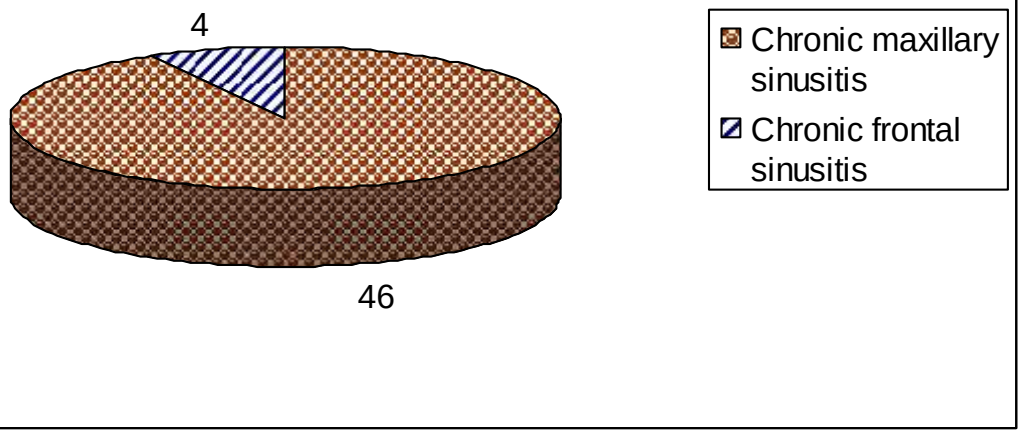


Table 5 : Results of Bacteriological culture in Chronic Sinusitis Cases (n=50)

	Types of isolates	Number	Percentage
1.	Pure aerobic and facultative anaerobic growth	17	34
2.	Pure anaerobic growth	9	18
3.	Mixed aerobic and anaerobic growth	7	14
4.	No growth	17	34

Graph No.5: Results of Bacteriological culture in Chronic Sinusitis Cases (n=50)

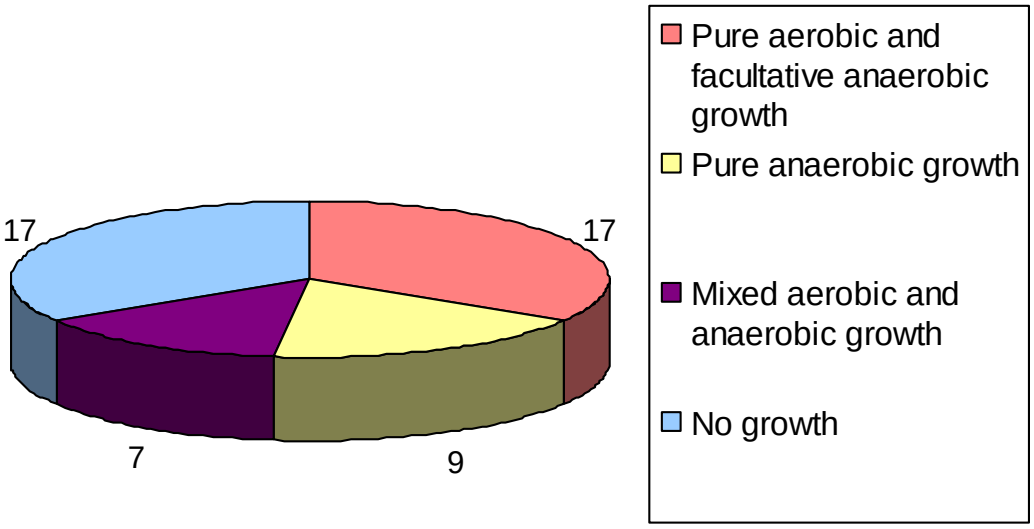


Table 6 : Bacterial isolates from cases of chronic sinusitis (n=50)

Bacteria	**Number**	**Percentage**
Anaerobic Bacteria		
Prevotella intermedia	4	8
Bacteroides fragilis	4	8
Prevotella loeschii	2	4
Prevotella melaninogenicus	1	2
Peptostreptococcus anaerobius	2	4
Porphyromonas species	2	4
Bacteroides urealyticus	2	4
Aerobic Bacteria		
Staphylococcus aureus	17	34
Klebsiella pneumoniae	3	6
Escherichia Coli	2	4
Coagulase negative Staphylococci	2	4

The above table shows that Staphylococcus aureus is the commonest isolate in aerobic organisms and Prevotella spp were the commonest isolates in anaerobic organisms followed by Bacteroides spp.

Table 7 : Antibiotic Sensitivity of the Aerobic & Facultative Anaerobic Organisms

Bacteria / **Antibiotics**	**S. aureus**		**CoNS**		**K. pneumoniae**		**E.coli**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Cefatoxime(Ce)	14	82	2	100	2	66	2	100
Amikacin(Ak)	9	52	2	100	3	100	2	100
Erythromycin(E)	6	35	1	50	1	3	1	50
Ciprofloxacin(Cf)	10	58	2	100	2	66	1	50
Cotrimoxazole(Co)	8	47	1	50	3	100	2	100
Amoxyclav(Ac)	10	58	2	100	2	66	1	50
Oxacillin(Ox)	11	64	2	100	-	-	-	-

No.: Number of isolates sensitive

%: Percentage of isolates sensitive

Table shows that most of the isolates were sensitive to cefatoxime, amikcacin and ciprofloxacin and methicllin sensitive Staphylococcus were seen in 64% of Staphylococcus aureus and 100% of CoNS.

DISCUSSION

The present work was undertaken to study the bacteriology of chronic sinusitis and the antibiotic sensitivity pattern of aerobic organisms.

Totally 50 patients with chronic sinusitis undergoing FESS during the period between Jan 2007 to Jan 2008 in ENT department of KLE's Prabhakar Kore Hospital and MRC Belgaum were included in the study. The pus samples collected during the surgery were studied for the isolation of organisms and sensitivity to easily available and commonly used antibiotics in the department of Microbiology, J.N. Medical College.

The age distribution in chronic sinusitis documents that the disease is more common in the third and fourth decades of life and in males compared to females[7,9,21]. However few studies reveal that chronic sinusitis can occur in any age group[35].

In the present study, a high incidence of chronic sinusitis was seen in the age group of 31-40 years, accounting for 13 (26%) of cases followed by 11(22%) cases in second decade of life. A least incidence was seen above the age of 50 years i.e. 2 cases (4%). Out of 50 cases of chronic sinusitis, there was a male preponderance accounting for 33 (66%) cases and females accounted for 17 (34%) cases.

Melen and colleagues observed that 40% of patients with chronic maxillary sinusitis in their study had a dental cause as a predisposing factor. Marginal periodonitis and periapical granuloma together accounted for 83% of their chronic odontogenic sinusitis[24]. Daley CL, describes obstruction or reduction in the patency of ostia as the most important predisposing factors. The size of the ostia decreases in

recumbency. In addition, mucosal inflammation and anatomic abnormalities (eg: DNS, nasal polyps, etc) can influence ostia size and predispose to sinusitis[51].

In our study dental infection was the most common predisposing condition accounting for 8 (16%) cases followed by obstructive conditions (DNS – 3 cases, nasal polyps- 2 cases) in 5 (10%) cases. However no predisposing conditions were seen in 29 (58%) of the cases. Other predisposing conditions like allergic rhinitis and upper respiratory tract infections were seen in 2 cases (4%) each. They seem to be less important in chronic sinusitis when compared to acute sinusitis.

The symptoms of chronic sinusitis are more or less similar to acute sinusitis but often less specific. Most of the patients usually present with facial pain or nasal obstruction which is maximal in the morning hours. Headache or heaviness of the head is more common in frontal sinusitis, that too in acute than in chronic. Fever is usually absent or low grade. Other frequently presenting complaints can be non-specific symptoms like malaise, generalized body weakness, post nasal discharge. Mucopurulent discharge presents frequently with acute exacerbation[52].

In our study, facial pain was the most common clinical symptom in chronic maxillary sinusitis cases observed in 30 (60%) of cases followed by nasal obstruction in 27(54%) of cases. Headache was the presenting complaint in 2 of the 4 chronic frontal sinusitis cases and few of the maxillary sinusitis cases i..e 10 (20%). Nasal discharge was seen in 16 (32%) of cases. Some patients had vague constitutional symptoms like malaise, altered sense of smell and taste. Low grade fever was seen in 3(6%) of patients.

The bacteria that are implicated in the causation of chronic sinusitis are varied and include a wide variety of aerobes, facultative anaerobes and obligate anaerobes.

The bacteriological study of antral aspirates of different authors has yielded culture positivity rates ranging from 54 to 92% [6,9, ,30, 32].

In our study the culture positivity rate was 66%. These findings are in accordance with findings of the above studies.

The isolation rate of aerobes and facultative anaerobes varied from 12% to 52% in different studies. Archana Thakur et al[31] documents an isolation rate of aerobes in 34% of cases followed by 39% by Karma et al[25], 44% by Chen Liu et al[53] and 52% by Hartog B et al[32]. A low isolation rate of 12% and 18% were documented by Erkan et al[30] and Itzhak Brook et al[7] .

Many authors have stressed the importance of anaerobes in chronic sinusitis[9, 16, 21, 32, 36]. Mixed growth was obtained in 36% by Erkan et al [30], 32% of cases by Itzhak Brook et al[7].

In our study of 50 cases of chronic sinusitis, 46 cases were of chronic maxillary sinusitis and in these pure aerobic growth was obtained in 16 (32%) cases. Pure anaerobic growth in 8 (16%) cases and mixed aerobic and anaerobic in 7 (14%) cases. In 4 of the frontal sinusitis cases one case showed only aerobic growth and another case only anaerobic growth. The other two cases did not yield any growth. Overall in 50 cases of chronic sinusitis anaerobic organisms were isolated in 16 (32%) of cases. This study clearly demonstrates the importance of anaerobic bacteria in the causation of chronic sinusitis and correlates with the studies of the above authors.

Among the aerobes, Staphylococcus aureus was the predominant organism isolated in various studies[6 ,29, 30,]. Alpha haemolytic Stretococci were the major organisms isolated in studies by Karma et al[25], Sener et al[41] and Itzhak Brook[2].

Coliforms were isolated in as high as 52% of cases of chronic maxillary sinusitis by Archana Thakur et al[31] of which Escherichia Coli was isolated in 11%, Klebsiella spp in 14% and Proteus spp in 18% of cases. Rajiv Arora et al[36] isolated Escherichia Coli in 40% of cases. Streptococcus pneumoniae was isolated in less number of chronic cases as compared to acute case as evidenced by its less frequency of isolation in various studies[6, 22, 29]. Other organisms isolated in various studies were Coagulase negative Staphylococci, Streptococcus, Haemophilus influenzae etc.[1, 2, 4,]. Pseudomonas aeruginosa is an important pathogen in chronic maxillary sinusitis of nosocomial origin and in patients suffering from cystic fibrosis[45].

In frontal sinusitis the rate of isolation of aerobic and anaerobic organisms and type of organisms isolated are similar to chronic maxillary sinusitis[9, 54].

Anaerobic bacteria have been implicated as significant pathogens in chronic sinusitis. They may alone or in combination with aerobes may lead to suppuration of sinuses causing considerable morbidity. Many studies have documented Peptostreptococcus spp as predominant anaerobe involved in the causation of chronic sinusitis. Erkan et al[30] isolated it in 72% of cases, Brook I et al[7] in 60% of cases, Frederick et al[6] in 34% of cases. Bacterioides spp was the next common isolate in many studies[6, 21, 31], but it was the predominant isolate in the study of Itzhak Brook[21]. Prevotella spp was the major anaerobic bacteria in the studies by Rajiv Arora et al[36] and Itzhak Brook et al[27]. Other anaerobes not infrequently isolated include Propioni-bacterium spp, Fusobacterium spp, Porphyromonas spp[7, 16, 30].

In our study among aerobic and facultative organisms, Staphylococcus aureus was the predominant organism isolated in 17(34%) of cases. The frequency of isolation is slightly higher when compared to the frequency rates described in

literature. The next common aerobic organism isolated was Klebsiella pneumoniae in 3 (6%) cases and Escherichia coli and Coagulase negative Staphylococci in 2 cases (4%) each. No other aerobic organisms were isolated which is in slight discord with other studies in literature were many different aerobic organisms were isolated[7, 30, 32].

The most common anaerobic organism isolated in our study was Prevotella spp, in 7 (14%) cases which correlates well with studies by Rajiv Arora et al[36] and Itzhak Brook et al[27]. Among the Prevotella spp, P. intermedia was the commonest isolate in 4 (8%) cases followed by P. loeschi in 2 (4%) and P. melaninogenicus in 1 (2%) case. Other anaerobic organisms isolated were Bacteroides fragilis in 4 (8%) cases, followed by Bacteroides urealyticus, Porphyromonas spp and Peptostreptococcus anaerobius in 2 (4%) cases each.

Antibiotic sensitivity testing of aerobic and facultative anaerobic isolates revealed that most bacterial isolates were sensitive to cefatoxime followed by to ciprofloxacin, amikacin, erythromycin and amoxyclav. Of the 17 isolates of Staphylococcus aureus, 5 (30%) strains were methicillin resistant. These strains were also seen to be resistant to most of the other antibiotics used.

Antibiotic sensitivity testing of anaerobic organisms was not carried out in this study. As anaerobic organisms are becoming increasingly resistant to commonly used antibiotics like metrinidazole, clindamycin etc, it becomes imperative that antibiotic susceptibility be carried out for all isolates to properly target these anaerobic organisms in the infection. Appropriate medical therapy during the initial phase of infection will reduce the likelihood of complications of sinusitis and thereby decrease the morbidity and mortality of this potentially life threatening infection.

Because quantitative culture of the specimens is helpful in distinguishing the small number of organisms resulting from specimen contamination and the large number of organisms consistent with sinus infection and because heavy growth in primary plate is regarded as a sign of causal relationship between the inflammatory process and the bacterial finding, further studies should include quantitative culture of the flora, and analysis of a control group would help in the interpretation of the clinical significance in this area.

SUMMARY

- Pus samples from 50 cases of chronic sinusitis undergoing FESS at the ENT department of KLE's Prabhakar Kore and MRC Belgaum were collected and processed in the department of Microbiology, JNMC, Belgaum.
- Age distribution shows that the disease is more common in the second and third decades of life with a male preponderance.
- Dental infections (16%) and obstructive conditions (10%) were the major predisposing factors. More than half of the patients had no predisposing conditions.
- The clinical symptoms commonly encountered were facial pain (60%), nasal obstruction (50%), nasal discharge (36%) and headache (20%).
- Aerobes and facultative anaerobes were the commonest isolates in 24 (48%) of the cases. Anaerobic growth was seen in 16 (32%) of cases. No growth was seen in 17 (34%) cases.
- Among aerobic organisms the commonest isolate was Staphylococcus aureus in 17 (34%) cases.
- In anaerobic bacteria Prevotella spp were the commonest isolates in 7 (14%) cases, of which Prevotella intermedia was the commonest species isolated. Bacteroides spp were the next common isolate in 6 (12%) cases, of which Bacteriodes fragilis was the commonest.
- Chronic maxillary sinusitis was the commonest sinusitis encountered in 46 cases (92%).
- Antibiotic susceptibility of aerobic organisms showed cefatoxime, ciprofloxacin and amikcacin as most effective antibiotics. MRSA was detected in 5 cases (30%) out of 17 cases of Staphylococcus aureus.

CONCLUSION

This study has shown that anaerobes are an important cause of chronic sinusitis along with aerobic and facultative anaerobic organisms and therefore their role should not be ignored in chronic sinusitis and an attempt should always be made to isolate the anaerobic organisms and their antibiotic susceptibility should be determined to start appropriate antibiotic therapy as early as possible to overcome the morbidity and mortality associated with this disease and further to prevent the development of resistant strains.

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ANNEXURE - I
PROFORMA

Name: Age: Sex:

Date: Hospital No.:

I Presenting Complaints:

- Fever:
- Headache:
- Facial pain:
- Foul smelling discharge:
- H/O trauma:
- Nasal congestion:

II Past history:

- Duration of illness / no. of episodes per year:
- Has the patient undergone any procedure?

Diagnostic:

Curative:

III H/O antibiotic intake:

IV Any other associated illness:

V Diagnosis:

VI Specimen:

- Direct- Macroscopy:
- Microscopy:

Aerobic culture

Growth on Mac Conkey agar:

- 5% BA:
- CA:

Gram stain of individual colonies:

Processing :

- Indole Test:
- H_2S production:
- Urea hydrolysation:
- Citrate utilization:
- Mannitol fermentation:
- TSI media:

Tests:

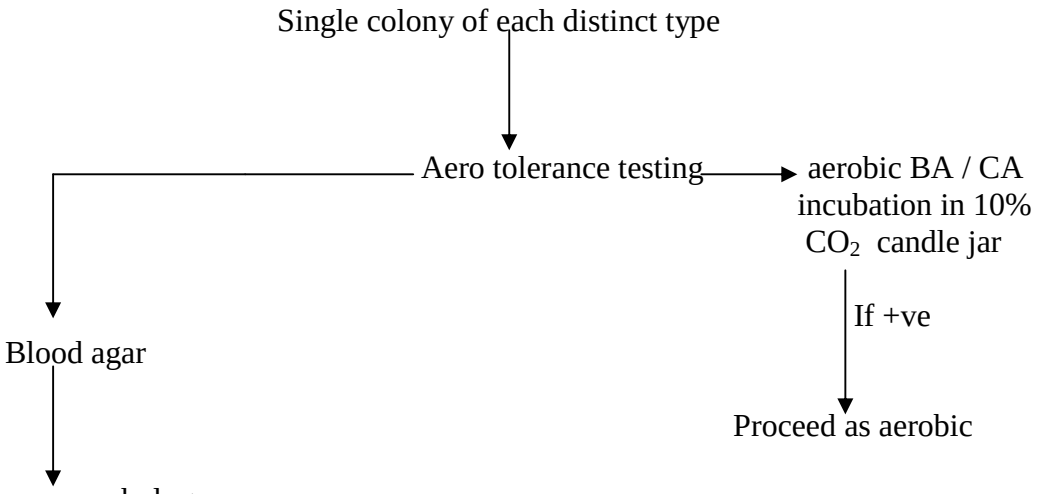
- Oxidase test:
- Catalase test:
- Coagulase test- Slide:
  Tube:
- Motility:
- OF:

Antibiotic sensitivity testing:

- Ciprofloxacin (Cf):
- Erythromycin(E)
- Amoxycillin / Clavulanic acid (Ac):
- Cefotaxime (Ce):
- Amikacin (A):
- Oxacillin(Ox)

Anaerobic culture

- Growth on blood agar supplemented with haemin & vitamin K:
- KVLB:
- BBE:



- Colony morphology:
- Pigment:
- Haemolysis:
- Fluoresence:
- Pitting:
- Antibiotic identification disc- SPS disc:
- Gram stain of individual colonies:
- Spot indole test:
- Catalase test:
- Nitrate test:
- Susceptibility to- Vancomycin:
  - Kanamycin:
  - Colistin:

ANNEXURE - II
CONSENT

Mr./Mrs. _____ we are requesting you to enroll yourself in a study titled "Bacteriological study of chronic sinusitis with special reference to anaerobes" conducted by Dr. D. E. Premalatha, postgraduate student in Microbiology under the guidance of Dr. Jyoti M. Nagmoti at J.N.M.C., Belgaum under KLE Academy of Higher Education and Research Centre, Belgaum.

You have been requested to participate in research because you are into the study group. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with J.N.M.C. If you decide to participate you are free to withdraw at any time.

The purpose of research is to isolate and identify the aerobic and anaerobic bacteria from clinically diagnosed cases of chronic sinusitis and to carry out antibiotic susceptibility testing for aerobic isolates.

PROCEDURE INVOLVED

Microbiological study of the material obtained during FESS will be done to detect the aerobic and anaerobic bacteria causing chronic sinusitis.

RISK AND BENEFITS

There are no extra risks involved and benefits are to be evaluated.

PRIVACY AND CONFIDENTIALITY

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except:

1. In emergency to protect your rights and welfare.
2. If required by law.

AUTHORIZATION TO PUBLISH RESULTS

When the results of the research are published or discussed, in a conference, no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not be paid/offered any free gifts for participating in the research. You will not be reimbursed for expenses.

CONSENT STATEMENT

I undersigned _____ have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions related to the study, you can contact Dr. Prematatha (Phone No. 9343834443).

In case you have any questions about your rights as a study participant, you can contact Dr. V. D. Patil (0831-2471350).

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name _____ Signature _____

Witness Name _____ Signature _____

Experimenter's Name _____ Signature _____

Date: _____

Place: _____

MASTER CHART

Sl. No.	Hospital No.	Age (in yrs)	Sex	Anaerobic organisms isolated	Tests										Aerobic organisms isolated	Antibiotic susceptibility						
					Gram's stain	Catalase test	Spot indole test	Nitrate test	Susceptibility				Pig production	Fluorescence	Pitting		Ce	Am	E	Ox	Cf	Co	Ac
									SPS disc	K	V	C											
1	230162	35	M	NOGC												NOGC							
2	230168	30	M	NOGC												S.aureus	S	S	R	R	S	R	R
3	281240	24	F	B. fragilis	GNB	Negative	Negative	Negative	-	R	R	R	-	-	-	NOGC							
4	284210	31	M	NOGC												NOGC							
5	394291	39	M	NOGC												NOGC							
6	367643	38	F	NOGC												NOGC							
7	332149	37	F	NOGC												NOGC							
8	333248	32	M	NOGC												S.aureus	S	R	R	R	R	R	S
9	331323	42	F	NOGC												S.aureus	R	S	R	S	R	R	S
10	540165	37	M	Porphyromonas Spp.	GN Coccobacilli	Negative	Positive	Negative	-	S	S	R	Brown	Negative	-	S.aureus	S	R	R	S	S	S	S
11	554210	37	M	B. urealyticus	GNB	Negative	Negative	Negative	-	S	R	S	Negative	Negative	Positive	S.aureus	S	R	S	S	S	S	S
12	632142	25	M	NOGC												S.aureus	S	R	R	R	S	S	R
13	641243	24	F	NOGC												NOGC							
14	659254	20	M	NOGC												NOGC							
15	659434	26	M	NOGC												NOGC							
16	659744	32	F	B. fragilis	GNB	Negative	Negative	Negative	-	R	R	R	Negative	Negative	Negative	E.coli	S	S	S	-	R	S	R
17	230450	28	F	NOGC												S.aureus	S	S	R	S	R	S	R
18	230582	45	F	NOGC												S.aureus	R	R	S	S	S	S	R
19	532840	44	M	NOGC												NOGC							

Sl. No.	Hospital No.	Age (in yrs)	Sex	Anaerobic organisms isolated	Tests										Aerobic organisms isolated	Antibiotic susceptibility						
					Gram's stain	Catalase test	Spot indole test	Nitrate test	Susceptibility				Pig production	Fluorescence	Pitting		Ce	Am	E	Ox	Cf	Co	Ac
									SPS disc	K	V	C											
20	231876	23	M	NOGC												K.pneumoniae	S	S	R	-	S	S	S
21	232393	27	M	P. loescheii	GNB	Negative	Positive	Negative	-	R	R	R	Black	Brick Red	-	S.aureus	S	S	S	S	S	R	R
22	624236	21	M	NOGC												NOGC							
23	671479	25	M	NOGC												NOGC							
24	244705	24	M	P. melaninogenica	GNB	Negative	Positive	Negative	-	R	R	S	Black	Brick Red	-	NOGC							
25	236212	45	M	P.intermedia &	GNB (beaded)	Negative	Positive	Negative	-	R	R	S	Black	Brick Red	-								
				B.fragilis	GNB	Negative	Negative	Negative	-	R	R	R	Negative	Negative	-	NOGC							
26	536504	55	M	P. intermedia	Pleomorphic GNB	Negative	Positive	Negative	-	R	R	S	Black	Brick Red	Negative	NOGC							
27	643282	38	M	B. fragilis	GNB	Negative	Negative	Negative	-	R	R	R	Negative	Negative	Negative	S.aureus	S	R	R	R	R	R	R
28	533285	32	F	B. urealyticus	GNB	Negative	Negative	Negative	-	S	R	R	Negative	Negative	Negative	NOGC							
29	634628	52	M	NOGC												E.coli	S	S	R	-	S	S	S
30	641026	42	M	NOGC												S.aureus	S	S	S	S	S	R	S
31	642204	27	M	P. loescheii	Pleomorphic GNB	Negative	Positive	Negative	-	R	R	R	Black	Brick Red	Negative	S.aureus	S	R	S	S	R	R	S
32	623346	35	M	P. anaerobius	GPC in clusters, chains	Negative	Positive	Negative	S	-	-	-	Negative	Negative	Negative	NOGC							

Sl. No.	Hospital No.	Age (in yrs)	Sex	Anaerobic organisms isolated	Tests										Aerobic organisms isolated	Antibiotic susceptibility						
					Gram's stain	Catalase test	Spot indole test	Nitrate test	Susceptibility				Pig production	Fluorescence	Pitting		Ce	Am	E	Ox	Cf	Co	Ac
									SPS disc	K	V	C											
33	723428	32	M	NOGC												S.aureus	S	R	S	S	S	S	R
34	763428	33	M	P. intermedia	Pleomorphic GNB	Negative	Positive	Negative	-	R	R	S	Black	Red	Negative	S.aureus	S	S	R	R	R	R	S
35	766734	32	F	P. anaerobius	GPC in clusters, chains	Negative	Positive	Negative	S	-	-	-	Negative	Negative	Negative	NOGC							
36	844362	44	M	NOGC												K.pneumoniae	S	S	S	-	R	S	R
37	873233	45	M	NOGC												S.aureus	R	S	R	R	S	R	S
38	882456	35	M	Porphyromonas Spp.	GNB	Negative	Positive	Negative	-	S	S	R	Brown	Negative	Negative	NOGC							
39	882611	48	F	NOGC												NOGC							
40	888201	28	F	NOGC												S.aureus	S	S	R	S	R	S	S
41	865342	30	M	NOGC												NOGC							
42	966582	28	F	NOGC												CONS	S	S	R	S	S	S	S
43	966595	32	F	NOGC												K.pneumoniae	R	S	R	-	S	S	R
44	966738	42	F	NOGC												NOGC							
45	966875	45	M	P. intermedia	GNB	Negative	Positive	Negative	-	R	R	S	Black	Brick Red	Negative	NOGC							
46	978428	25	M	NOGC												CONS	S	S	S	S	S	R	S
47	321642	32	M	NOGC												S.aureus	R	S	R	S	S	R	S
48	342621	30	F	NOGC												NOGC							
49	343623	36	M	NOGC												NOGC							
50	347628	28	F	NOGC												NOGC							

KEY TO MASTER CHART

Ac	-	Amoxyclav
Am	-	Amikacin
C	-	Colistin
Ce	-	Cefatoxime
Cf	-	Ciprofloxacin
Co	-	Co-Trimoxazole
CoNS	-	Coagulase negative staphylococcus
E	-	Erythromycin
GNB	-	Gram Negative Bacilli
R	-	Resistant
S	-	Sensitive
SPS	-	Sodium Polyanethol Sulphonate