"BACTERIOLOGICAL STUDY OF ORAL AND MAXILLOFACIAL INFECTIONS WITH SPECIAL REFERENCE TO ANAEROBES"

REG. NO. BI0111002

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka
In partial fulfillment
of the requirements for the degree of

DOCTOR OF MEDICINE (M.D)

in

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
BELGAUM - 590010.

APRIL - 2014

KLE UNIVERSITY BELGAUM,

KARNATAKA

Endorsement by the HOD, Principal/

Head of the Institution

This is to certify that the dissertation entitled **"BACTERIOLOGICAL STUDY OF ORAL AND MAXILLOFACIAL INFECTIONS WITH SPECIAL REFERENCE TO ANAEROBES"** is a bonafide research work done by **REG. NO. BI0111002**.

Dr. M. B. NAGMOTI **M.D, Ph. D**
Professor & Head
Department of Microbiology,
J. N. Medical College, Nehru Nagar,
Belgaum-590010

Date:

Place: Belgaum

Dr. A. S. GODHI **M.S.FICS**
Principal
J. N. Medical College,
Nehru Nagar,
Belgaum-590010

Date:

Place: Belgaum

LIST OF ABBREVIATION

Ac	-	Amoxyclav
Ak	-	Amikacin
Cip	-	Ciprofloxacin
E	-	Erythromycin
GNB	-	Gram negative bacilli
GPC	-	Gram positive cocci
SPS	-	Sodium polyeanethol sulfonate
Cu	-	Cefuroxime
Amp	-	Ampicillin
Pt	-	Piperacillin –tazobactam
V	-	Vancomycin
Cl	-	Colistin
K	-	Kanamycin
Mt	-	Metronidazole
Cd	-	Clindamycin

ABSTRACT

INTRODUCTION:

Most of the Oral and Maxillofacial infections are of odontogenic origin which arises as a sequel to caries, trauma and peridontitis. Morbidity seen with it shows how an ignored or ill treated decayed tooth can turn into a life threatening condition. Bacteriological flora of these infections are considered to be mixed with both aerobes and anaerobes.

OBJECTIVE:

1. To isolate and identify aerobes and anaerobes from oral and maxillofacial infections.
2. To perform antibiotic susceptibility pattern of the isolates from oral and maxillofacial infections.

MATERIALS AND METHODS:

The study was carried out from January 2012- December 2012 in the Department of Microbiology, Jawaharlal Nehru Medical College, Belgaum.

The study was conducted on 50 patients who had moderate to severe oral and maxillofacial infection with abscess in the orofacial region. Pus was aspirated and collected in freshly prepared thioglycollate broth.

Isolation, Identification of aerobes and anaerobes was done employing standard bacteriological methods. Antibiotic susceptibility of anaerobes was carried out by using agar dilution methods.

RESULTS:

Among the 50 cases, 33(66%) were females and 17 (34%) males. Submandibular space was most commonly involved 23 (46%) followed by buccal space in 10 (20%). Polymicrobial infection was present in majority of cases 36 (72%) as compared to monomicrobial 14 (28%).Mixed infection involving both aerobes and anaerobes was present in 27 (54%) cases. Out of total 41 (75.9%) anaerobic isolates, anaerobic *streptococci* was most common anaerobes (44%) followed by *Porphyromonas* (16.27%). Among aerobic isolates *Staphylococcus* aureus was the commonest aerobes 16 (39%) followed by *Streptococcus pyogens* 9 (21.9%). All anaerobic isolates were found to be sensitive to metronidazole with MIC range of 0.5-2µ/ml. While 28 (65.11%) anaerobic isolates were sensitive to clindamycin and 6 (13.9%) resistant.

CONCLUSION:

Microbiological flora of orofacial abscess consists of complex mixture of aerobic and anaerobic bacteria and polymicrobial in nature. Aerobic isolates had a high sensitivity to Ciprofloxocin, Amoxyclave and cephalosporins. All anaerobic isolates was found to be sensitive to metronidazole while 28 (65.11%) were susceptible to clindamycin.

As anaerobes are emerging as major causes of oral and maxillofacial infections, their identification and antibiotic susceptibility have assumed importance. The emergence of resistance pattern in anaerobes as reported by many studies is also reflected in this study. Hence knowing the susceptibility pattern of these pathogenic bacteria will help in appropriate and effective treatment and thereby prevent complications.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

INTRODUCTION

Oral and maxillofacial infections are among the most commonly encountered problems in dental practice. In health, oral microorganisms and human immune system are in ecological balance which is a pressure for sustaining a barrier against ingested pathogenic organism. However, pathogens can invade and initiate an infectious process when host immunity is compromised or there is increased virulence of these microorganisms

These infections are mostly of odontogenic origin, as a sequal to pulp necrosis due to caries, trauma, periodontal infections and pericoronitis because some of these infections resolve with little consequences, while some may spread to facial spaces adjacent to the oral cavity and spread aggressively leading to more severe infections [1]. If these infections are ignored or not treated properly, complications such as airway obstruction, infection of carotid sheath, meningitis, septicemia, cavernous sinus thrombosis, mediastinitis and distant metastatic foci have been reported[2] . The management of these severe infections includes determination of severity of infection, surgical treatment, & appropriate antibiotic selection for pathogens.

Oral and maxillofacial infections have overwhelmed our community constantly, despite a quest for better oral hygiene. Our knowledge of these pathogenic microorganisms and their role in disease has increased during the past decades due to better diagnostic techniques in microbiology laboratory to isolate and identify these microorganisms.

Oral and maxillofacial infections are usually polymicrobial and it is the microbial interactions of pathogenic species that cause tissue destruction. The underlying microflora of orofacial odontogenic infection predominantly involve strictly anaerobic gram positive cocci and gram negative rods, along with aerobic and facultative anaerobic bacteria[3] .

Bacteriological evaluation of these infections has shown contradictory results in relation to proportion of aerobic & anaerobic bacteria in many studies because selection, proper collection and transportation of specimens are important factors in formative the usefulness of laboratory results.

According to newer studies there is significant difference in antibiotic sensitivity pattern of microbes with time to a lesser or greater degree. Same is observed clinically while treating facial space infections in hospitals in our community. We are constantly challenged by new and improved versions of pathogens that we once subjugated and were familiar with. This alteration in antibiotic sensitivity is now the expected result of wide spread use of antibiotics in daily practices. The risk to the individual patient from a single recommendation of antibiotic is small, but altered bacterial flora represents a present and future risk of antibiotic resistance to our community. In order to achieve this, there has been a consistent effort by microbiologists to evaluate the bacterial strains and their antibiotic sensitivity pattern from time to time.

In the everyday practice of oral and maxillofacial surgeons, empirical antibiotics are prescribed in the face of uncertainty which requires the knowledge of likely pathogenic microbes that may cause orofacial infections and their antibiotic

susceptibility pattern in the confined environment as a guide for rational choice of antibiotic therapy.

Therefore this study was decided to carry out to re-evaluate the presumed pathogens involved in oral and maxillofacial infections and their susceptibility to the routinely used antibiotics in this part of the world.

OBJECTIVES OF THE STUDY

1. To isolate and identify aerobes and anaerobes from oral and maxillofacial infections.
2. To perform antibiotic susceptibility pattern of the isolates from oral and maxillofacial infections.

REVIEW OF LITERATURE

Oral and maxillofacial infection is most prevalent disease worldwide and is principle reason for seeking dental care. These infections are a public health concern and are most common in under-served patients lacking access to health care.

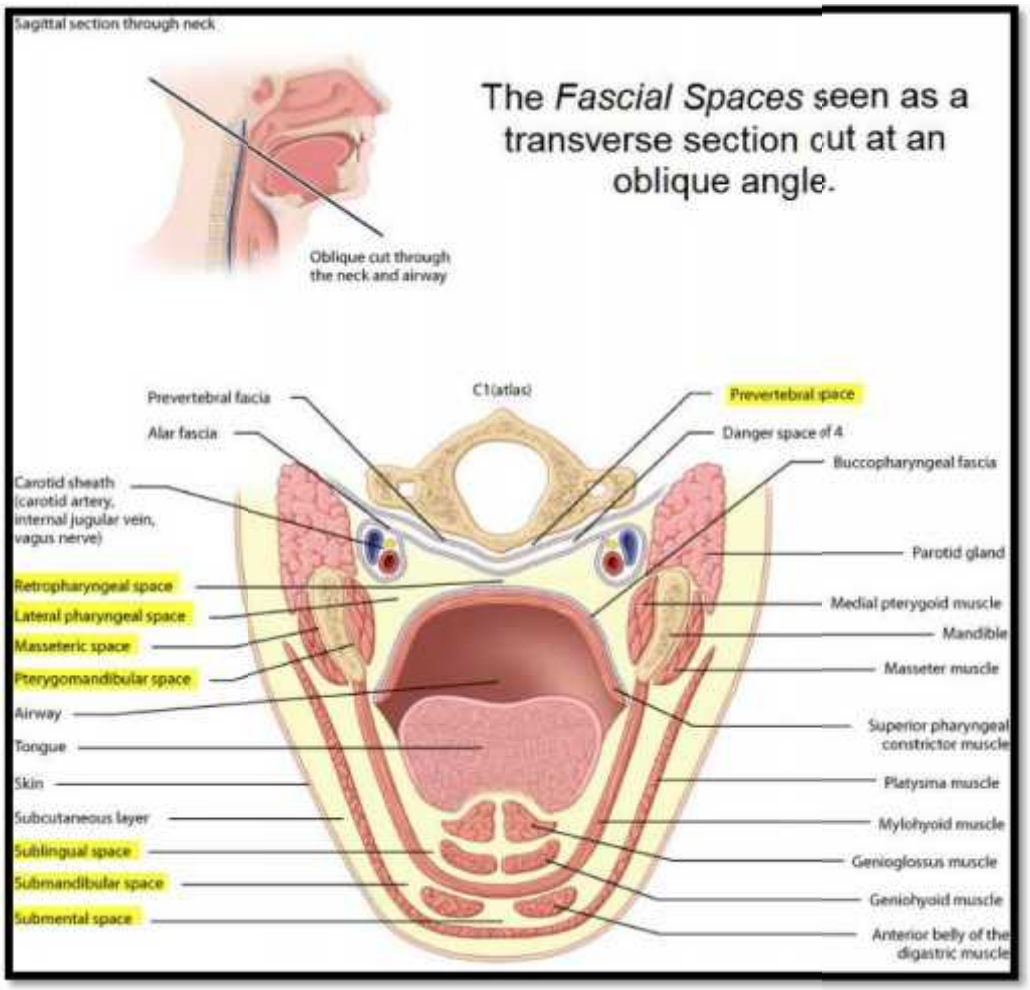
Spreading odontogenic infections are the most common type of serious oral and maxillofacial infections encountered by oral and maxillofacial surgeons. These infections range from periapical abscess to superficial and deep neck abscess.[1] Spreading odontogenic infections represent the transformation from a localized dentoalveolar infection ; usually a periradicular abscess to a destructive infection that can spread rapidly through the tissue planes, resulting in significant incidence of mortality. Infection from the original focus can spread along the tissue spaces and lead to facial cellulitis involving deeper fascial spaces. The infections generally spread by following the path of least resistance through connective tissue and along facial planes [4] .

Shapiro defined that Maxillofacial facial spaces are present between the layers of fascia .They are normally filled by loose connective tissue but when the space is invaded by infection, the connective tissue breaks down and abscess formation occurs[5]. Spread of infection from the teeth as well as associated oral tissues because the pathogenic microorganism can travel within the fascial planes, from one infected space to another distant space, via the spread of the associated inflammatory exudates.

Facial spaces are divided into primary and secondary on the basis of direct and indirect involvement of infection of the spaces from the original focus[6,7] -:

1) Primary spaces- The spaces those are directly adjacent to the origin of the odontogenic infections , which are :-
   a) Primary mandibular spaces - Submandibular , Submental and Sublingual
   b) Primary maxillary spaces - Buccal , canine and Infratemporal.

2) Secondary spaces- Fascial spaces that become involved subsequent spread of infection from the primary spaces, which are : Pterygomandibular ,Masseteric, Lateral pharyngeal , Superficial and deep temporal, Retropharyngeal, Parotid and Prevertebral space.



Anatomy of Facial spaces –

Submandibular space -

Compartment containing submandibular salivary gland and lymph nodes beneath the investing layer of deep cervical fascia and platysma and the inferior border of the mandible. Mylohyoid muscle lies superiorly, anteriorly communicates with the submental space and anteromedially with the sublingual space.

Involvement - It is involved most frequently by infections from mandibular molars. When this space becomes infected, the swelling begins at the inferior lateral border of the mandible and extends medially to the digastric area and posteriorly to the hyoid bone.

Submental space -

Present between mylohyoid muscles superiorly and the investing layer of deep cervical fascia, below enclosed by superficial fascia and skin; laterally bounded by the anterior bellies of the digastric muscle .The space contain submental lymphnodes.

Involvement- It is involved most frequently by the infection originating from the six anterior mandibular teeth; then perforate the cortical plate below the origin of mentalis musle labially; and mylohyoid lingually.

Sublingual space -

This space is V- shaped through lying lareral to muscles of tongue ,including hypoglossus,genioglossus and geniohyoid.

Involvement- The teeth which frequently give rise to sublingual are the mandibular , incisors, canines, premolars and sometimes first molars. The infection perforates lingual plate below the floor of the mouth and passes in to sublingual space.

Buccal Space -

Anteriomedially bounded by the buccinator muscle and posterio medially by the masseter and anterior border of the ramus of the mandible. Lateral cover is from fascia & skin. It contains buccal fat pad . It is a potential space and recurrently swollen with oedema or blood after surgical removal of wisdom tooth .

Involvement -The buccal space becomes involved from the maxillary teeth when the infection erodes through the bone superior to the attachment of the buccinator muscle.

Submasseteric Space

Beneath the masseter muscle on the lateral aspect of the mandible with the ascending ramus of the mandible on the medial aspect.

Involvement- Infection usually originates from the lowert third molars ;either resulting from pericoronitis related to vertical and distoangular third molars, or if a periapical abscess spread subperiosteally in a distal direction.When the pus accumulates between the ramus of the mandible and the masseter muscle , it produces a submasseteric abscess.

Canine space –

Bounded superiorly by levator labii superioris nasi and zygomaticus minor muscles ,inferiorly by caninus muscles , anteriorly by orbicularis oris and posteriorly by buccinators muscles.

Involvement- The teeth which frequently give rise to abscess are the canine and premolars sometimes roots of first molars. The periapical abscess discharges buccaly superior to the origin of the canine muscles and pus accumulates in canine fossa.

Infratemporal space –

It is partly situated behind the zygomatic bone. The space is continuous with upper part of pterygomandibular space anteriorly. However it is separated from it by lateral pterygoid muscle posteriorly.

Involvement- Infection of this space arise from infection of the buccal roots of second and third maxillary molars, mostly unerupted third molars and local anaesthesia injections with contaminated needle in the area of tuberosity.

Parotid Space -

The superficial layer of deep cervical fascia forms the parotid space as it splits to surround the parotid gland [7] .

Involvement - Infection to this space is generally spread from non odontogenic source ,either blood borne or by involvement of stenson duct. However , the gland is continuous to submasseteric and lateral pharyngeal space.

Pterygomandibular space –

It is bounded laterally by medial surface of ramus of mandible , medially by medial pterygoid muscle, anteriorly by pterygomandibular raphae.

Involvement - Infection is mostly related to disease of the maxillary third molar.

Lateral pharyngeal space-

It is a potential cone shaped space with its base at the base of skull .The space is divided by styloid process in to anterior and posterior compartment.

Involvement- It is involved from abscess extending from third molar teeth and by backward spread from sublingual, submandibular and tonsillar abscess.

Superficial and deep temporal space –

The superficial temporal pouch lies between the temporal fascia and temporalis muscle .The deep pouch lies deep to the temporalis muscle and the skull.

Involvement- It is secondary to the initial involvement of pterygopalatine and infratemporal space.

Retropharyngeal space –

The retropharyngeal space, extending from the skull base to the mediastinum at the tracheal bifurcation, refers to the lymph node and connective tissue containing potential space between the middle and deep layers of deep cervical fasciae.

Involvement - It typically becomes involved by direct spread from the parapharyngeal space or lymphatic spread from paranasal sinuses or nasopharyngeal region.

Prevertebral space –

The prevertebral space is enclosed by the prevertebral fascia, vertebral bodies and transverse processes, and extends from the clivus of the skull base to the coccyx. It contains the vertebral artery and vein, brachial plexus, and phrenic nerve [7].

Involvement-The main pathways of spread to the prevertebral space are from infection of the vertebral bodies and penetrating injuries[7].

Distribution of facial spaces abscess -: Characteristic of oral and maxillofacial infections has become more complex during past decades. The most common facial space infections effected are the submandibular (20.3-68 %) and buccal space (8.5-96%) followed by lateral pharyngeal space, submental space , sublingual space and canine space [8]. The lateral pharyngeal space and retropharyngeal space are a more commonly affected deep facial spaces at present.

Children have been shown to more likely develop maxillary space infection then mandibular space infection [9]. In addition multi space involvement (50.6-61.2%) is at present more common than single space involvement compared to past[10].

Etiology - Oral and maxillofacial infection usually originate from following sources-

1) Odontogenic : The majority of oral and maxillofacial infections belong to this group. They can be classified as those arising as a sequel from -
   a) Pulp disease b) Peridontal disease c) Secondary infected cysts
   d) Remaining root fragment e) Pericoronal abscess
2) Traumatic: Occasionally, trauma from penetrating wound of soft or hard tissues of the face lead to orofacial infections.
3) Complication of implant and reconstructive surgery.
4) Others – This group includes instances oral and maxillofacial infections arising from other factors such as infected antrum , salivary gland afflictions and secondary to oral malignancies .[4]

Factors influencing spread [4] – Progession of these infections depends on the factors that includes –:

1) Compromised host defences -

   a) Uncontrolled metabolic diseases : malnutrition, severe diabetes mellitus, Uraemia,

   b) Suppressing diseases : Leukaemia , lymphoma, malignant tumours.

   c) Suppressing drugs : cancer chemotherapeutic & immunosuppressive drugs.

   - Host resistance depends upon:

      - Humoral factors involve immunoglobulins derived from B lymphocytes or plasma cells and complement.
      - Cellular factors include polymorphonuclear leucocyte ,lymphocytes and tissue macrophages.

2) Virulence of microorganisms : It is determined by invasiveness of the causative microorganisms.

Pathogenesis of Oral and maxillofacial Infection[11,12]

Deep dental caries as well as acute peridontitis initiates inflammation of the dental pulp tissue. While on the other hand vasodilatation and oedema creates pressure in the pulp chamber, which cause severe dental pain as the rigid walls of the tooth prevent swelling . If these conditions remain untreated ,the augmented pressure leads to strangulation of the blood supply to the tooth through the apex resulting in necrosis of the pulp. The necrotic pulp creates a ideal environment for bacterial invasion into the bony tissue. Entry of bacteria into deep tissues to cause an infection results from virulent aerobic bacteria entered through a necrotic dental pulp. Local environment for anaerobic bacteria is initiated by aerobic bacterial infection.

The anaerobic becomes predominant afterwards since the reduced oxidation potential favours the growth of anaerobic bacteria.

Once the bacteria have invaded the bone, the infection spreads similarly in all direction until a cortical plate is encountered. The patient usually experience sufficient pain to seek treatment when there is intraosseous spread. The extent and direction of spread of infection from the tooth apex depends on the thickness of the overlying bone as well as connection of the bone's perforation site to the jaws muscle attachments . Once the dental infection erodes the bony boundary it spreads through fascial planes to different well-defined fascial spaces and make abscess.

Clinical features [4,7]- The symptoms of the space infection are determined by generalized inflammatory process with localizing symptom at the site of infection-

- Inflammatory symptoms such as pain, fever, swelling and redness are common.There may be enlargement of corresponding draining lymph nodes.
- Symptoms such as dysphagia, odynophagia, drooling, "hot potato" voice, hoarseness, dyspnea, trismus, and ear pain offer further clues about the location of the inflammatory process as well as its potential severity.
- Recent events such as dental work, upper airway surgery or intubation, intravenous drug use, sinusitis, pharyngitis, otitis, or blunt or penetrating soft tissue trauma that preceded worsening symptoms should be identified.

Bacteriology of oral and maxillofacial infections[4,7] –

It has long been recognised that dental infections are usually polymicrobial and a wide variety of facultative and obligate anaerobic bacteria have been isolated from them. The causative organism of these infections are-

1) Aerobic bacteria – Gram positive cocci mainly *Staphylococcus aureus, Staphylococcus epidermidis* , group A beta-hemolytic *Streptococcus (Streptococcus pyogenes), Streptococcus viridans ,group B Streptococcus and Enterococcos spp.*

- Gram negative bacilli – *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aerruginosa*, *Haemophilus* species and others enterobactericae
- Occasionaly Gram negative cocci – *Neisseria sp.*

2) Anaerobic bacteria- Gram negative bacteria mainly *Porphyromonas endodontalis* ,*P. gingivalis* and other *Porphyromonas* spp., *Prevotellolla intermedius, P.melaninogenicus* and *Fusobacterium* , *Bacteriodes spp*. are also responsible for infection.

- Gram positive cocci – *Peptococcus* and *Peptostreptococcus* spp. are common
- Occasionaly Gram negative cocci- *Vellionella spp*.

Aerobic bacteria[13]

Staphylococcus aureus-

- Gram positive cocci about 1 µ in diameter usually arranged in grape like clusters. Nonmotile, non sporing and usually non capsulated except for young cultures. Grows on temperature range of 10-42^0 C optimum being 37^0 C and pH 7.4-7.6 .
- After 24 hrs incubation, colonies are 1-2 mm diameter, circular, convex, smooth, opaque and easily emulsifiable on nutrient agar. Most strains produces golden yellow pigment. Colonies are beta heamolytic on blood agar. On MacConkeyagar, they form lactose fermenting colonies.
- Selective media are media containing 8-10% NaCl (Salt milk agar, salt broth), Lithium chloride and tellurite (Ludlam's medium) and polymyxin. For primary isolation sheep blood agar is suggested. On mannitol salt agar, most strains of staphylococcus aureus ferment manitol and colonies surrounded by yellow zones due to acid production.
- Biochemical reactions- Coagulase positive, catalase positive, hydrolyse urea, reduce nitrates to nitrites, liquefy gelatin, MR and VP positive, indole negative. They produce phosphatases and thermostable nucleases.
- Virulence factors are cell associated polymers eg. cell wall polysaccharide peptidoglycan, teichoic acid, capsular polysaccharide, cell surface proteins like protein A, clumping factor or bound coagulase, extracellular enzymes (coagulase, lipases, hyaluronidase, nuclease, protein receptors), toxins (cytolytic toxins- Alpha, Beta, Gamma, Delta haemolysins, leucocidin, enterotoxin, toxic shock syndrome toxin, exfoliative toxin).

Coagulase negative *Staphylococci* (CONS)

These are skin commensals that cause opportunistic infections. These are Gram positive cocci arranged in clusters and give negative coagulase test . *Staphylococcus epidermidis is* major pathogens of human infections

On nutrient agar, colonies are whitish opaque, small, smooth, circular, low convex. As the don't ferment mannitol , they form small orange colonies surrounded by red or purple medium on mannitol salt agar.

Group A beta haemolytic *Streptococcus*

Spherical or oval cocci 0.5-1 μ in diameter and arranged in pairs and chains. Some strains have capsules composed of hyaluronic acid. It is exacting in nutritive requirements, growth occurs in media containing fermentable carbohydrates or enriched with blood or serum.

- On blood agar, colonies are 0.5-1mm in size, circular, semi transparent, low convex discs with beta haemolysis after incubation for 24 hrs.

  They are catalase negative, not soluble in 10% bile like *pneumococci*. Differentiation from other *streptococci* is done by hydrolysis of pyrrolidonyl naphthylamide and failure to ferment ribose
- Virulence factors include haemolysins (streptolysin O and S), pyrogenic exotoxin, streptokinase(fibrinolysin), DNAse , NADase, hyaluronidase and serum opacity factor.

Group B *Streptococcus*

Colonies are larger in size than Group A *Streptococci* ; translucent , flat and glossy; They have narrow zone of beta haemolysis; some strains are non haemolytic.

- It hydrolyses hippurate. It may be identified by CAMP reaction which can be demonstrated as an accentuated zone of haemolysis when *Streptococcus agalactiae* is inoculated perpendicular to a streak of Staphylococcus aureus grown on blood agar. They are usually bacitracin resistant.
- Human pathogenic Group B strains possess a polysaccharide capsule which appears to confer virulence.

***Streptococcus viridans*-**

This bacteria is not categorised under the Lancefield antigenic group. It produces greenish (alpha lysis) on blood agar –hence name *viridans*. It is catalase negative and optochin resistant. It is resident flora of mouth and respiratory tract but has been isolated in oral and maxillofacial abscess with anaerobes.

***Enterococcus* species**

The commonest species infecting humans is *Enterococcus faecalis*. Others include *Enterococcus faecium* and *Enterococcus durans*.

- Typically appear as pairs of oval cocci arranged at an angle to each other. On blood agar, usually non haemolytic, may show alpha or beta haemolysis. On MacConkey agar, tiny deep pink (magenta) colonies seen.
- They possess several distinctive features separating them from streptococci. They have ability to grow in 40% bile, 6.5% NaCl, at pH 9.6, at 45 ^{0}C and in 0.1%

methylene blue milk. *Enterococcus faecalis* can be identified by its ability to ferment mannitol, sucrose, sorbitol, esculin and to grow on tellurite blood agar producing black colonies.

- *Enterococcus* species produces cytolysin, aggressive substances, gelatinase, extracellular superoxide and surface protein which help to cause abscess formation.

Klebsiella pneumonia- Gram negative straight bacilli about 1.2x0.8µ in size, nonsporing, nonmotile and capsulated.

Colonies on nutrient agar and blood agar are large, mucoid, greyish white. While on MacConkey agar they form mucoid, large, dome shaped, lactose fermenting colonies.

- They are Indole and Methyl red negative, Voges Proskauer and citrate positive. They ferment sugars (glucose, lactose, sucrose, mannitol) with production of acid and abundant gas. *Klebsiella oxytoca* has similar reactions except that indole is positive.

Escherichia coli

Gram negative bacilli measuring 1-3µ x 0.4-0.7µ, motile, non sporing and some strains possess capsule and fimbriae .They possess somatic(O), flagellar (H) and capsular(K) antigens .Grows on ordinary media at 37 ^{0}C in 18- 24 hrs.

- Colonies on nutrient agar are 2-3mm, circular, low convex, greyish white, moist, smooth, opaque or partially translucent. Many pathologic strains are haemolytic

on blood agar. On MacConkey agar, colonies are bright pink due to lactose fermentation.

- They are Indole and Methyl red positive, Voges proskauer and citrate negative. Gelatin is not liquefied, H_2S not formed, urea is not split and growth does not occur in KCN medium.

Anaerobic bacteria[13,14]-

***Peptostreptococcus anaerobius*-**

It is a gram positive coccus about 0.8 mm in diameter , typically arranged in long chains .On blood agar colonies are about 1.5 -2 m in diameter after 3-4 days incubation ,circular entire ,convex ,smooth and pearly grey in colour. The organism is nonhemolytic.

- It grows abundantly in relative simple fluid media such as peptone water and cooked meat broth with the production of gas. Most strains ferment glucose and fructose. It is susceptible to SPS as compared to other anaerobic gram positive cocci. It is entirely proteolytic, indole is not formed but hydrogen sulphide is produced.
- The major product of metabolism is acetic acid with small amounts of propionic, isobutyric, butyric and isocaproic acid.

***Peptococcus asaccharolyticus*-**

- Colony morphology of gram positive cocci is variable .It produces gray, transluscent colonies about 1 mm in diameter. In gram staining usually 0.5-1 μm in diameter and occouring singly ,in pairs and in irregular clusters.

- It is resistant to SPS and produces indole. Catalase tests is variable in this species. It does not ferment glucose, maltose and sucrose. The major product of metabolism is acetic acid and butyric acid.

Peptostreptococcus magnus

It has been found as a part of the normal flora of the mouth, vagina and skin. Spherical Gram positive cocci 0.8-1 μ diameter. Colonies on blood agar after 24hrs are very small, round, convex, shiny, opaque , gray and nonhaemolytic.

- They don't ferment carbohydrates and are negative for aesculin hydrolysis, nitrate reduction, indole formation. They liquefy gelatin.
- Gas chromatography shows a single, large acetic acid peak is characteristic of *Peptostreptococcus magnus*.

Bacteroides fragilis-

In clinical specimens there are five species which has been described – *B. fragilis, B.vulgates, B. diastasonis, B. ovatus* and *B. thetaiotaomicron*. Subspecies *fragilis* is one most implicated.

- It is a gram negative bacillus of about 0.4x 3-5 μ in size, regularly shaped, with a strait or slightly curved axis and rounded ends.-In glucose broth cultures pleomorphism is usually marked with filamentous and curved form and staining is quiet irregular.
- It is an obligate anaerobe that grows well on horse blood agar. Colonies are low convex domes, 1-2 mm in diameter after 24-48 hrs of incubation, and semitransluscent or greyish white in colour. Most strains are non haemolytic but

occasional strains may be frankly haemolytic.-In fluid media growth is not inhibited by 20% bile .Failure of bile to inhibit its growth is an important feature that distinguish *B.fragilis* from most other members of the genus.

- Strains of *B. fragilis* is resistant to kanamycin, Vancomycin and colistin. It does not produces catalase and indole. Produces acid from glucose, lactose, sucrose, maltose & usually xylose but do not ferment trehalose, arabinose, mannitol, salicin.
- The major products of glucose metabolism are acetic and succinic acid; lesser amount of lactic, isovaleric and propionic acid are also produced.

Fusobacterium –

The *Fusobacteria* are strict anaerobes, and causing variety of infections in man. The four species most commonly implicated as significant human pathogens are *F. necrophorum* ,*F. nucleatum* , *F. mortiferum* and *F. varium*

- It is a gram negative bacillus with a tendency to pleomorphism and irregular staining. Cells are about 0.5 -5-10 μm in size, the larger ones commonly being curved; filamentous form is not uncommon. Fusiform swelling of bacilli sometimes seen but spheroids are rarely developed.
- Colonies are 1-2 mm in diameter after 48h of incubation, circular with scalloped or diffuse margin, high convex or umbonate, with a ridged surface and semitransluscent or opaque ,and yellowish in colour. Many strains of the organism are haemolytic, and many are lipolytic on egg yolk agar.
- The organism is sensitive to kanamycin and colistin and resistant to Vancomycin. Catalase is negative. Nitrate is not reduced but indole and hydrogen sulphide are produced in some strain. They have weakly saccharolytic activity.

- Major product of metabolism are butyric acid ,and lesser amounts of acetic acid and propionic acid.

Pigmented *Prevotella* and *Porphyromonas*

Nine of the atleast 12 species in this group have been isolated from human clinical material; the others are associated with animals. Those most frequently encountered are *Porphyromonas asachrolytica, Porphyromonas gingivalis, Prevotella intermedia* and *Prevotella melaninogenica.*

Most strains of *Porphyromonas* and occasional strains of *Prevotella* appear as pale sataining coccobacilli. Young cultures may appear gram variable.

- The organisms produce a variety of colony types with varying amounts of pigment. *Prevotella intermedia, Prevotella nigrescens* and *Prevotella corporis* produce dark brown to black dry colonies. *Porphyromonas* colonies are usually more mucoid and dark brown to black. *Prevotella melaninogenica* group produces tan to light brown, smooth colonies. Pigment production may not always be readily detected and may require prolonged incubation (>5 days), especially with the *P. melaninogenica* group. Pigment production is most reliably demonstrated on laked rabbit blood agar. With the exception of *Porphyromonas gingivalis*, colonies fluoresce brick red under ultraviolet light. After pigment is produced. The fluorescence may not be detectable .

Most *Porphyromonas* species are difficult to grow in broth and on some selective agars containing 7.5 µg /ml Vancomycin (eg. Kanamycin –vancomycin-laked blood). Addition of serum, laked rabbit blood, or hemin may enhance growth in

broth. Reduction of the Vancomycin concentration to 2 μg/ml allows for growth of most *Porphyromonas* while still inhibiting many gram-positive organisms.

- The pigmented anaerobic gram-negative bacilli are all sensitive to bile and form three groups based on the antimicrobial disk identification pattern, indole reaction, and colony morphology :
  1) *Prevotella intermedia, P. corporis* and *P. nigrescens* are indole positive.
  2) The *Prevotella melaninogenica* group (*P. loescheii, P. denticola, P. melaninogenica*) are indole negative, often resistant to all antibiotics identification disks (colistin, kanamycin, Vancomycin), and their colonies do not develop dark pigmentation.
  3) *Porphyromonas* species are indole positive, sensitive to Vancomycin and resistant to kanamycin and colistin and their colonies are often mucoid, shiny , smooth, and dark brown to black.

Virulence factors of anaerobic bactria-[15,16]

Anaerobic infections are endogenous in origin, arising adjacent to mucosal surfaces where the resident anaerobic flora is prominent. Microbial factors that determine whether infection with anaerobes will occur are the virulence of the infecting organisms as well as their ability to relate synergistically with other aerobic and anaerobic bacterial species. There are important virulence mechanism in anaerobic bacteria described below-

Capsule - The capsule is involved in adhesion, abscess formation, and impaired phagocytosis of microorganisms. Support for the importance of encapsulated *Bacteroides spp*. and anaerobic and facultative Gram positive cocci has been provided

by their higher recovery rate in oropharyngeal infections, compared with their number in the normal oral flora[16,17]. Encapsulated *Bacteroides, Fusobacterium*, and facultative gram-positive cocci generally induce abscesses, whereas non-encapsulated organisms do not[18] . The majority of clinical *P. gingivalis* isolates are encapsulated and encapsulation is associated with the presence of a K antigen[19]. The presence of K antigen in *P. gingivalis* correlates with serum resistance, low chemiluminescence, resistance to phagocytosis and the need for opsonization with specific antibodies for complement-mediated killing[20].

Adhesion- Bacterial fimbriae play an important role in the interaction between bacteria and host cells. *P. gingivalis* fimbriae have a variety of biologic activities including immunogenicity, binding to various host proteins, stimulation of cytokine production and promotion of bone resorption[21,22].

F. nucleatum agglutinates human erythrocytes and attaches to human oral epithelial cells, gingival fibroblasts and polymorphonuclear leukocytes[23]. *P. magna* strains isolated from localized suppurative infections bind human serum albumin (HSA), whereas commensal isolates bind neither Ig nor HSA[24]. Fimbriae of *P. intermedia* induce hemagglutination reaction, those of *P. loescheii* cause coaggregation with other bacteria[22]. *P. intermedia* can invade an oral epithelial cell line and that the type C fimbriae and a cytoskeletal rearrangement are required for invasion.[25]

Toxins and Enzymes- These factors are responsible for invasion in host tissue and degradation of host tissue.

- Lipopolysaccharide (LPS) endotoxins formed by *Prevotella* and *Phyromonas* organisms are less potent than conventional endotoxins and are less likely to produce classic manifestations of endotoxic damage[26].
- However, it has been shown that the LPS of *P. endodontalis* plays an important role in the initiation and magnification of maxillofacial abscess formation acting as a virulence factor by stimulating the production of inflammatory cytokines[27]. On the other hand, LPS from *Fusobacteria* has a structure similar to that of gram negative enteric rods; the LPS of *F. necrophorum*, in particular, displays toxicity comparable to that of enterobacterial LPS, accounting for the high virulence of this organism[28] .
- Anaerobic bacteria produce volatile sulfur compounds, such as methyl mercaptan and the highly cytotoxic hydrogen sulfide, which are responsible for the putrid smell of pus found in anaerobic infections [29,30].
- *P. micros* has an exceptionally high capacity to produce hydrogen sulfide from glutathione found in high concentrations in most tissue cells and also released from damaged PMNs. Although, as a rule, GPAC express their pathogenicity via a synergistic interaction with facultative organisms or other anaerobes, this capacity of P. micros combined with its strong proteolytic activity may indicate a crucial role in abscess development in the course of orofacial odontogenic infections [31].
- The ability to survive in the oxygen tension of the living tissues- Superoxide dismutase produced by moderate anaerobes renders them by tolerant of oxygen levels of 2% to 8% and it is considered a prerequisite to their pathogenicity[32,33].

- Collagenase - Collagenase is a potential virulence factor expressed by *P. gingivalis* associated with periodontal disease . They assist in destroying extracellular structures which helps in spreading of infection. It has a main role in infection by *Actinomycetemcomitans*[34] .
- Proteinases - The cysteine proteinases of *P. gingivalis* are generally considered as the major virulence factors of dental infection. Proteases from odontopathogenic bacteria may act as direct proteolytic activators of human procollagenases and degrade collagen fragments[35]. The direct extracellular matrix proteolytic activity exhibited by the *Peptostreptococcus spp*. is limited. However, Krepel et al. suggested that *peptostreptococcal* production of proteolytic enzymes may have an important adjunctive effect on the pathogenesis of certain soft tissue infections[36].

- In recent years there has been an increased approval of the frequency of occurrence and importance of strict anaerobes in pus from oral and maxillofacial abscess.

The review of literature is alarmed with account of oral and maxillofacial infections from early times to the current status.

Staphyloccus aureus and *Streptococcal species* were established as predominant pathogens in abscesses in most studies until 1970[37] . Contrary to that, in a survey of 1000 consecutive specimens of pus from oral lesions, by Sims (1974), *Viridans streptococci* were found in 90% of the cases, whereas anaerobic gram negative rods (*Bacteroides spp*. and *Fusobacterium spp*.) were only reported in 2.6% cases . However, the author pointed out that isolation frequency of strict anaerobes using routine clinical laboratory methods, was probably a gross underestimate which

could be improved by the use of strict anaerobiasis at all stages in the handling of specimens[38].

The role of capsulated *Bacteroides* species in orofacial space infections was first described by Brook in 1986. Brook's study supported the study by John Barlett, who was first person to bring anaerobic predominance (66%) into the picture. He mentioned that predominant organisms were *Peptostreptococci*, *Fusobacterium*, *Prevotella spp.* Metronidazole was found to be the most effective antibiotic in them.[28]

Skalavounous (1986) was the first person who took into consideration the use of antibiotics taken by patients prior to incision and drainage. He studied 40 orofacial abscess cases in which patients had taken antibiotics for several days. Aerobic or facultative anaerobic bacteria were present in 21 cases (67.1%) and anaerobic bacteria in 17 cases (33%). Again *Staphylococci* and *Streptococci* were the predominant gram positive aerobic isolates; *Peptostreptococci*, *Prevotella melaninogenica, Bacteroides fragilis* and *Bacteroides oralis* being the anaerobic isolates[39]. This study showed results opposite to those of Lewis et al because of aerobic predominance in the study.[40]

To understand the microbiology and antibiotic sensitivity of orofacial infections T. kuuriyama et. al had done a lot of work from 1991 to 2000. First in between 1991 and 1996 they gave information about bacteriology and antibiotic sensivity of anaerobes then in 1997 they reported the beta lactamase production among gram negative anaerobes [41]. In 2002 they determined the bacteriology and antibiotic sensitivity of anaerobes other than gram negative bacilli mainly gram positive cocci isolated from the same specimen. *Peptostreptococci, Prevotella spp* and *Eubacterium spp* were commonest recovered anaerobes along with *Staphylococci*[42].

Many studies has been done to find out changing trends in the bacteriological flora of maxillofacial space infections . Indian study by Yuvraj V. and Alexander M. (2007) stated that aerobes were still dominating the microflora as they found 76.3% cases positive with the same, against 17.6% anaerobes. *Streptococcus* spp. and *Peptostreptococcus* were the most commonly isolated among aerobic and anaerobic organisms [43].

In recent years a study conducted on 80 patients with orofacial infection yield growth of total of 109 micro-organisms. Pure aerobes were identified in 28(35%) of cases, pure anaerobes in 18(22.5%), mixed aerobes and anaerobes in 10(12.5%), mixed aerobes in 15(18.75%) and mixed anaerobes were isolated in 6 (7.5%) cases[2]. This study was supported by another Indian study in 2012.Out of the 64 patient of orofacial infections a total of 64 aerobic and 87 anaerobic strains were isolated. The predominant bacteria were *Streptococci viridans* (64%), *Prevotella* (43%), *Peptostreptococcus* (26%), *Porphyromonas* (7%), and *Fusobacterium* (14%)[44].

Therefore after evaluating the research for oral and maxillofacial infections we have found polymicrobial infection involving both aerobes and anaerobes.

- Both clinical and investigational evidence indicate synergy between certain aerobes and anaerobes do exist. Most probable mechanisms of synergy are-
    a) Some anaerobes can inhibit phagocytic killing of aerobes in vitro. Ex*: B. Fragilis* inhibits uptake of *E.coli*. Other studies have demonstrated the ability of anaerobes to inhibit phagocytosis by measuring the uptake of [3H] thymidine-labelled bacteria by polymorphonuclear leucocytes in the presence of an inhibiting anaerobe.

b) Bacteria may also interact by providing nutrients for each other. Ex: Vitamin K is an important growth factor for *B. melaninogenicus*. This vitamin is produced by various diphtheroids. *Klebsiella* spp. produces succinate, which is a growth factor for *B.asaccharolyticus*, a heat labile growth factor produced by *S. aureus* will stimulate growth of microaerophillic *Streptococci*.

c) One species of bacteria optimizes the local environment for the other. Pasteur stated "aerobic organisms can utilize the available oxygen to allow obligate anaerobes to proliferate."This also inhibits leucocytic oxidative function, thus further enhancing susceptibility to infection.

d) Protection of bacteria against antibiotics through bacterial interaction may result in enhancement of virulence. Enzymatic degradation of the antibiotic, such as beta lactamases that are derived from one bacterium can protect other bacteria in mixed infections. Ex- Protection of *F. necrophorum* from penicillin when this organism is combined with *B. fragilis*, a beta lactamase producer and chloramphenicol appears to be inactivated through conversion to its aminophenyl derivative in the presence of *B. fragilis* and *Clostridium perfringens*[45].

Management of Oral and maxillofacial infections-

1) **Airway Maintenance -** Patients should be carefully monitored for airway obstruction. Inflammation in airway, tenderness of tissues, tissue rigidity and trismus make the maintenance of airway becomes difficult. The need for tracheostomy/ intubation should be assessed carefully and done promptly to prevent further damage to already inflammed tissues.

2) Other investigations -

- Complete blood cell count with differential count.
- C reactive protein and ESR to monitored as predictors of clinical response.
- Radiological investigations- Plain radiographs-lateral cervical films are faster and show retropharyngeal soft tissue widening and gas shadow suggestive of abscess particularly in children.
    - CT scan aids in diagnosis as well as surgical planning. Contrast enhanced CT (Coronal and axial Planes) is preferred. CT is highly sensitive for abscess. Contrast enhancement of abscess wall, the relationship to adjacent fascial planes and soft tissue oedema can be elicited
    - Ultrasound is a cost effective tool. Ultrasound can differentiate involving abscess and cellulites. It is not reliable due to the difficulty in diagnosing Deep Seated Infections. Ultrasound can guide in aspiration.
    - MRI gives soft tissue imaging and can recognize internal Jugular Vein thrombosis.

3) Surgical treatment-The abscess is drained surgically and simultaneously dental treatment also should be instituted to achieve quick resolution. Incision and drainage helps in

- To get rid of the toxic material which facilitates microbial growth
- To decompress the edematous tissues.
- To allow better perfusion of blood ,containing antibiotics and defence elements.

4) Medical treatment – consist of supportive therapy and antibiotic treatment-

- Supportive care – Hydration, soft or liquid diet, analgesics and use of antiseptic mouthwash to maintain the oral hygiene.
- Antibiotic therapy –Combination of antibiotics for both aerobes and anaerobes is given.

Antimicrobial Treatment in the Oral and maxillofacial Infections

Antimicrobial therapy has an essential role in the management of these infections. It shorten the period of infection and minimize associated risks.

Penicillins and Cephalosporins- Historically, the penicillins have been used as first-line agents in the treatment of odontogenic infections. The penicillins exert their antibacterial effect by inhibiting a number of bacterial enzymes (the penicillin binding proteins) that are essential for peptidoglycan synthesis [46].

Increasing rates of penicillin resistance and treatment failures have been reported. The highest rates of penicillin resistance have been observed with the members of the genus *Bacteroides* and *Prevotella*.[47,48] Penicillin resistance in these pathogens has been correlated with ß-lactamase production. Heimdahl et. al. reported it on a series of patients with orofacial infections who failed to respond to penicillin therapy due to ß-lactamase producing *Bacteroides*.[48] Witcher et. al. described a series of patients who, after treatment with penicillin, developed mandibular osteomyelitis caused by ß-lactamase producing P. melaninogenicus.[49] Using an animal model, ß-lactamase production by strains of *P. melaninogenicus* in a mixed infection has been shown to protect both *P. melaninogenicus* and other bacteria from penicillin.[50]

Due to the increasing prevalence of penicillin resistance, the Sanford Guide to Antimicrobial therapy recently replaced penicillin V with clindamycin as the drug of choice in treating odontogenic infections.[51]

Macrolides- The currently available macrolides include erythromycin, clarithromycin, and azithromycin. Macrolides are bacteriostatic agents that inhibit bacterial RNA-dependent protein synthesis.[46] Erythromycin was recommended for the treatment of dental infections as the antibiotic of first choice for patients with known hypersensitivity to penicillins. This is no longer valid because of widespread resistance to this drug among oral anaerobes, especially *Fusobacteria* and GPAC[52] .

Azithromycin has been found to be the most active macrolide antibiotic against oral gram-negative anaerobes, while showing activity against oral *Streptococci* comparable to that of erythromycin and is probably the most suitable agent of this group for orofacial odontogenic infections.

However, macrolides should not be well thought out as firstline therapy in treating odontogenic infections and should be reserve for patients of penicillin allergy.

Fluoroquinolones -The fluoroquinolones currently available are ciprofloxacin, oflo-xacin and levofloxacin. These agents are bactericidal, and exert their antibacterial effect by inhibiting DNA gyrase and topoisomerase IV. The fluoroquinolones have potent gram negative activity, including activity against Pseudomonas sp. Their activity against gram positive bacteria is marginal, however unlike the earliest fluoroquinolones, ‘‘third-’’ and ‘‘fourth-generation’’ fluoroquinolones have enhanced gram-positive and anaerobic spectrum [53].

For trovafloxacin, good in vitro activity against clinically important anaerobic bacteria was shown to correlate with in vivo efficacy which led to its approval for the treatment of anaerobic infections, but its use has been limited because of hepatotoxicity[54].

The methoxyfluoroquinolones gatifloxacin and moxifloxacin also have anaerobic activity but have not been clinically tested[55]. Nevertheless, moxifloxacin has shown remarkably good in vitro activity against dental pathogens, including most anaerobic bacteria. The in vitro activity of moxifloxacin has been studied against anaerobic bacteria isolated from odontogenic abscesses and periodontal infections. The MIC90s were 0.5 mg/mL for anaerobic isolates from periodontal infections, which included *Porphyromonas gingivalis, Prevotella* species, *Actinomyces* species, *Fusobacterium nucleatum,* and *Peptostreptococcus* species[56]. It is not recommended for children, and appropriate use is necessary if this group of agents is to remain clinically useful.

Metronidazole- Metronidazole is only effective against obligate anaerobes, because its molecule must enter the bacterial cell before it is reduced to form the active antibacterial agent and this reduction takes place effectively only under anaerobic conditions.[57] The combination of penicillin (or an aminopenicillin) with metronidazole adequately covers the microbial flora of odontogenic abscesses since metronidazole compensates for limited activity of penicillin against β-lactamase producing strains of anaerobic bacteria[58,59] .

Furthermore, metronidazole levels in abscess fluid exceed those necessary to kill most obligate anaerobic gram-negative rods, and in contrast with β -lactams killing rates are not affected by the inoculum size or growth rate of the bacteria[60].

Prevotella spp., *Peptostreptococcus* spp., *Bacteroides* spp. and *Porphyromonas* spp., all of which previously implicated in odontogenic disease have been found highly sensitive to metronidazole.

Clindamycin - Clindamycin has excellent activity against gram positive organisms, including anaerobes and ß-lactamase producing strains. Low concentrations of the drug are bacteriostatic, but bactericidal activity is achieved clinically with the usual recommended doses. Clindamycin binds to the 50S ribosomal subunit of suscep-tible bacteria and interferes with protein synthesis [46]. Over 90 per cent of clindamycin is absorbed following oral administration. Absorption is delayed but not decreased with the ingestion of food. The principle side effect associated with clindamycin is diarrhea, with a reported incidence ranging from 0.1 per cent to 17 per cent. Clindamycin has also been related with pseudomembranous colitis also[61].

Clindamycin has excellent activity against aerobic gram positive cocci, such as *Staphylococcus aureus, Streptococcus spp,* and most anaerobes, including penicillin resistant strains of *Bacteroides, Prevotella*, and *Porphyromonas spp*.[62]

Gilmore et al demonstrated comparable activity between clindamycin and penicillin V in the treatment of moderate to severe odontogenic infections[63]. Clindamycin has broad spectrum of coverage and excellent clinical efficacy in oral and maxillofacial infections [64].

METHODOLOGY

The present study was conducted at the Department of Microbiology, JNMC, Belgaum.

Source of Data : Fifty patients presenting with signs and symptoms of abscess of Oral and maxillofacial infections reporting to department of Oral and Maxillofacial Surgery, K.L.E.S. Vishwanath Katti Institute of Dental Sciences and K.L.E.S. Prabhakar Kore Hospital, Belgaum over a period of one year from Jan 2012 to Dec 2012 were included in this study.

Pus samples collected from the abscess were studied in the department. Detailed clinical history regarding age, sex, chief complaints, past history and treatment history was obtained from each patient.

Method of Collection of data

Inclusion Criteria:

1. All the patients of oral and maxillofacial infection with clinical evidence of abscess formation.
2. Patients who were not on antibiotics 48 hours prior to sampling.

Sample size calculation : Sample size was calculated by using formula-

Sample size (n) -$4pq/d^2$

Where n – Sample size

p - Prevalence [46]

q - 100-p

d – Absolute error which is 5%

Hence sample size is -

$$4\times97\times3/5\times5$$

$$=47 \text{ approx } 50$$

Collection of sample - The pus from the abscess was collected aseptically with the help of syringe and needle and when abscess is deep and site is difficult to reach a sterile cotton swab was used to collect the sample. After collection, the sample was immediately transferred to thioglycollate broth.

Sample processing - Direct smear was made by using a loop full of sample. 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 (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.

1. **Gram stain Procedure: [Hucker's modification][64] :**

a) A thin smear of the material was made on a clean glass slide and allowed to air dry.

b) 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.

c) Slide was placed on staining rack and the smear overlayed with crystal violet solution.

d) After 1 minute the slide was washed thoroughly with distilled water.

e) Then the smear was overlayed with Gram's iodine solution for 1 minute and washed again with water.

f) The smear was held between the thumb and fore finger and the surface flooded with a few drops of acetone- alcohol decolorizer, until no colour washes off.

g) 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.

h) The smear was placed in a upright position in a staining rack, allowing excess water to drain off and smear to dry.

i) The stained smear was examined under 100 X (oil) immersion objective lens.

2. Culture

Culture was done for both aerobic & anaerobic bacteria.

a) Aerobic culture:

For this purpose sample was inoculated onto –

1) 5% Sheep blood agar

2) Chocolate agar

3) Mc Conkey's agar

- The inoculated culture plates were incubated at 37^0 C for 24 hrs aerobically (Mac Conkey agar and Blood agar).
- All the isolates were identified and characterized biochemically by standard procedures[64].

b) Antibiotic sensitivity testing for Aerobic bacteria -

The antimicrobial susceptibility testing was done for aerobic 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) - 30 mcg
2) Ampicillin(Amp) - 10mcg
3) Carbenicillin(Cb) -100mcg
4) Ciprofloxacin(Cf) - 5 mc
5) Amoxicillin / clavulanic acid (Ac) - 30 mcg
6) Ceftazidime(Caz) - 30 mcg 5)
7) Erythromycin (E) - 15 mcg
8) Cephoxitin (Cn) - 30 mcg
9) Co-Trimoxazole(Cot) -1.25/23.75mcg
10) Vancomycin(V) -30mcg-

Method - For antimicrobial sensitivity testing a single colony was inoculated in peptone water and incubated at 37 ^{0}C for 4 to 6 hrs 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 ^{0}C . Zone of inhibition was measured. Interpretation was recorded according to the Kirby Bauer chart.

- Control stain used was Staphylococcus aureus ATCC 25923.

c) Culture for anaerobic organisms[65] - Each sample was inoculated onto:

Blood agar supplemented with Haemin and Vit K.

⇩

Single colony of each distinct type

⇩

Aerotolerance testing

⇩ ⟹ -ve Aerobic (CA) incubation in 5-10% candle jar

Purity blood agar plate

⇩

⇩	⇩	⇩
Add antibiotic	**Perform**	**Record**
Identification discs	1. Gram stains	1. Colony Morphology
- Sodium polyanethol	2. Spot indole test	2. Pigment
Sulphonate (SPS) disc	3. Catalase test	3. Haemolysis
- Nitrate disc		4. Fluorescence
- Susceptibility to		5. Pitting
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.
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. 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 autoclaving. Laked blood was prepared by freezing whole blood overnight and then thawing.

3) Bacteroids 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.

Method used for obtaining anaerobiosis - in the jar was "internal gas generating system" described by Lakshminarayana and Vaidhyalingam[66].

Catalyst:

Cold catalyst consist of pellets of alumina coated with finely devided Palladium (Baker platinum Ltd.,London)that is reactivated every time before use by drying at 150^0C -160^0 C for 1-2 hrs was used .

Indicator for anaerobiasis-

Fildes and Mcintosh indicator - was prepared by mixing equal volumes of –

a. 6% glucose solution in distilled water
b. Sodium hydroxide solution prepared by diluting 6ml of 0.1 N NaOH to 100 ml of distilled water.
c. Methylene blue prepared by adding 3ml of 0.5% w/v solution of methylene blue diluted to 100 ml of distilled water.
    - Mixture is placed in anaerobic jar after it is made colourless by heating in a boiling water bath.
    - If anaerobic conditions are secured and maintained the indicator solution remains colourless. If on the other hand solution colour becomes blue.

- **Bacteriological indicator-** Pure aerobic organism *'Pseudomonas aeruginosa'* ATCC -25922 is used as negative control. If anaerobiasis is mentioned then it will not grow inside the jar.

Principle of internal gas generating system-

- 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 $\Longrightarrow$ sodium citrate + carbon dioxide

$C_6H_8O + 3NaHCO_3 \Longrightarrow Na_3C_6H_5O_7 + 3H_2O + 3CO$

(2) Sodium metaborate + hydrogen $\Longrightarrow$ Sodium borohydride + hydrogen

$NaBH_4 + 2H_2O \Longrightarrow NaBO_2 + 4H_2$

Operation of the gas generator system-

a) 1g sodium borohydride was taken in 30 ml test tube
b) 1g sodium bicarbonate and 1g citric acid were taken in the 5ml 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 funnel dips into 5 ml test tube. Entire unit was kept inside the jar with the indicator. 20 ml 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 liberating CO2. Carbon di oxide 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 CO2.

- 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.

After 72 hours of incubation at 37^0 C 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), general appearance (eg: mucoid transparent, opaque) and colony count
- 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 5 µg 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.

- **Aerotolerance test-**

- Inoculated Chocolate agar plate incubated in candle jar at 37 ^{0}C to test for aerotolerance.
- If there was no growth on plates after 72 hours of anaerobic incubation, plates were reincubated for an additional period of 48 hours .

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 colour change or a pinkish colour.

- **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 colour indicated nitrate had been reduced to nitrite. If no colour developed in 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.

Preparation of 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

d) Anarobic isolates were stored in Robertson cooked medium.

e) **Antibiotic susceptibility of anaerobes**- It was performed by Agar dilution method for the following antibiotics[67]-

1) Clindamycin 2) Metronidazole

Background information –

Antibiotic tested: Clindamycin (hydrochloride)[68]

- ✓ Potency: 91 µg/mg, Solvent: Distilled water, Diluent: Distilled water
- ✓ Therefore, number of petri plates required per dilution is: 1
- ✓ Volume of culture medium per plate: 20 ml we have prepared 40 ml medium for each dilution
- ✓ Number of dilutions for the MIC range 0.125 to 32 is

a) Weighing Antimicrobial Powders- Using this information, a standardized solution can be formulated.

$$\text{Weight (mg)} = \frac{\text{volume (mL)} \times \text{concentration (}\ \mu\text{ g/mL)}}{\text{assay potency (}\mu\text{ g/mg)}}$$

Example- To prepare 10 mL of a stock solution containing 1,000 µ g/mL of a particular antibiotic with a potency of 869 µg/mg, perform the following calculation-

$$\text{Weight (mg)} = \frac{10\text{ (mL)} \times (\ 1000\mu\text{ g/mL})}{91\text{ (}\mu\text{ g/mg)}}$$

$$= 109.89\text{ mg}$$

Preparation of stock solution[69]

B) Preparation of 1,000 µg/ml stock solution:

Required volume is 0.6 ml but prepare a stock solution of 1,000 µg/ml in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	10.99
2 ml	21.98
3 ml	32.97
4 ml	43.96
5 ml	54.95
10 ml	109.89

C) Preparation of 100 µg/ml stock solution:

Required volume is only 0.35 ml but prepare a stock solution of 100 µg/ml in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	1.1
2 ml	2.2
3 ml	3.3
4 ml	4.4
5 ml	5.49
10 ml	10.99

- ✓ Weigh out the required amount of antibiotic, dissolve antibiotic powder in small volumes of solvent (water) and make it up to final volume with diluent (water).

Preparation of antibiotic dilution in plates

Prepare 40 ml of Brucella blood agar with vitamin K and hemin, sand allow it to cool in a water bath to between 45°C and 50°C. Add antibiotic from the stock solution using micropipette with sterile tips.

Dilution µg/ml	Volume taken (ml)	Stock solution
32	1.28	1,000 µg/ml
16	0.64	1,000 µg/ml
8	0.32	1,000 µg/ml
4	0.16	1,000 µg/ml
2	0.08	1000 µg/ml
1	0.04	1000 µg/ml
0.5	0.2	100 µg/ml
0.25	0.1	100 µg/ml
0.125	0.05	100 µg/ml

- ✓ Swirl the flask to mix thoroughly and pour into the petri plates on a level surface to a depth of 3–4 mm .Let the plates solidify at room temperature and label the plate of its antibiotic concentration.

Procedure for MIC determination by agar dilution

Bring all the plates with antibiotic range to room temperature and allow the agar surface to dry. While performing dilution test, a plain medium without antibiotic must be used as a control.

- Prepare a suspension of test organism in Thioglycollate broth with vitamin K and hemin with out indicator and adjust its turbidity to 0.5 McF standard.
- Dilute this suspension 10 times (1 in 10 dilution) using sterile saline. Within 15 minutes this suspension must be tested.
- Arrange the plates in increasing concentrations and arrange the order of test tubes as per grid markings.
- Transfer 1–2 µl of this inoculum on the agar plate; it should form a spot of 5-8 mm. The final inoculum on plate is 10^4 cfu/ml.
- Allow the spots on plates to dry (10 minutes); invert them and incubate them in Mc Intosh Fildes jar for 48-72 hrs.
- Following incubation, there must be no growth on all spots in the aerobic control plate. Record the control organism growth in each plate.
- The end point, the first negative, is to be read at the point where a marked change in growth appears as compared with growth on control plate.
- The concentration of antibiotic that has completely inhibited bacterial growth is taken as MIC.
- Bacteroides fragilis ATCC 25285 strains was used as quality controls.
- Susceptibility MIC range of this ATCC strain for clindamycin is 0.5-2µg/ml and for metronidazole is 0.25 -1µg/ml.

Background information

- ✓ Antibiotic tested : Metronidazole {68}

Potency: 94 µg/mg ,Solvent: DMSO(Dimethyl sulfoxide), Diluent: distilled water

Therefore, number of petri plates required per dilution is: 1

- ✓ Volume of culture medium per plate: 20 ml But we have prepared 40 ml medium for each dilution, Number of dilutions for the MIC range 0.125 to 32 is: 9

Therefore, prepare 9 sets of culture medium with 40 ml each Total number of petri plates required for entire MIC range: 9

a) By the formula described before required amount of antimicrobial powder is weighed.

Preparing stock solution

b) Preparation of 1,000 µg/ml stock solution: Required volume is 0.6 ml but you may prepare a stock solution of 1,000 µg/ml in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	10.64
2 ml	21.28
3 ml	31.91
4 ml	42.55
5 ml	53.19
10 ml	106.38

c) Preparation of 100 µg/ml stock solution:

Required volume is only 0.35 ml but you may prepare a stock solution of 100 µg/ml in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	1.06
2 ml	2.13
3 ml	3.19
4 ml	4.26
5 ml	5.32
10 ml	10.64

- ✓ Weight out the required amount of antibiotic and dissolved in corresponding volume of the solvent and made it up to final volume with diluent (water).

Preparation of antibiotic dilution in plates-

Dilution µg/ml	Volume taken (ml)	Stock solution
32	1.28	1,000 µg/ml
16	0.64	1,000 µg/ml
8	0.32	1,000 µg/ml
4	0.16	1,000 µg/ml
2	0.08	1000 µg/ml
1	0.04	1000 µg/ml
0.5	0.2	100 µg/ml
0.25	0.1	100 µg/ml
0.125	0.05	100 µg/ml

- ✓ Prepare 40 ml of Brucella blood agar with vitamin K and hemin allow it to cool in a water bath to between 45°C and 50°C
- ✓ Add antibiotic from the stock solution using micropipette with sterile tips.
- ✓ Procedure was followed same as described above.

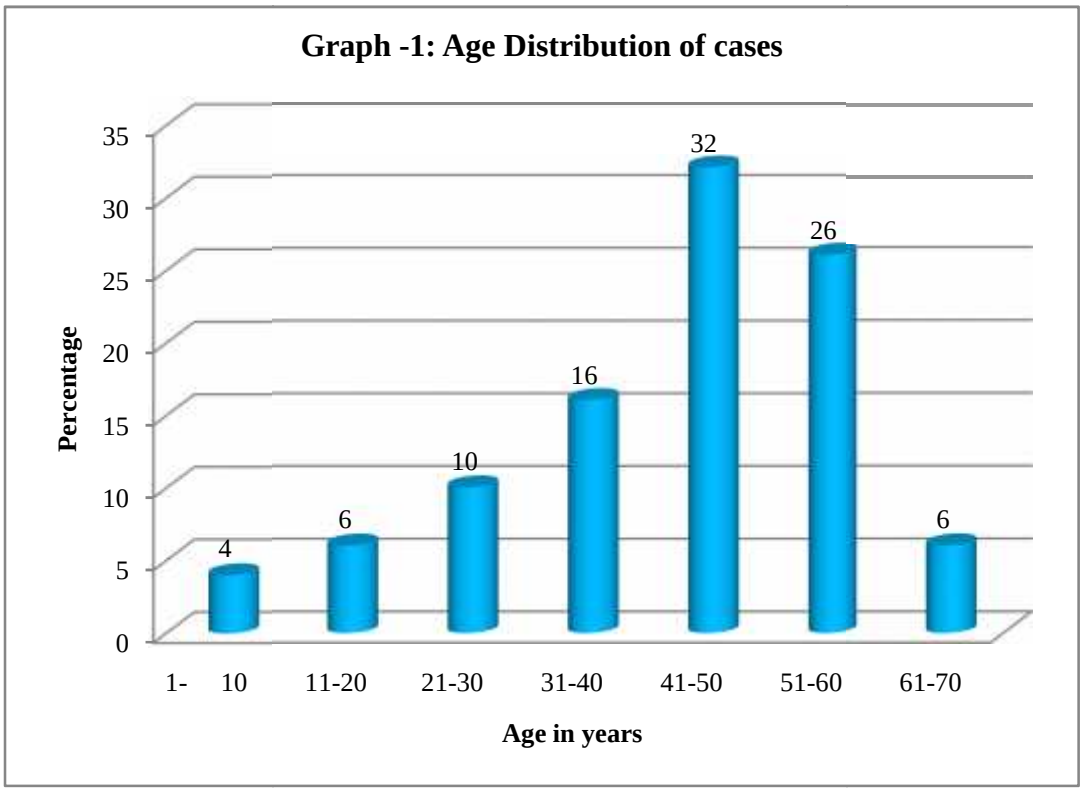
RESULTS

A total of 50 samples were collected from patients presenting with maxillofacial abscess at KLE V.K. Institute of Dental Sciences, Belgaum (Karnataka) from January 2012 – December 2012

The samples were processed for the isolation, identification and antibiotic sensitivity of aerobes and anaerobes.

TABLE 1: AGE DISTRIBUTION

Age group (In years)	Number of cases N=50	Percentage (%)
1- 10	2	4
11-20	3	6
21-30	5	10
31-40	8	16
41-50	16	32
51-60	13	26
61-70	3	6



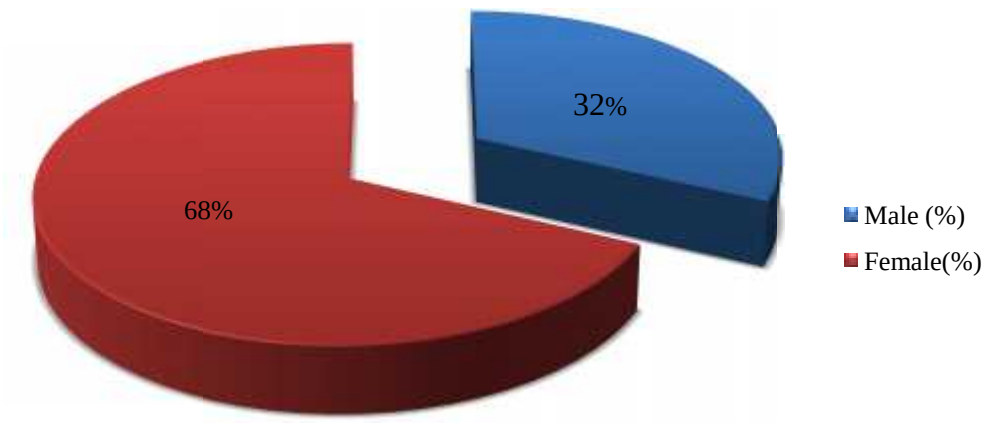
In the present study -

- Our cases ranged between 1-70 years.
- The frequency of maxillofacial infections was highest in the age group of 31-50 years.
- 5 (10%) patients were in the age group of 1-20 years

TABLE 2: SEX DISTRIBUTION

Gender	Number of cases	Percentage
Male	16	32%
Female	34	68%
Total	50	100%

Graph -2: Sex Distribution



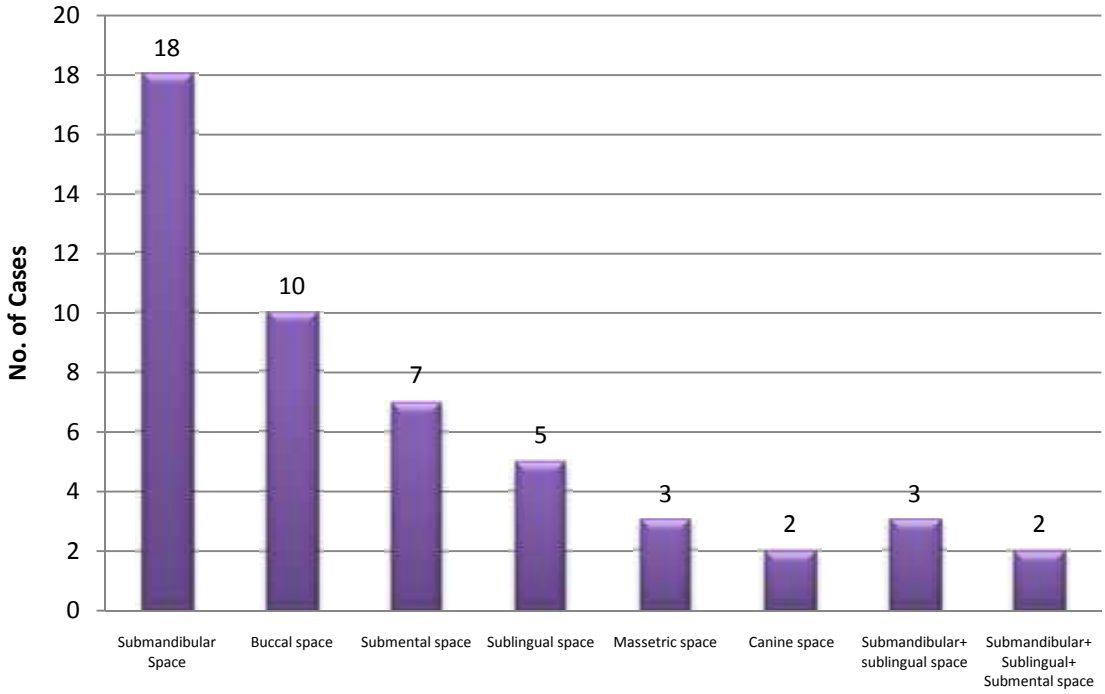
In the present study -

- 16 (32%) patients were males.
- 34 (68%) patients were females.

TABLE 3: DISTRIBUTION OF CASES ACCORDING TO MAXILLOFACIAL SPACE INVOLVED

Site of abscess	Cases N=50	Percentage
Submandibular Space	18	36
Buccal space	10	20
Submental space	7	14
Sublingual space	5	10
Massetric space	3	6
Canine space	2	4
Submandibular+ sublingual space	3	6
Submandibular+ Sublingual+ Submental space	2	4

Graph - 3: Distribution of cases according to maxillofacial space involved



In the present study

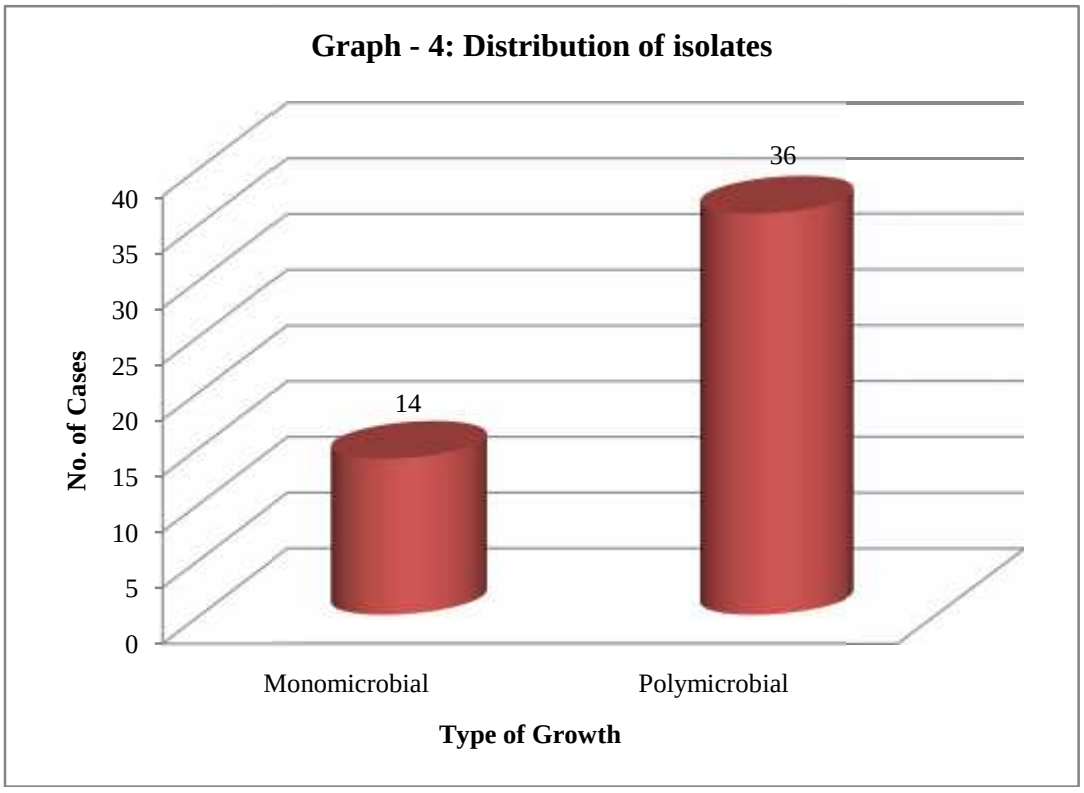
- The submandibular space was the most common space involved 23(46%) in maxillofacial infections.
- The least common space involved was canine space 2(4%).
- While multiple space involvement was seen in 5(10%) of cases.

ISOLATES OBSERVED - Pus samples from all 50 cases yielded growth on culture.

A total number of 97 isolates were obtained from 50 samples.

TABLE 4-DISTRIBUTION OF ISOLATES

Type of Growth	Number(50)	Percentage (%)
Monomicrobial	14	28
Polymicrobial	36	72

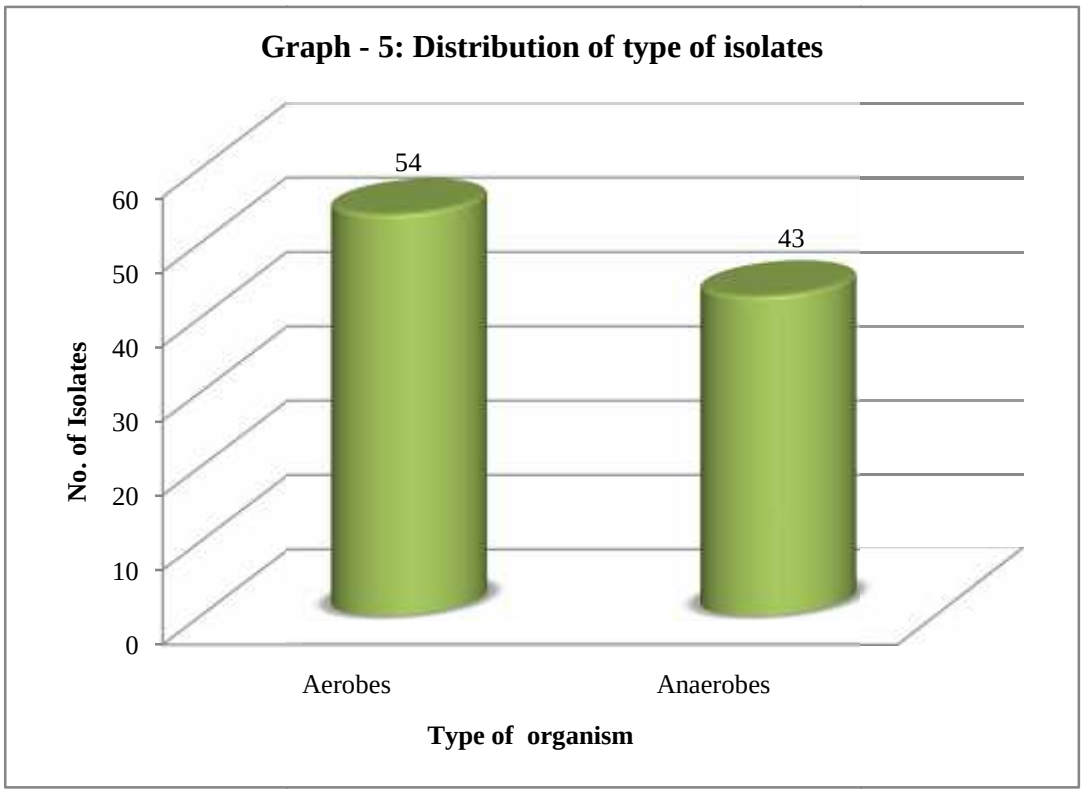


In the present study –

- Polymicrobial growth was present in 36(72%) samples.
- Monomicrobial growth was present in 14(28%) samples.

TABLE 5: TYPE OF ISOLATES

Type of organism	Number (97)	Percentage (%)
Aerobes	54	52.38
Anaerobes	43	47.61



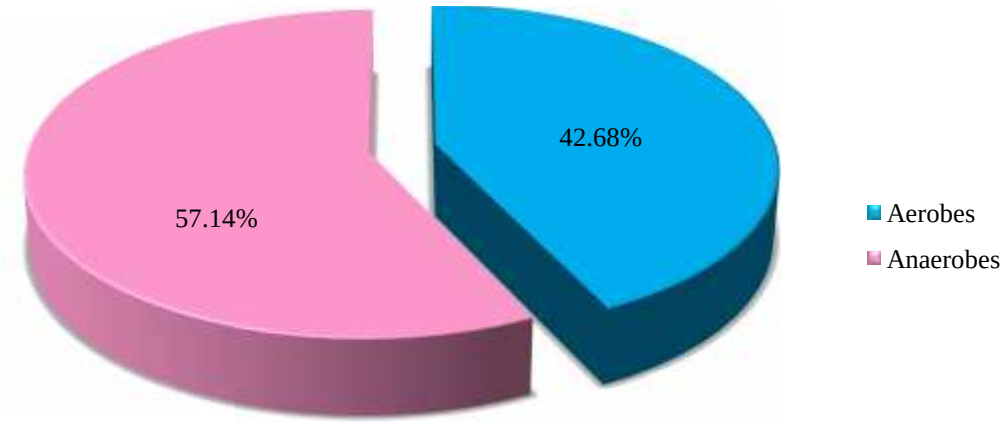
In the present study

- Among 50 samples aerobes had grown in 54(52.38%) cases
- While anaerobes was present in 43(47.61%) cases.
- The mean number of isolates per positive culture was almost 1.9

TABLE 6: DISTRIBUTION OF MONOMICROBIAL ISOLATES

Distribution of monomicrobial isolates	Number (14)	Percentage
Aerobes	6	42.86
Anaerobes	8	57.14

Graph - 6: Distribution of monomicrobial isolates

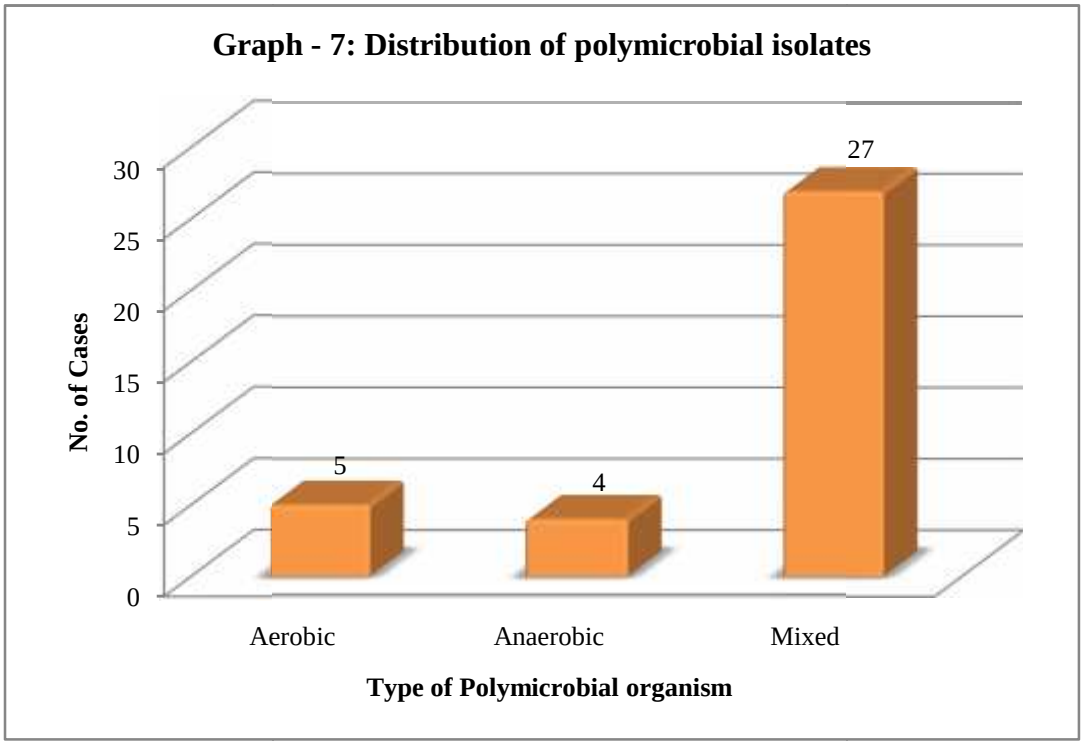


In the present study

- Out of total 14 monomicrobial samples 8 (57.14%) samples had shown only anaerobic isolates.
- In 6 (42.16%) samples only aerobes had grown.

TABLE 7: DISTRIBUTION OF POLYMICROBIAL ISOLATES

Type of Polymicrobial organism	Number (36)	Percentage
Aerobic	5	13.88
Anaerobic	4	11.11
Mixed	27	75.0



In the present study-

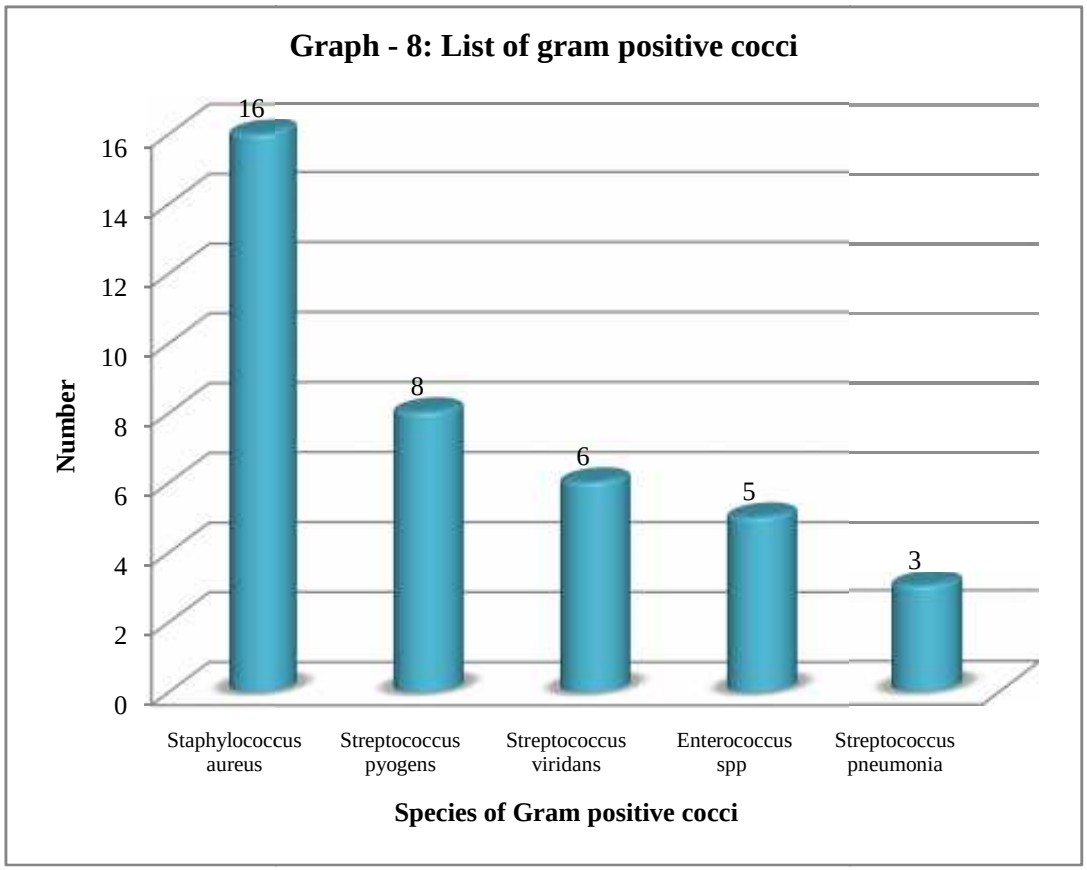
- Polymicrobial growth – Out of 36 cases of which only anaerobes and aerobes was present in 4 and 5 samples respectively with synergy of two isolates.
- In 27 (75%) samples there was mixed infection by both aerobes and anaerobes. Out of these 27 samples, 17(62.9%) samples had two isolates and 10(27.03%) samples had three isolates three.
- In only 1 case four isolates was present.

CULTURE OF AEROBIC ISOLATES –

Out of total 50 samples, 54 aerobic organisms were isolated .Gram positive cocci were in 43(75.9%) samples and gram negative bacilli were 13(24.07%).

TABLE 8: DETAILS OF GRAM POSITIVE AEROBIC ISOLATES

Species of Gram positive cocci	Number N=41	Percentage (%)
Staphylococcus aureus	16	39.0
Streptococcus pyogens	9	21.9
Streptococcus viridans	8	19.5
Enterococcus spp	5	12.19
Streptococcus pneumoniae	3	7.31

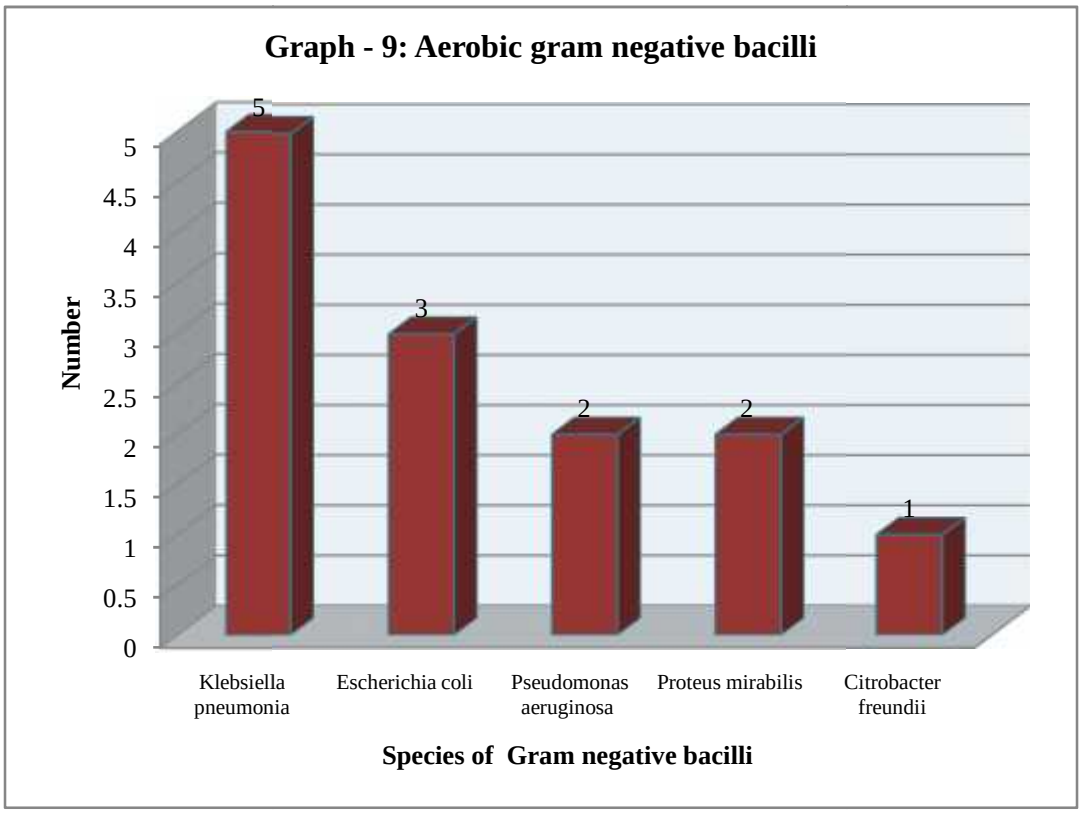


In the present study

- Among gram positive isolates *Staphylococcus aureus* was the *commonest organism* isolated followed by *Streptococcus pyogens.*
- Among these facultative anaerobes *S.aureus* (8), *Streptococcus pyogens (6)* and *Streptococcus viridians* (4) were common species found in synergism with obligate anerobes.

TABLE 9: AEROBIC GRAM NEGATIVE BACILLI

Species of Gram negative bacilli	Number N= 13	Percentage
Klebsiella pneumonia	5	38.4
Pseudomonas aeruginosa	3	23.0
Escherichia coli	2	15.3
Proteus mirabilis	2	15.3
Citrobacter freundii	1	7.69

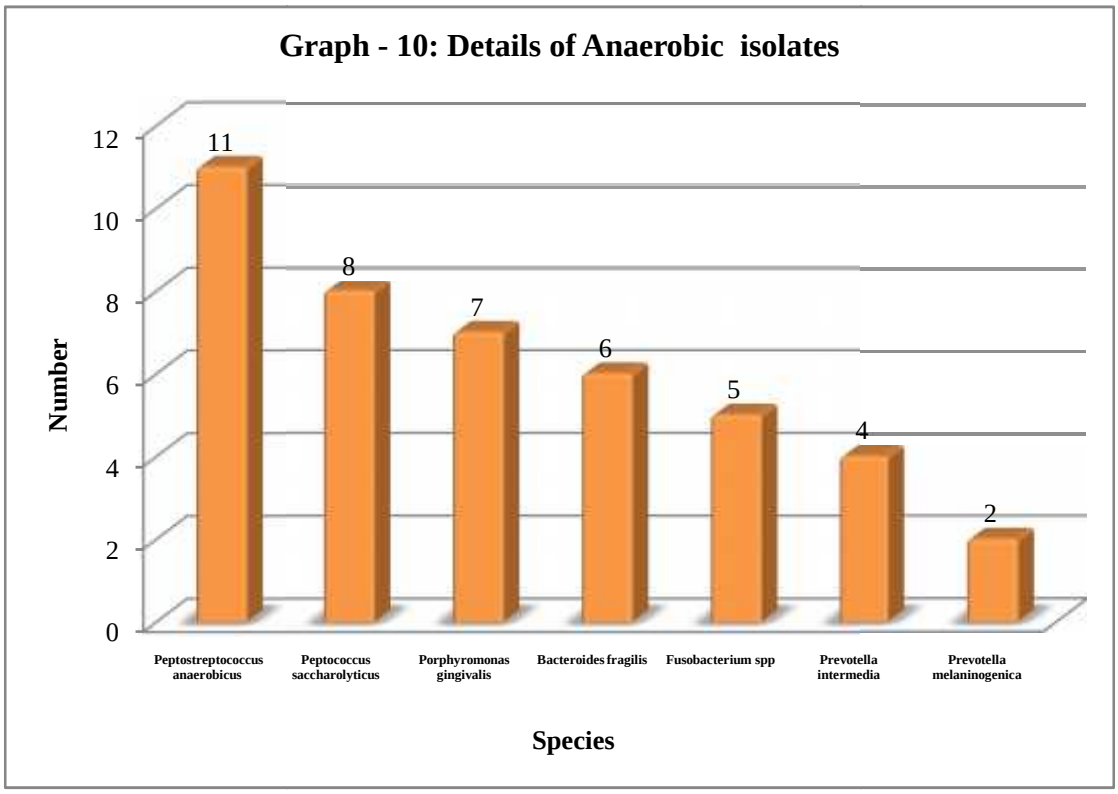


In the present study-

- *Klebsiella pneumonia* was the commonest gram negative bacilli isolated 5(38.4%) followed by Pseudomonas aeruginosa 3(23%).

TABLE 10 - CULTURE OF ANAEROBIC ISOLATES

Species	Number (43)	Percentage %
Peptostreptococcus anaerobicus	11	25.58
Peptococcus saccharolyticus	8	18.6
Porphyromonas gingivalis	7	16.27
Bacteroides fragilis	6	13.9
Fusobacterium spp	5	11.62
Prevotella intermedia	4	9.30
Prevotella melaninogenica	2	4.65



In the present study

- The most common organism was *Peptostreptococcus spp (43.1%)* followed by *P. gingivalis (6.27%) and B fragilis(13.9%).*
- Synergy was most commonly found in *Peptostreptococcus spp(10), P. gingivalis(6) and Fusobacterium(3)* with aerobic bacteria.

ANTIBIOTIC SUSCEPTIBILITY TESTING OF AEROBES-

Antibiotic susceptibility testing for aerobes was done by Kirby Bauer disc diffusion method for commonly used antibiotics whose results are as shown in the table 11a &11b.

TABLE 11a- ANTIBIOTIC SENSIVITY PATTERN OF AEROBIC GRAM POSITIVE COCCI–

		Antibiotic Sensitivity															
Isolate	**No. of Isolate**	**Amp**		**Amx**		**Cip**		**Ac**		**Caz**		**Cu**		**E**		**V**	
		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
S.aureus	16	5	11	7	9	13	3	14	2	11	5	9	7	6	10	-	-
S pyogenes	9	2	7	3	6	7	2	7	2	6	3	4	5	5	4	-	-
S viridans	8	3	5	2	6	6	2	5	3	6	2	4	4	3	5	-	-
Enterococcus spp.	5	2	3	3	2	4	1	5	0	4	1	1	4	3	2	4	1
S. pneumoniae	3	2	1	2	1	2	1	3	0	2	1	2	1	1	2	-	-

In the present study-

- *Staphylococcus aureus* has shown high resistance to Ampicillin (68.75%) and Amoxicillin (43.75%).
- Amoxicillin and Clavulanic acid combination performed better, as 87.5% Staphylococcus strains were sensitive to it.
- *Staphylococcus aureus* isolates had a high sensitivity to third generation cephalosporin ceftazidime (68.75%) and ciprofloxacin(81.25%).
- Results showed very low sensitivity to macrolide group. Only 43.1% gram positive cocci were found sensitive to Erythromycin.
- Only 1 (20%) of isolates of *Enterococcus spp* was found to be resistant to Vancomycin.

TABLE 11b- ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE BACILLI –

Organism	Antibiotic Sensitivity	Amp		Cu		Ak		Caz		Ac		Cip		Pt	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
K. pneumoniae	5	1	4	3	2	4	1	4	1	4	1	4	1	-	-
P aeruginosa	3	1	2	0	3	1	2	3	0	3	0	2	1	2	1
E coli	2	1	1	2	0	1	1	2	0	2	0	2	0		
P mirabilis	2	1	1	1	1	2	0	1	1	2	0	1	1	2	0
Citrobacter sps	1	1	0	0	1	1	0	1	0	1	0	1	0	-	-

In the present study

- 70.3% of Gram negative bacteria was found to be sensitive for ampicillin.
- Gram negative bacteria had higher sensitivity (84.6%) to ceftazidime than to cefuroxime in 46.1% isolates.
- Only 1 isolate of *P. aeruginosa* was resistant to Piperacillin-tazobactam.
- Gram negative isolates had higher sensitivity to Amikacin and amoxyclave.

ANTIBIOTIC SENSIVITY OF ANAEROBES -

Susceptibility to Metronidazole and clindamycin was done by agar dilution method. *Bacteroides fragilis* ATCC 25285 was used as a control strain .MIC of this strain for clindamicin is 0.5 - 2µ/ml and for metronidazole is 0.25 -1µ/ml.

TABLE 12a- SUSCEPTIBILITY PATTERN OF METRONIDAZOLE

Organism	Sensitive 8 µg/ml	Intermediate 16µg/ml	Resistant 32µg/ml
Peptostreptococcus anaerobicus n =11	11	0	0
Peptococcus saccharolyticus n = 8	8	0	0
Porphyromonas gingivalis n = 7	7	0	0
Bacteroides fragilis n = 6	6	0	0
Fusobacterium spp n = 5	5	0	0
Prevotella intermedia n = 4	4	0	0
Prevotella melaninogenica n = 2	2	0	0

In the present study-

- None of the isolates was found to be resistant to metronidazole. Most of the isolates was sensitive at mic of range 0.5 -2 µg/ml.
- 2 isolates of *Fusobacterium spp* and *Peptostreptococcus anaerobicus* had higher mic 4 µg/ml

TABLE 12b - SUSCEPTIBILITY OF ISOLATES TO CLINDAMYCIN

Organism	Sensitive 2µg/ml	Intermediate 4µg/ml	Resistant 8µg/ml
Peptostreptococcus anaerobicus n =11	7	2	2
Peptococcus saccharolyticus n = 8	5	2	1
Porphyromonas gingivalis n = 7	5	1	1
Bacteroides Fragilis n = 6	3	1	2
Fusobacterium spp n = 5	4	1	0
Prevotella Intermedia n = 4	3	1	0
Prevotella melaninogenica n = 2	1	1	0

In the present study

- 28(65.11%) anaerobic isolates were sensitive to clindamycin while susceptibility of 9 (20.9%) isolates was intermediate.
- Only 6(13.9%) isolates were resistant to clindamycin
- Among the resistant isolates *Bacteroides fragilis* (2out of 6) followed by *Peptostreptococcus spp*. (3 out of 19) and *Porphyromonas gingivalis* (1 out of 5)
- Resistance was not found in *Fusobacterium spp*, and *Prevotella spp*.

DISCUSSION

Maxillofacial infections are a public health concern, mainly related with odontogenic origin, if ignored or ill treated at early stage can rapidly develop and spread to neighbouring anatomic structures, leading serious complications like airway obstruction, mediastinitis, septicemia, cavernous sinus thrombosis, jugular vein thrombosis, carotid aneurysm and shock. So prevention and prompt management is necessary in country like India where healthcare providers are inadequate in number and facilities are less.

Incision and drainage is the prime treatment for sure, but understanding of involved bacteriology and sensitivity pattern constitutes an important part of it [16]. Many a times even after proper surgical treatment patient condition fails to improve, one of the important reasons for this is resistant bacterial strains and selection of wrong antibiotics.

In the present study, the age of patients varied from 5 to 70 years with mean age 33.77 years . Reja et al studied 103 patients with facial space infections with an age range of 7 to 93 years with mean age of 33.3 years [8]. While Lee et al, found these infections were in the age range of 1 to 89 years with mean age of 33.4 years [70]. In another study by Suehara et al , mean age of facial space infections was 37.6 years[71] . Our studies correlate well with the findings of these studies.

Most of the patients24 (48%) in our study were adults in the age group of 31–50 years. This confirms the reports of previous studies that although children may acquire maxillofacial infections (10%), the majority occurs in adults in this age group.

The probable reason for adults being at higher risk is the neglect of oral health and the higher prevalence of systemic diseases that compromise immunity.

Children were found to have relatively less occurrence of infection of the primary facial spaces because the erupting permanent teeth resorb their roots making their length short. That is why primary teeth usually present odontogenic infection in the form of a gum boil rather than spreading to the fascial spaces.

Among the 50 cases ,33(66%) was females and 17(34%) males with male to female ratio of 1:1.94 approx. Rehman et al reported in his study that 63.1% males and 36.9% females had Odontogenic maxillofacial infections with male to female ratio of 1.7:1 [72] . In another study by Poeschl et al males were predominant, having primary facial space infections more than females with the ratio of 1.4:1 [73]. The female predominance in the present study may be due to their high pain threshold, socioeconomic reasons and cultural restrictions where people have reluctance to take their females patients to the dentist in this part of world. The illtreated or ignored dental disease leads to abscess formation in them.

Rehmann et al studied odontogenic infections and found that these infections occurred most commonly in the 3rd decade followed by 4th decade of life[72].While Seuhara et al observed that most of the patients with facial space infections presented in the 3rd decade followed by 5th and 4th decade [71]. Our findings are also consistent with these studies.

In the present study submandibular space was most commonly involved space infected in 23(46%) cases followed by buccal space in 10 (20%). Multi space involvement was present in 5 (10%) of the cases in which submandibular space was

commnest as found in single space involvement. Rega et al in his study reported that submandibular space was involved in 30% cases followed by buccal space which was involved in 27.5% cases [8].

A different pattern was observed by Bridgeman et al, where buccal space (52.6%) was the most common space followed by submandibular space (24%) in the study[74]. Labriola et al reported 24% of their patients presented with submandibular space infections and 20% with buccal space infections [75].Thus in majority of studies submandibular space was most commonly infected spece.

Maxillofacial infections are considered to be polymicrobial in nature caused by both aerobes and anaerobes [74,76] . Our study also showed polymicrobial infections in 36 (72%) of cases as compared to monomicrobial in 14(28%) cases. Among 14 (28%) monomicrobial isolates , 8(16%) pus samples yielded only anaerobic bacteria and 6(12%) only aerobic bacteria. Among 36 polymicrobial samples 27 samples showed mixed infection caused by both aerobes and anaerobes. While synergy between two anaerobes and two aerobes was found in 4 (8%) and 5(10%) of cases respectively. Earlier studies from other parts of the world have reported about mixed anaerobic flora in orofacial infections as found in our study.[2,43,43]

According to literature available aerobic predominance was seen initially which later on turned towards anaerobes[37,54]. Results by various studies are contradictory till today. In our study aerobic isolates were 54 (55.67%) and anaerobes 43(44.32%) . A recent study by Anthony J. Reja et.al. demonstrated that aerobic organisms outnumbered anaerobes by almost 2:1 ratio[8] . All most same result was obtained by Kohli M showing 66% aerobes in the study[2]. These results was

contradictory to many studies who showed anaerobic predominance in the study[42,43,77,78] .

Our study showed out of 54 aerobes isolates , 41(75.9%) gram positive cocci were present in pus samples . Looking at aerobic population it was found that *Staphylococcus aureus* was the most commonly16 (39%) isolated species followed by *Streptococcus pyogens* 9(21.9%) and *Streptococcus viridans* 8(19.19%). The frequent isolation of staphylococci in pus samples from odontogenic infections have been reported in previous studies [2,10]. *Staphylococcus aureus* is not a normal flora of oral cavity and may be important pathogenic organism in supprative non odontogenic infection of maxillofacial region. In contrast to our sudy mutiple studies demonstrated *Streptococcus viridans* as their predominant species[10,75] . Sakaguchi et al. and Kohli M reported 13.8%and 11% of *Streptococcus pyogenes* respectively in their study respectively[2,79] . In our study *Streptococcus pyogens* was the second commonest gram positive cocci (20.9%)isolated in pus samples. Among these aerobic most commonly synergism was found in *Staphylococcus aureus* (8), *Streptococcus pyogens* (6) and *Streptococcus viridians* (4) with the anaerobic bacteria .

Gram negative bacilli isolated were 13(24%) out of total 54 aerobic isolates. *Klebsiella pneumonia* 5(38.4%) was most the common gram negative bacilli followed by *Pseudomonas aeruginosa* 3 (23.0%). Other gram negative bacilli isolated were *Proteus mirabilis*, *Escherichia coli* and *Citrobacter freundii* .These gram-negative bacilli isolated in orofacial infections are likely key players in synergism with other bacterial species. *Klebsiella pneumonia* (4) was the most common aerobic gram negative bacteria found in synergism with anaerobes. In other studies also variable number of gram negative bacilli has been isolated [2,80]. Drug resistant pathogen like

Pseudomonas aeruginosa was Isolated from two canine space and one buccal space infection which may be due to inadvisable use of antibiotics in dentistry .

Isolation rate of anaerobes was 43(44.32%) from pus samples in our study. The detection rate of anaerobes from patients with deep space infections was relatively lower than that of observed by , Kuriyama T et al(2000) , Yuvraj V(2004), Ndukwe (2004), Pathak A (2012) and Osazuwa F (2010).[41,43,76,78,80] The isolation of anaerobic bacteria was less in the study could be because of the isolation of anaerobic bacteria requires adequate methods for collection, transportation and cultivation of clinical specimens.Second reason could be that they are fastidious in nature, anaerobes are hard to isolate and are often not recovered from the infected sites .However the different studies has shown 30-90 % isolation rate of anaerobes in their studies .[2,8,43,43,83]

Thus our study shows the coinciding results demonstrating isolation rate of 44.3%.The anaerobic flora isolated was predominantly of anaerobic streptococci in 19 isolates of (44%) along with 7 (16.27%) isolates of *Porphyromonas gingivalis*, (13.9%) of *Bacteroides fragilis* , 5(11.62%) of *Fusobacterium spp* and 6(14%) of *Prevotella spp* .

Mixed infection was found in 27(63%) cases involving both aerobes and anaerobes. Many investigators have demonstrated the mixed infections of Streptococcus *spp*, *Peptostreptococcus*, *Prevotella*, *Porphyromonas*, and *Fusobacterium* which were frequently isolated from orofacial odontogenic infections[57,78,81,82,83]. We have also found synersigm most commonly in anaerobic isolates of *Peptostreptococcus spp (10) Porphyromonas (6) and Fusobacterium (3),* with aerobic bacteria. The interactions of organisms within the microbial mixtures

leads to production of virulence factors such as hemolysins, proteases and collagenases that cause inflammation, healing and contribute to the chronicity of infection. In the study 8(16%) pus samples yielded pure anaerobic isolates. In the abscess this pure anaerobic organisms produce in the late stage of abscess formation[82].

The primary therapeutic modality for orofacial infections is surgical drainage of any pus collection, followed by extraction or endodontic therapy of the responsible tooth[57]. Although antibiotics are generally considered adjunctive therapy, their value should not be underestimated, especially when drainage cannot be achieved or the infection shows signs of local extension or systemic involvement. In clinical infections the consequences of inadequate antibiotic coverage may be both serious in nature and rapid in onset and hence it is essential that the possibility of the responsible organisms being resistant to the chosen antibiotic is minimized. Therefore, when orofacial odontogenic infections are treated with antibiotics for both aerobes and anaerobes due to their polymicrobial and mixed in nature.

Antibiotic susceptibility testing-

In our study susceptibility of aerobic isolates was tested for commonly used antibiotics in daily practise. While susceptibilty testing of anaerobic isolates was done for metronidazole and clindamycin .

Antibiotic susceptibility testing for aerobes-

Penicillin - Historically, the penicillins have been used as first-line agents in the treatment of odontogenic infections. We found that GPC were resistant to ampicillin and amoxicillin in 65.8% cases and 43.9.3% cases respectively. Gram

negative bacteria was found to be 70.3% resistant to ampicillin. In contrast to our study recently a study in 2006 quoted penicillin resistance to be seen in 19% of all strains[8]. Most common resistant strains was staphylococcus group in the study. Only 68.7% isolates of *Staphylococcus aureus* was sensitive to ampicillin and 56.25% isolates for amoxicillin. Anthony J Rega in (2006) and Kohli M (2009) showed resistant *Staphylococcus* 72.6% and 93.75% respectively to penicillin in the study[2,8]. Other gram positive cocci had susceptibility in the range of 50 -68%. Susceptibility pattern of other gram positive cocci was comparable to other studies showing the range of 40-75% .[2,83,84] .

Semisynthetic penicillins like amoxicillin with clavulanic acid have been proved to be better than penicillin alone. Our study found the same as 83.0% gram positive isolates were susceptible to this combination. These results was supported by the findings of Anthony J. Reja et.al. (2006)[8] and Chunduri NS (2012)[83].

Since 1980s the effectiveness of erythromycin has decreased and their use in the treatment of maxillofacial infections are very limited. We found only 43.1 % of gram positive cocci responding to erythromycin. This coincides with the susceptibility results available in the literature [2,76].

Starting from first generation, the cephalosporins have travelled a long distance reaching fifth generation in the present time. The reason of this evolution is obvious: resistant infections. This study saw a difference in the sensitivity of second and third generation cephalosporins too. Cefuroxime (second generation) had very low sensitivity (48.7%) as compared to ceftazidime (70.7%) (third generation) in gram positive isolates. Gram negative isolates showed susceptibility 46.1% and 84.6% to cefuroxime and ceftazidime respectively . The first and second generation

oral cephalosporins, while having a significantly broader spectrum of activity, do not offer any advantage over penicillin in treating odontogenic infections. Because they are generally unpredictable in their activity against anaerobic gram-negative rods.[57]

One of the first drugs from the quinolone group, ciprofloxacin has been replaced by its advanced versions like gatifloxacin and moxifloxacin these days. Still, the studies done in last five years show their relevance in current scenario [8,10,83] We too found ciprofloxacin as potent as third generation cephalosporins. In our study 78% gram positive organism were sensitive to this antibiotic. Gram negative isolates showed sensitivity of 76.9%. Anthony J. Rega et.al. (2006) and Kohli M (2009) showed the sensitivity of 95% and 100% respectively to aerobic isolates[2,18]. Looking at all these datas in support, the importance of this drug can not be ignored.

Resistant infections of hospital ICUs are being treated by higher antibiotics like amikacin and pipracillin- tazobactam these days. In case of maxillofacial infections when we encounter life threatening infections, one has to switch to these options as a last resort. . A while to Same has been postulated from past observations by series of case reports of lethal infections like cervical necrotizing fasciitis[79,82]. This study revealed 100% sensitivity of all microbes to pipracillin- tazobactam and 10 (76.9%) sensitivity to Amikacin. Vancomycin is also used for resistant gram positive isolates specially for *Staphylococcus aureus* and *Enterococcus* in hospitals. In our study 4 (80%) isolates of *Enterococcus spp*. were sensitive to Vancomycin. Reja et al and Kohli M showed that 100% and 56.25% of gram positive cocci were sensitive to Vancomycin respectively[2,8] .

Antimicrobial susceptibility of anaerobes-

Metronidazole- Metronidazole is a bactericidal agent that is highly active against most of the anaerobes, but it lacks activity against aerobic bacteria. The combination of penicillin (or an aminopenicillin) with metronidazole adequately covers the microbial flora of odontogenic abscesses, since metronidazole compensates for the limited activity of penicillin against b-lactamase producing strains of anaerobic bacteria[85]. Resistance among anaerobic pathogens is still generally low; however, the susceptibility patterns of anaerobic bacteria are undergoing changes, and decreases in in vitro susceptibility to various antimicrobials have been reported in recent years.

In our study none of the isolates was found to be resistant to metronidazole. Most of the isolates was sensitive at mic of range 0.5 -2 µg/ml. Two isolates of *Fusobacterium spp* and one isolate of Bacteroides *fragilis* had higher mic of 4 µg/ml**.**

The first metronidazole-resistant *Bacteroides* strain was reported in 1978 [86]. Metronidazole resistance among this group is generally lower than 1%, but levels up to 7.5% were reported in the United Kingdom in 1998 [87]. Compared with metronidazole resistance rates of 1.9% in 1995 and 3.8% in 1997, the 7.5% rate could have represented a possible increase in resistance in *B. fragilis* that was achieved with a MIC _32 mg/L. Reduced susceptibility to metronidazole with MICs of 4– 16 mg/L is more frequent (up to 4.5%), indicating the presence of resistance mechanisms [88]. Despite the low levels of resistance to metronidazole, treatment failures attributed to metronidazole resistance have been reported, and multi drug resistant strains have been identified[89,90]. Kuriyama T et al showed 100 % sensitive *Peptostreptococcus* spp.isolates with the mic range of 0.5– 2 mg/L[44] .Our study showed the same result in *Peptostreptococcus spp*. isolates [42].

The practice in many laboratories of identifying obligate anaerobes by susceptibility to metronidazole is a reason that contributes to possible underestimation of true resistance rates. Subsequently, the treatment of anaerobic infections is generally empirical and is based on published information of susceptibility rates, which emphasizes the importance of reference laboratories providing valid and updated information [83].

Clindamycin-

Clindamycin has excellent activity against gram positive organisms, including anaerobes and ß-lactamase producing strains Clindamycin's broad spectrum of coverage and excellent clinical efficacy, coupled with the increase in both penicillin resistance and the reports of treatment failures with penicillin, has prompted the Sanford Guide to Antimicrobial Therapy to replace penicillin V with clindamycin as the drug of choice in treating odontogenic infections.[51]

In our study the 28 (65.11%) anaerobic isolates were sensitive to clindamycin. While susceptibility of 9 (20.9%) isolates were intermediate with mic of 4µg/ml .Only 6(13.9%) isolates were resistant to clindamycin. Among these resistant isolates *Bacteroides fragilis* was most common (2 out of 6) followed by *Peptostreptococcus* spp (3 out of 19) and *Porphyromonas gingivalis* (1 out of 5).

Tomoari Kuriyama et al performed clindamycin susceptibility testing by agar dilution and showed that all anaerobic isolates was susceptible to clindamycin with the mic of 0.5 -1µg/ml. They also showed that some of isolates of *Peptostreptococcus spp*. had increased mic of 2 µg/ml[42] . Although CLSI has recommended Agar diltion test as a standard test for susceptibility testing of anaerobes studies were done by disc

diffusion method. In one of the study of Negria all the anaerobic isolates, *Peptostreptococcus spp, Prevotella spp., Fusobacterium spp and Porphyromonas spp* was sensitive to clindamycin antibiotic done by disc diffusion method [76] . In another Indian study disc diffusion method was performed for anaerobic antibiotic susceptibility testing. They have found 34 (92%) isolates of *Prevotella*, 23 (100%) of *Peptostreptococcus*, 11 (92%) of *Fusobacterium*, and 6(100%) of *Porphyromonas spp* were sensitive to clindamycin.[83]

CONCLUSION

Our study showed that the microbiological flora of orofacial abscess consists of complex mixture of aerobic and anaerobic bacteria and polymicrobial in nature. Aerobes were dominated bacterial population over anaerobes, in contrast to the recent studies .Gram positive cocci were more common in aerobic isolates while in anaerobic isolates gram negative bacilli were common. Most commonly isolated organisms were Peptostreptococcus and Staphylococcuc aureus in anaerobes and aerobes respectively.

Aerobic isolates showed high sensivity to Ciprofloxocin , Amoxyclave and cephalosporins. Penicillin resistance was within the expected limits as mentioned before. Metronidazole and clindamycin are still effective to treat anaerobic infections. The study clearly indicated when orofacial odontogenic infections are treated with antibiotics, an antimicrobial spectrum against both aerobes and anaerobes may be required.

Importance of meticulous drainage of the infected site can not be ignored and this should be supported by proper antibiotic therapy based on culture & sensitivity reports. These measures helps the clinician to provide an evidence based therapy for the patient improvement & also avoid going for the empirical therapy which is resulting in over usage of the drugs & emergence of resistance to various antibiotics. Time to time analysis of bacterial strains and resistance pattern should be a continuous process, so that we do not lag behind at the latest changes. Considering the inadequate data on bacteriological flora in the Indian population, further studies for assessment of bacteriological profile and sensitivity pattern in various forms of maxillofacial infections should be carried out.

SUMMARY

The present study was conducted on 50 patient of maxillofacial abscess in the department of Microbiology, J.N. Medical College, Belgaum for a period of 1 year from January 2012 to December 2012.

- The samples were collected from outpatient of Oral and Maxillofacial department of V.K. Institute of Dental Sciences, Belgaum.
- Our cases ranged between 5 to 70 years. Maximum numbers of cases were in the age group of 31-50 years and minimum number of cases seen in 1-10 years. Male to Female ratio observed in our study is M: F- 1:1.94.
- Submandibular space was most commonly involved in 46% followed by buccal space in 20% cases. 50 samples yielded 97 isolates of which 72% were polymicrobial, 28% were monomicrobial.
- Out of 97 isolates 44.3% were anaerobic, 55.37% were aerobic.
    - In aerobic population, Staphyloccus aureus was the most commonly (39%) isolated species followed by Streptococcus pyogens (21.9%) among gram positive cocci. While among gram negative bacilli, most common bacilli was Klebsiella pneumoniae 5(38.4%) followed by Pseudomonas aeruginosa 3(23.0%).
- In anaerobic population, Peptostreptococcus spp. was most common 19(44%) followed by Porphyromonas gingivalis 7(16.27%).

- Staphylococcus aureus isolates had a high sensitivity to third generation cephalosporin ceftazidime (68.75%), amoxyclave (83%) and ciprofloxacin (75%). Other gram positive isolates had high susceptibility to Amoxyclave, ciprofloxacin and ceftazidime.

- All anaerobic isolates was susceptible to metronidazole with the mic range of 0.5-2 µg/ml. In only two isolates of Bacteroides fragilis and Fusobacteriim spp. mic was up to 4 µg/ml.
- In the study 28 (65.11%) anaerobic isolates was susceptible to clindamycin while 9 (20.9%) isolates had intermediate susceptibility with mic of 4 µg/ml. Only 6(13.9%) anaerobic isolates were resistant .Among these resistant isolates Bacteroides fragilis (2) was the commonest followed by Peptostreptococcus spp.
    - In this study we saw maxillofacial space infection are polymicrobial and mixed involving both aerobes and anaerobes. Other findings such as predominance of Gram positive bacteria and sensitivity patterns were almost the same reflecting the relevance of ciprofloxacin, ceftazidime and amoxyclave in the treatment of maxillofacial infections.
    - Metronidazole and clindamycin are effective antibiotic to treat anaerobic infection and should be given in combination to cover aerobic bacteria.

BIBLIOGRAPHY

1. Simpson AJH. Rational antibiotic therapy. Surgery 2002;20(8):177–9.
2. Kohli M, Mathur A, Kohli M, Siddiqui SR. In Vitro evaluation of microbiological flora of orofacial infections. J Maxillofac Oral Surg 2009;8(4):329–33.
3. Moenning JE, Nelson CL, Kohler RB. The microbiology and chemotherapy of odontogenic infection. J Oral Maxillofac Surg 1989;7:976-85.
4. Topazian RG, Goldberg MH, Hupp JR. Oral & Maxillofacial Infections. 4th edition. W.B. Saunders company. 2002.
5. Singh T.P., Sanju B, Kalsey G., Singla RK. Applied Anatomy of Fascial spaces in Head and neck. J Anat Soc. India. 2000;49(1): 78-88.
6. Akinbami BO. Aetio-Pathogenesis and Clinical Pattern of Orofacial Infections. Maxillofacial Surgery. Prof. Leon Assael (Ed.) InTech. 2012.
7. Oliver ER, Gillespie MB. Deep neck infections. Cummings otolaryngology-head and neck surgery. 5th edition. Philadelphia. Elsevier. 2010.
8. Reja AJ, Shahid RA, Ziccardi VB. Microbiology and sensitivity of Head and neck space infections of odontogenic origin. J Oral Maxillofac Surg 2006;64:1377-80.
9. Wang J . Ahani A. Pogrel MA. A five year retrospective study of odontogenic maxillofacial infections in a large urban public hospital. Int . Oral Maxillofac Surg. 2005 ;34:646-9.
10. Storoe W, Haig RH, Lillich TT. The changing face of the odontogenic infections. J Oral Maxillofac Surg 2001;59:739-48.
11. Fehrenbach MJ. Spread of dental infection. Practical Hygiene. 1997;13-18.

12. Mahmmod M, Mahmood S. Odontogenic Neck Infections . TAJ 2005; 18(1):55-9.

13. Forbes BA, Sahm DF, Wiessfield AS. Bailey and Scott's Diagnostic Microbiology. 11th edition. St. Louis Missouri. Mosby Inc. An affiliate of Elsevier Science. 2002.

14. Willis AT, Anaerobic Bacteriology. Clinical and Laboratory Practice. 4th edition.

15. Dahlen G, Olsen I. Salient virulence factors in anaerobic bacteria, with emphasis on their importance in endodontic infections. Endodontic Topics 2004;9:15–26.

16. Panagiotis K, Stefanopoulos DDS, Alexandros E. The clinical significance of anaerobic bacteria in acute orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:398-408.

17. Brook I. Isolation of capsulate anaerobic bacteria from orofacial abscesses. J Med Microbiol 1986;22:171-4.

18. Brook I. The role of encapsulated anaerobic bacteria in synergistic infections. FEMS Microbiol Rev 1994;13:65–74.

19. Laine ML, Appelmelk BJ, van Winkelhoff AJ. Prevalence and distribution of six capsular serotypes of Porphyromonas gingivalis in periodontitis patients. J Dent Res 1997;76:1840–44.

20. vanWinkelhoff AJ, Appelmelk BJ, Kippuw N, de Graaff J . K-antigens in Porphyromonas gingivalis are associated with virulence. Oral Microbiol Immunol 1993;8:259–65.

21. Xie H, Kozlova N, Lamount RJ. Porphyromonas gingivalis genes involved in fim A regulation. Infect Immun 2004;72:651–8.

22. Agani G, Tricot-Doleux S, Houalet S, Bonnaure-Mallet M. Epithelial cell surfaces involved in the polyvalent adherence of Porphyromonas gingivalis: a convincing role for neuraminic acid and glucuronic acid. Infect Immun 2003;71:991–6.

23. Bolstad AI, Jensen HB, Bakken V. Taxonomy, biology and periodontal aspects of Fusobacterium nucleatum. Clin Microbiol Rev 1996;9:55–71.

24. Chateau M, Holst E, Bjorck L. Protein PAB, an albumin-binding bacterial surface protein promoting growth and virulence. J Biol Chem 1996;271: 26609–15.

25. Dorn BR, Leung KL, Progulske-Fox A. Invasion of human oral epithelial cells by Prevotella intermedia. Infect Immun 1998;66:6054–7.

26. Duerden BI. Virulence factors in anaerobes. Clin Infect Dis 1994;18(4):S253-9.

27. Murakami Y, Hanazawa S, Tanaka S, Iwahashi H, Yamamoto Y, Fujisawa S. A possible mechanism of maxillofacial abscess formation: involvement of Porphyromonas endodontalis lipopolysaccharide via the expression of inflammatory cytokines. Oral Microbiol Immunol 2001;16:321-5.

28. Bartlett JG. Anaerobic bacteria in Infectious diseases. Gorbach SL, Bartlett JG, Blacklow NR, editors. 2nd ed. Philadelphia: Saunders; 1998. p. 1888-901.

29. Persson S, Edlund MB, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. Oral Microbiol Immunol 1990;5:195-201.

30. Carlsson J, Larsen JT, Edlund M-B. Peptostreptococcus micros has a unique high capacity to form hydrogen sulfide from glutathione. Oral Microbiol Immunol 1993;8:42-5.

31. Murdoch DA. Gram-positive anaerobic cocci. Clin Microbiol Rev 1998;11:81-120.

32. Tally FP, Goldin BR, Jacobus NV, Gorbach SL. Superoxide dismutase in anaerobic bacteria of clinical significance. Infect Immun 1977;16:20-5.

33. Hofstad T. Pathogenicity of anaerobic gram-negative rods: possible mechanisms. Rev Infect Dis 1984;6:189-99.

34. Odell LJ, Baumgartner JC, Xia T, David LL. Survey for collagenase gene prtC in Porphyromonas gingivalis and Porphyromonas endodontalis isolated from endodontic infections. J Endod 1999; 25: 555–8.

35. Sorsa T, Ingman T, Suomalainen K, Haapasalo M, Konttinen YT, Lindy O, Saari H, Uitto VJ. Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast type interstitial collagenases. Infect Immun 1992;60:4491–5.

36. Krepel CJ, Gohr CM,Walker AP, Farmer SG, Edmiston CE. Enzymatically active Peptostreptococcus magnus: association with site of infection. J Clin Microbiol 1992;30:2330–4.

37. Goldberg MH. The changing biologic nature of acute dental infection. JADA 1970;80:1048.

38. Sims W. The clinical bacteriology of purulent oral infections. Br J Oral Surg 1974; 12: 1-12.

39. Sklavounos A, Legakis NJ, Ioannidou H, Patrikiou A. Anaerobic bacteria in dentoalveolar abscesses. Int J Oral Maxillofac Surg 1986;15:288-91.

40. Lewis MAO, MacFarlane TW and McGowan DA. Quantitative bacteriology of acute dentoalveolar abscesses. J Med Microbiol 1986; 21: 101- 4.

41. Kuriyama T, Karasawa T, Nakagawa K. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90(5):600-8.

42. Kuriyama T, Karasawa T, Nakagawa K. Bacteriology and antimicrobial susceptibility of gram-positive cocci isolated form pus specimens of orofacial odontogenic infections. Oral Microbiol Immunol 2002; 17(2):132-5.

43. Yuvraj V, Alexander M. Is the microflora of Oral & Maxillofacial infections changing? Int J Oral Maxillofac Surg 2007; 8(285): 1040.

44. Mathew G, Rangnathan L, Gandhi S. Odontogenic maxillofacial space infections at a tertiary care center in North India: a five-year retrospective study. Int J Infect Dis 2012;16:296–302.

45. Rotstein OD, Pruett TL, Simmons RL. Mechanism of Microbial Synergy in polymicrobial surgical infections. Review of infectious disease 1985;7 (2); 151-7.

46. Murray P.R., Rosenblatt J.E. Penicillin resistance and penicillinase production in clinical isolates of Bacteroides melaninogenicus. Antimicrob Agents Chemother 1977; 11:605-8.

47. Edson RS, Rosenblatt JE, Lee DT. Recent experience with antimicrobial susceptibility of anaerobic bacteria: increasing resistance to penicillin. Mayo Clin Proc 1982;57:737-41.

48. Heimdahl A, VonKonow L, Nord CE. Isolation of ß-lactamase producing Bacteroides strains associated with clinical failures with penicillin treatment of human orofacial infections. Arch Oral Biol 1980;25:689-92.

49. Witcher BL, Beirne OR, Smith RA. ß-lactamase producing Bacteroides melaninogenicus and osteomyelitis of the mandible. J Oral Med 1983;38:17-20.

50. Hackman AS, Wilkins TD. Influence of penicillinase production by strains of Bacteroides melaninogenicus and Bacteroides oralis on penicillin therapy of experimental mixed anaerobic infection in mice. Arch Oral Biol 1976;21:385-9.

51. Sanford JP, Gilbert DN, Moellering RC. Selection of initial empirical antimicrobial therapy on clinical grounds. The Sanford Guide to Antimicrobial Therapy. Sanford JP, Gilbert DN, Moellering RC, eds. 1996.

52. Karlowsky J, Ferguson J, Zhanel G. A review of commonly prescribed oral antibiotics in general dentistry. J Can Dent Assoc 1993;59:292-4,297-300.

53. Drlica K, Zhao X. DNA gyrase, topoisomerase IV and the 4-quinolones. Microbiol Mol Biol Rev 1977;61:377-92.

54. Greenberg RN, James RB, Marier RL. Microbiologic and antibiotic aspects of infections in the oral and maxillofacial region. J Oral Surg 1979;37:873-84.

55. O'Donnell JA, Gelone SP. Fluoroquinolones. Infect Dis Clin North Am 2000;14:489-513

56. Sobottka I, Cachovan G, Sturenburg E. In vitro activity of moxifloxacin against bacteria isolated from odontogenic abscesses. Antimicrob Agents Chemother 2002;46:4019-21.

57. Edwards DI. Nitroimidazole—mechanism of action and resistance mechanisms. J Antimicrob Chemother 1993;31:9-20.

58. Flynn TR. The swollen face: severe odontogenic infections. Emerg Med Clin North Am 2000;18:481-519.

59. Kuriyama T, Karasawa T, Nakagawa K, Yamamoto E, Nakamura S. Incidence of b-lactamase production and antimicrobial susceptibility of anaerobic gram-negative rods isolated from pus specimens of orofacial odontogenic infections. Oral Microbiol Immunol 2001;16:10-5.

60. Finegold SM. Metronidazole. Mandell, Douglas and Bennett's principles and practice of infectious diseases. Mandell GL, Bennett JE, Dolin R, editors. 4th ed. Edinburgh. Churchill Livingstone; 2000. p. 361-6.

61. Sands T, Pynn BR. Odontogenic infections and clindamycin. UTDJ 1995; 32-3.

62. Kinder SA, Holt SC, Korman KS. Penicillin resistance in subgingival microbiota associated with adult periodontitis. J Clin Microbiol 1986;23: 1127-33.

63. Gilmore WC, Jacobus NV, Gorbach SL. A prospective double blind evaluation of penicillin versus clindamycin in the treatment of odontogenic infections. J Oral Maxillofac Surg 1988;46:1065-70.

64. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. (Eds). Mackie and Mc Cartney Practical Medical Microbiology, 14th Edn, Singapore: Churchill Livingstone 1989

65. Sutter VL, Citron DM, Edelstein M, Finegold SM. Wadsworth Anaerobic Bacteriology Manual. 4th edition. Star publishing company, California. 1985

66. Vaidhyalingam K, Laxminarayana CS. Internal Gas Generator System suitable for creating Anaerobiosis. Ind J Surg 1980;42:154-9.

67. Methods for antimicrobial susceptibility tests for anaerobic bacteria 6th edition. Approved standard NCCLS documents .M11-A6.Villanova:National committee for clinical laboratory standards;2004.

68. Glenmark, Mumbai.

69. Schwalbe R, Moore L, Goodwin A. Antimicrobial Susceptibility Testing Protocols. CRC press, Taylor and Francis group, New York.

70. Lee JK, Kim HD, Lim SC. Predisposing factors of complicated deep neck infection: An analysis of 158 cases. Younsei Med J 2007; 48: 55-62.

71. Sueharal AB, Gonçalves AJ, Alcadipani FAMC, Kavabata NK, Menezes MB. Deep neck infection: Analysis of 80 cases. Rev Bras Otorhinolaringol 2008; 74: 253-9.

72. Rahman ZA, Hamimah H, Bunyarit SS. Clinical patterns of orofacial infections. Annal Dent Univ Malaya 2005; 12: 18-23.

73. Poeschl PW, Spusta L, Russmueller G, Seemann R, Hirschl A, Poeschl E et al. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010; 110: 151-6.

74. Bridgeman A, Wiesenfeld D, Hellyar A, Sheldon W. Major Maxillofacial infections: An evaluation of 107 cases. Aust Dent J 1995; 40: 281-8.

75. Labriola DJ, Mascaro J, Alpert B. The microbiologic flora of orofacial abscesses. J Oral Maxillofac Surg 1983; 41: 711-4.

76. Ndukwe KC, Okeke IN, Akinwande JA, Aboderin AO, Lamikrah A. Bacteriology and antimicrobial susceptibility profile of agents of orofacial infections in Nigerians. Afr J Clin Exp Microbial 2004; 5(3): 272-7.

77. Huang TT, Tseng FV, Yeh TH, Hsu CJ, Chen YS. Factors affecting the bacteriology of deep neck infection: A retrospective study of 128 patients. Acta Otolaryngol 2006;126:396-401.

78. Pathak A, Gupta MK, Pillai A. Incidence of Anaerobic Bacteria in 118 Patients with Deep-space Head and Neck Infections from the People's University Hospital of Maxillofacial Surgery, Bhopal, India. J Orofac Res 2012;2(3): 121-6.

79. Sakaguchi M, Sato S, Ishiyama T, Katsuno S, Taguchi K. Characterization and management of deep neck infections. Int J Oral Maxillofac Surg 1997;26(2): 131–3.

80. Osazuwa F, Adebayo AO, Alli OAT, Osazuwa EO. Bacteriology Of Orofacial Infections In Gombe, Nigeria. Academia Arena 2010;2(12):82-4.

81. Peterson: Contemporary Oral and Maxillofacial Surgery. 4th ed. Chapt. 15; 344–55.

82. Flynn TR, Shanti RM, Levi MH. Severe odontogenic infections, part 1: Prospective report and part 2: Prospective outcomes study. J Oral Maxillofac Surg 2006; 64: 1093-1113.

83. Chunduri NS, Madasu K, Goteki VR, Karpe T, Reddy H. Evaluation of bacterial spectrum of orofacial infections and their antibiotic susceptibility. Ann Maxillofac Surg. 2012;2(1):46-50.

84. Flynn TR, Halpern LR. Antibiotic selection in head and neck infections. Oral Maxillofac Surg Clin North Am 2003;15:17-38.

85. Ingham HR, Eaton S, Venables CW, Adams PC. *Bacteroides fragilis* resistant to Metronidazole after long-term therapy. Lancet 1978; 1:214.

86. Brazier JS, Stubbs SL, Duerden BI. Metronidazole resistance among clinical isolates belonging to the *Bacteroides fragilis* group: time to be concerned? J Antimicrob Chemother 1999; 44:580–1.

87. Hedberg M, Nord CE. Antimicrobial susceptibility of *Bacteroides fragilis* group isolates in Europe. Clin Microbiol Infect 2003; 9:475–88.

88. Rotimi VO, Khoursheed M, Brazier JS, Jamal WY, Khodakhast FB. *Bacteroides* species highly resistant to metronidazole: an emerging clinical problem? Clin Microbiol Infect 1999; 5:166–9.

89. Wareham DW, Wilks M, Ahmed D, Brazier JS, Millar M. Anaerobic sepsis due to multidrug-resistant *Bacteroides fragilis:* microbiological cure and clinical response with linezolid therapy. Clin Infect Dis 2005; 40:e67–8.

90. Hecht DW. Anaerobes: antibiotic resistance, clinical significance, and the role of susceptibility testing. Anaerobe 2006; 12:115–21.

ANNEXURE – I

CONSENT FOR PARTICIPATION RESEARCH

We are requesting you to enrol yourself in a study **–"Bacteriological Study of Oral and Maxillofacial Infections with special reference to anaerobes"** conducted by **Dr. _____,** PG student in microbiology, Belgaum KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, KLE University, Belgaum.

You are 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 Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

The purpose of research is to guide clinicians for making rational decisions over the choice of antibiotics in the management of these infections.

PROCEDURE INVOLVED:

The Sample from the abscess shall be collected aseptically with the help of syringe and needle .When abscess is deep seated and site is difficult to reach, a sterile cotton swab shall be used to collect the sample.

RISKS AND BENEFITS:

There are no 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

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed in a conference, no information will be displaced 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 PARTICIP

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 to Dr. _____ (mobile no. _____)

In case you have any questions about your rights as a participant, you can contact Dr _____ Professor and Head of Department and Chairman, J.N. Medical College Institutional Ethical Committee for Human Subjects Research, Ph. _____ at J.N. Medical College, Belgaum.

CONSENT STATEMENT

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

Participants Name _____ Signature_____

Witness Name _____ Signature_____

Experimenters Name _____ Signature_____

Date :

Place:

ANNEXURE – II: PROFORMA

BACTERIOLOGICAL STUDY OF ORL AND MAXILLOFACIAL INFECTONS WITH SPECIAL REFERENCE TO ANAEROBES''

Name	:	Sex	:
Age	:	I.P. No.	:
DOA	:		
Address	:		

Presenting complaints

Extra oral examination:

Swelling

Mouth opening

Lymph nodes involved

Intraoral examination:

Oral hygiene

Teeth

Laboratory investigation:

Gram stain

Culture:

Blood agar

Mc conkey agar

A.Gram positive cocci:

ANTIBIOGRAM-

Gram negative bacilli:

Catalase **- Oxidase**

Indole **-citrate**

H_2S: **Mannitol fermentation**

Urease **-TSI media**

ANTIBIOGRAM

Anaerobic Culture

-Growth on Brucella Blood Agar with witamin K and Hemin-

-Colony morphology

-Pigment

-Haemolysis

-Pitting

-Antibiotic identification disc- Kanamycin - , Vancomycin-

Colistin - , SPS -

-Gram stain of individual colony

-Spot Indole test

-Catalase test

ANTIBIOGRAM-

Clindamycin-

Metronidazole-

MASTER CHART

S.n.	I.P. NO.	AGE	SEX	INFECTED SPACE	AEROBIC ISOLATE	Antibiotic susceptibility of aerobes										Anaerobes Isolate	Antibiotic susceptibility of anaerobes	
						Amp	V	Amx	Cip	Ac	Caz	Cu	E	Pt	Ak		Mt	Cd
1	452349	59	F	SL	S.aureus, Enterococcus	S/R	S/R	R/R	R/R	S/S	R/R	R/R	R/R	-	-	P.intermedia	S	S
2	452559	51	F	SMB	S.aureus, S.viridans	R/S	-	S/R	S/R	S/S	S/S	S/R	S/S	-	-	-		
3	452881	34	F	massetric	-											P.anaerobius	S	R
4	455731	45	F	SM	S.pyogens	S	-	R	S	S	R	R	S	-	-	-		
5	457209	42	F	SMB	S.aureus, Enterococcus	R/S	-/S	R/S	S/S	R/S	R/S	R/S	R/S	-	-	-		
6	457517	47	F	buccal	-											P.saccharolyticus	S	S
7	458312	12	M	buccal	-											P.anaerobius+B.fragilis	S	S/S
8	462230	49	M	SMB	,S.viridans	R	-	R	S	R	R	R	R	-	-	P.saccharolyticus	S	S
9	400628	58	M	buccal	-											P.saccharolytica+ P.intermedia	S	S/S
10	452310	48	M	SMB	k.pneumoniae+ Enterococcus	S/R	-/S	S/R	R/R	S/S	S/S	S/R	/R	-	S/-	Pophyromonas.sps	S	S
11	467331	52	M	SM	S.pyogens	S	-	R	R	S	S	R	R	-	-	Fusobacterium.sps	S	S
12	401077	55	F	SL	S.aureus,S.viridans	R/R		S/S	S/S	R/R	S/R	R/	R/R	-	-	P.anaerobius	S	S
13	473165	53	M	SMB	E.coli+S.aureus	R/R	-	S/S	S/R	S/S	S/R	S/R	S/S	-	S/-	P.anaerobius	S	I
14	473171	41	F	massetric	-											Fusobacterium.sps	S	I

15	474805	30	M	buccal	S.aureus,S.viridans	R/S		R/S	R/S	S/S	S/S	R/S	S/S	-	-	P.saccharolyticus	S	I
16	474987	62	F	SMB	-											-	S	
17	474807	30	M	SMB,SM,SL	S.aureus+S.pneumoniae	S/R	-	R/S	S/S	S/S	S/S	R/S	R/S	-	-	P.anaerobius	S	S
18	473633	41	F	SM	-											B.fragilis	S	S
19	474123	13	M	SMB,SM,SL	K.pneumoniae	R	-	-	S	R	S	R	-	-	R	Fusobacterium.sps	S	S
20	483677	41	F	buccal	S.aureus	R	-	R	S	S	S	R	S	-	-	-		
21	484031	42	F	SMB	S.pyogens+entercoccus	R/R	-	S/S	R/S	S/S	R/S	R/R	S/S	-	-	P.intermedia	S	R
22	485751	51	F	SL	-											P.anaerobius	S	S
23	486264	47	M	SMB	S.pyogens	R	-	S	S	R	R	S	R	-	-	Pophyromonas.sps	S	R
24	486879	52	M	SM	citrobacter freundii	S	-	-	S	S	S	R	-	-	S	P.anaerobius	S	I
25	2198501	32	F	SMB	P.aeruginosa	R	-	-	S	S	S	R	-	-	R	-		
26	489062	44	F	SMB,SM,SL	E.coli	S	-	-	S	S	S	S	-	-	R	B.fragilis	S	I
27	495135	28	M	SMB,SM,SL	-											B.fragilis+P.melaninogenica	S	S/I
28	495108	43	F	submental	S.pyogens	R	-	S	R	R	R	S	R	-	-	Pophyromonas.sps	S	S
29	495135	14	F	SMB,SM,SL	S.viridans	R	-	R	S	S	S	S	S	-	-	-		
30	425425	11	F	buccal	K.pneumoniae+S.aureus	R/R	-	R/S	S/R	S/S	R/S	R/R	S/-	R/-	-	-		
31	496744	40	F	SM	S.aureus	R	-	S	S	S	S	S	R	-	-	Fusobacterium.sps	S	S
32	497975	28	F	SMB	P.aeruginosa	S	-	-	R	S	S	R	-	-	S	P.saccahrolyticus	S	S
33	498591	39	M	SMB,SM,SL	-											P.intermedia+B.fragilis	S	S/R
34	499413	12	M	buccal	S.aureus	S	-	S	S	S	S	S	R	-	-	Pophyromonas.sps	S	I
35	502046	23	F	SMB	-											P.anaerobius	S	R

36	507215	40	M	buccal	S.aureus	R	-	R	S	S	S	R	S	-	-	S.saccharolyticus	S	I
37	507760	55	M	SM	S.pyogens+entercoccus	R/S	-/R	S/S	S/S	S/S	S/S	R/R	R/S	-	-	Pophyromonas.sps	S	S
38	509107	16	F	SMB	S.viridans	S	-	R/S	S	R	S	R	R	-	-	P.anaerobius	S	S
39	512043	46	F	SMB	P.mirabilis	R	-	-	R	S	R	R	-	-	S	-		
40	512050	11	F	SL	S.aureus+S.pneumoniae	S/S	-	R/S	S/S	S/S	R/S	R/S	R/S	-	-	Pophyromonas.sps	S	S
41	443863	54	F	buccal	S.pneumoniae	R	R	S	R	S	R	S	R	-	-	P.anaerobius	S	S
42	446150	56	F	SMB	S.pyogens	R	-	R	S	S	S	R	S			S.saccharolyticus	S	S
43	446321	48	F	cannine	S.aureus	R	-	S	S	S	S	S	R	-	-	Fusobacterium.sps	S	S
44	447152	8	F	SMB	-											P.melaninogenica	S	S
45	438417	42	F	submassetric	S.pyogens+P.mirabilis S	R/S	-	R/-	S/R	S/S	S/S	S/S	S/-	-	-/S	-		
46	453218	63	F	SM	P.aeruginosa	R	-	-	S	S	S	R	-	S	R	P.anaerobius	S	S
47	444993	56	F	SMB	K.pneumoniae	R	-	-	S	S	S	S	-	-	S	B.fragilis	S	R
48	445407	9	F	SL	S.aureus	R	-	R	S	S	S	S	S	-	-	Pophyromonas.sps	S	S
49	443456	43	F	buccal	S.pyogens	R	-	S	S	S	S	S	S	-	-	P.anaerobius	S	S
50	450759	66	F	SMB	S.viridans	S	-	R	S	S	S	R	S	-	-	P.saccharolyticus	S	R

KEY TO MASTER CHART

Ac	-	Amoxyclav
Ak	-	Amikacin
Cip	-	Ciprofloxacin
E	-	Erythromycin
Cu	-	Cefuroxime
Amp	-	Ampicillin
Pt	-	Piperacillin –tazobactam
V	-	Vancomycin
Mt	-	Metronidazole
Cd	-	Clindamycin
SMB	-	Submandibular space
SM	-	Submental space
SL	-	Sublingual space
S	-	Sensitive
I	-	Intermediate
R	-	Resistant

Introduction

Objectives

Review of Literature

Methodology

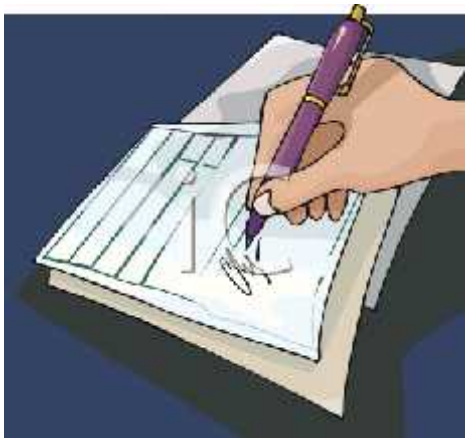
Results

Discussion

Conclusion

Summary

Bibliography



Annexure I:

Consent Form

Annexure II:

Proforma

Annexure III:

Master Chart