

Original Research Article

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Bacteriological study of Orofacial Space Infections and their Antibiotic Sensitivity Profile

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ABSTRACT

Orofacial space infections are among the most commonly encountered problems in dental practice. Spreading odontogenic infections are the most common type of serious oral and maxillofacial infections and range from the periapical abscess to superficial and deep neck abscess. The purpose of this study was to identify and perform antibiotic susceptibility pattern of aerobes and anaerobes isolates from oral and maxillofacial infections. Fifty patients with space infection of odontogenic origin were selected irrespective of their age and gender. Pus samples were collected and processed in the microbiology laboratory for the growth of anaerobic and aerobic bacteria and antibiotic sensitivity profile. Demographic profile of the patients showed that male patients were more commonly involved and most patients fell into the third and fourth decade of age groups. Submandibular space was most commonly involved in 46% followed by buccal space in 20% cases. *Staphylococcus aureus* was the frequent aerobic bacterial isolate and among anaerobes, *Peptostreptococcus* spp. was most common 19(44%) followed by *Porphyromonas gingivalis* 7(16.27%). Clindamycin, Gentamycin, Linezolid, Imipenem were the most effective antibiotics. 20 % of the aerobes were resistant to penicillin. There is the predominance of Gram positive bacteria and sensitivity patterns were almost the same reflecting the relevance of ciprofloxacin, ceftazidime and amoxiclav 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.

Keywords

Odontogenic infection,
Submandibular space,
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Orofacial Space
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Introduction

Orofacial space infection is most prevalent disease worldwide and is principle reason for seeking dental care. In health, oral microorganisms and human immune system are in ecological balance which is a pressure for sustaining a barrier against ingested pathogenic organism. These infections are mostly of odontogenic origin, as a sequel 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, 2] 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. [3, 4]. 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 a 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.

Materials and Methods

The present study was conducted at the Department of Microbiology at a tertiary care hospital. Fifty patients presenting with signs and symptoms of abscess of Oral and maxillofacial infections reporting to the department of Oral and Maxillofacial Surgery, over a period of one year from Jan 2012 to Dec 2012 were included in this study. All the patients of oro-maxillofacial infection with clinical evidence of abscess formation and not on antibiotics 48 hours prior to sampling were included.

The pus from the abscess was collected aseptically with the help of syringe and needle and when the abscess is deep and the site is difficult to reach sterile cotton swab was used to collect the sample. After collection, the sample was immediately transferred to thioglycollate broth. Direct smear was made by using a loop full of sample. The smear was stained by Gram's method [Hucker's modification] for an immediate presumptive diagnosis of the number and type of microorganisms present in the sample.

The culture was done for both aerobic and anaerobic bacteria. For aerobic culture sample was inoculated onto 5% Sheep blood agar, Chocolate agar and McConkey's agar. All the isolates were identified and characterised biochemically by standard procedures. [5] The antimicrobial susceptibility testing was done for aerobic isolates by disc diffusion method as described by Kirby and Bauer for Amikacin (30 mcg), Ampicillin (10mcg), Carbenicillin (100mcg), Ciprofloxacin (5 mcg), Amoxicillin / clavulanic acid (30 mcg), Ceftazidime (30 mcg), Erythromycin (15mcg), Cephoxitin (30mcg), Co-Trimoxazole (1.25/23.75mcg), Vancomycin (30mcg). Control strain used was *Staphylococcus aureus* ATCC 25923.

For culture for anaerobic organisms,[6] each sample was inoculated onto Blood agar supplemented with Haemin(5mcg/ml) and Vit K (10mcg/ml). Blood agar plates used for anaerobic isolation were prepared with Brucella agar base. Inoculated Chocolate agar plate incubated in a candle jar at 37 °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. Method used for obtaining anaerobiosis in the jar was "internal gas generating system" described by Lakshminarayana and Vaidhyalingam. [7] Cold catalyst consist of pellets of alumina coated with finely divided Palladium (Baker platinum Ltd., London) that is reactivated every time before use by drying at 150°C - 160°C for 1-2 hrs was used.

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 clindamycin is 0.5 - 2µ/ml and for metronidazole is 0.25 - 1µ/ml.

Results and Discussion

A total of 50 samples were collected from patients presenting with maxillofacial abscess from January to December 2012. The ages of the study population ranged between 5 and 100 years with male to female ratio of 1:1.94. Maximum numbers of cases were in the age group of 31-50 years and minimum number of cases seen in 1-10 years.

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%) (Figure 1). While multiple space involvement was seen in 5(10%) of cases. Pus samples from all 50 cases yielded growth on culture. A total number of 97 isolates were obtained from 50 samples. Polymicrobial growth was present in 36(72%) samples. Out of total 14 monomicrobial samples, 8 (57.14%) samples had shown only anaerobic isolates (Figure 2). 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. In only 1 case four isolates was present.

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%). Among gram positive isolates *Staphylococcus aureus* was the commonest organism isolated followed by *Streptococcus pyogenes*. Among these facultative anaerobes *S. aureus* (8), *Streptococcus pyogenes* (6) and *Streptococcus viridians* (4) were common species found in synergism with obligate anaerobes (Table 1). *Klebsiella pneumonia* was the commonest gram negative bacilli isolated 5(38.4%) followed by *Pseudomonas aeruginosa* 3(23%). The most common anaerobic organism was *Peptostreptococcus spp*

(25.58%). Synergy was most commonly found in *Peptostreptococcus spp.* (10), *P. gingivalis* (6) and *Fusobacterium* (3) with aerobic bacteria.

Staphylococcus aureus has shown high resistance to Ampicillin (68.75%) and Amoxicillin (43.75%). *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. 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 Amoxyclav.

In the present study none of the isolates was found to be resistant to metronidazole (Table 2). Most of the isolates were sensitive at MIC of range 0.5 -2 µg/ml. 2 isolates of *Fusobacterium spp* and *Peptostreptococcus anaerobicus* had higher MIC 4 µg/ml. 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 (Table 3). Among the resistant isolates *Bacteroides fragilis* (33.33%) followed by *Peptostreptococcus spp.* (15.79%) and *Porphyromonas gingivalis* (20%). Resistance was not found in *Fusobacterium spp.* and *Prevotella spp.*

Maxillofacial infections are a public health concern, mainly related with the odontogenic origin if ignored or ill-treated at the 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 are necessary for countries like India where healthcare providers are inadequate in number and facilities are less. Incision and drainage are the prime treatment for sure, but the

understanding of involved bacteriology and sensitivity pattern constitutes an important part of it. [8] Many 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.

Table.1 Culture of anaerobic isolates

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

Table.2 Susceptibility pattern of metronidazole

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

Table.3 Susceptibility pattern of clindamycin

Organism	Sensitive 2µg/ml	Intermediate 4µg/ml	Resistant 8µg/ml
<i>Peptostreptococcus anaerobicus</i> n =11	7	2	2
<i>Peptococcus saccharolyticus</i> n = 8	5	2	1
<i>Porphyromonas gingivalis</i> n = 7	5	1	1
<i>Bacteroides fragilis</i> n = 6	3	1	2
<i>Fusobacterium spp.</i> n = 5	4	1	0
<i>Prevotella intermedia</i> n = 4	3	1	0
<i>Prevotella melaninogenica</i> n = 2	1	1	0

Fig.1 Distribution of cases according to maxillofacial space involved

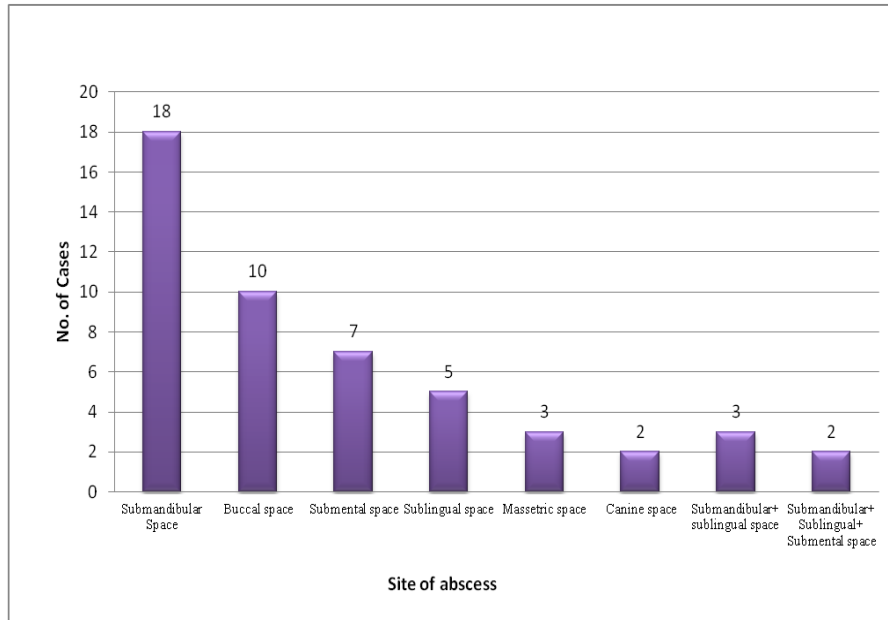
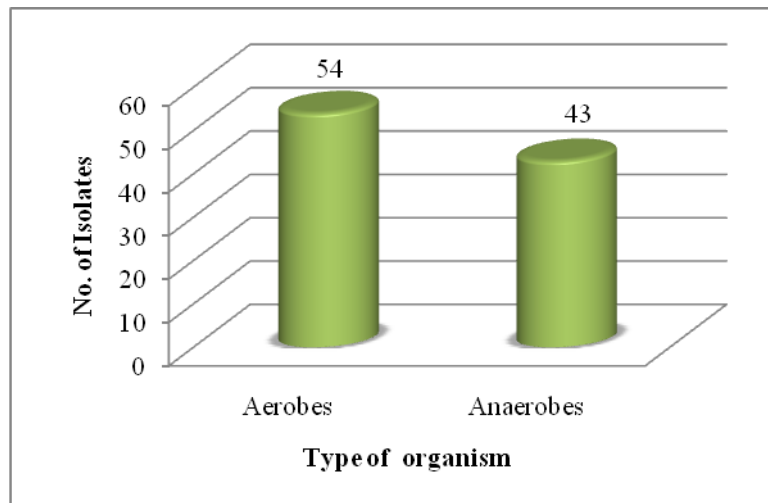


Fig.2 Distribution of type of isolates



In the present study, the age of patients varied from 5 to 70 years with mean age 33.77 years. Reja *et al.*, studied [9] patients with facial space infections with an age range of 7 to 93 years with mean age of 33.3 years. While Lee *et al.*, found these infections were in the age range of 1 to 89 years with mean age of 33.4 years. [10] In another study by the Suehara *et al.*, mean age of facial space infections was 37.6 years. [11] Our studies correlate well

with the findings of these studies. Most of the patients 24 (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.

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 the comment as found in single space involvement. Reja *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. [9] 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. [12] Maxillofacial infections are considered to be polymicrobial in nature caused by both aerobes and anaerobes. [12, 13] Our study also showed polymicrobial infections in 36 (72%) of cases as compared to monomicrobial in 14(28%) cases. Among 14 (28%) monomicrobial isolates, 16% pus samples yielded only anaerobic bacteria and 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 8% and 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, 14]

According to the literature available, aerobic predominance was seen initially which later on turned towards anaerobes. [15, 16] Results by various studies are contradictory till today. In our study, aerobic isolates were 55.67%

and anaerobes 44.32%. A recent study by demonstrated that aerobic organisms outnumbered anaerobes by almost 2:1 ratio. [9] These results were contradictory to many studies who showed anaerobic predominance in the study. [14, 16, 17, 18, 19]

Our study showed out of 54 aerobes isolates, 75.9% Gram positive cocci were present in pus samples. Looking at aerobic population it was found that *Staphylococcus aureus* was the most commonly 39% isolated species followed by *Streptococcus pyogenes* 21.9%. *Staphylococcus aureus* is not a normal flora of oral cavity and may be an important pathogenic organism in suppurative non-odontogenic infection of the maxillofacial region. In contrast to our study, multiple studies demonstrated *Streptococcus viridans* as their predominant species. [20, 21]

Isolation rate of anaerobes was 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 *et al.*, (2000), Yuvraj (2004), Ndukwe (2004), Pathak (2012) and Osazuwa (2010). [13, 14, 17, 19, 22] The isolation of anaerobic bacteria was less in the study could be because they are fastidious in nature, anaerobes are hard to isolate and are often not recovered from the infected sites. However, the different studies have shown 30-90 % isolation rate of anaerobes in their studies. [2, 9, 14, 23] Thus our study shows the coinciding results demonstrating isolation rate of 44.3%. 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. [19, 23, 24] We have also found synergism 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 a production of virulence factors such as hemolysins, proteases and collagenases that cause inflammation, healing and contribute to the chronicity of infection. In the study, 16% pus samples yielded pure anaerobic isolates. In the abscess, this pure anaerobic organisms produce in the late stage of abscess formation. [24]

In our study, none of the isolates was found to be resistant to metronidazole. Most of the isolates were 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.

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. Our study showed that the microbiological flora of orofacial abscess consists of the complex mixture of aerobic and anaerobic bacteria and polymicrobial in nature. 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 *Staphylococcus aureus* in anaerobes and aerobes respectively. 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.

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