Detection of Bacterial Flora in Orofacial Space Infections and Their Antibiotic Sensitivity Profile

Nitin Suresh Fating · D. Saikrishna ·
G. S. Vijay Kumar · Sujeeth Kumar Shetty ·
M. Raghavendra Rao

Received: 18 March 2013 / Accepted: 21 August 2013 / Published online: 1 October 2013
© Association of Oral and Maxillofacial Surgeons of India 2013

Introduction Most odontogenic infections arise as a sequel of pulp necrosis caused by caries, trauma, peri-odontitis, etc. They range from periapical abscesses to superficial and deep infections in neck. Some resolve with little consequence and some lead to severe infections of head and neck region. The purpose of this study was to identify microbial flora present in orofacial space infection of odontogenic origin and thereby provide better perspective in management of odontogenic infection.

Materials and Methods Twenty-six 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.

Results Demographic profile of the patients showed that male patients were more commonly involved and most patients fell in to the third and fourth decade of age groups. Most common site of involvement was submandibular space. Alpha hemolytic streptococci were the frequent aerobic bacterial isolate and among anaerobes, anaerobic streptococci followed by bacteroids were the major pathogens. Clindamycin, Gentamycin, Linezolid, Imipenem were the most effective antibiotics. 20 % of the aerobes were resistant to penicillin.

Conclusion Streptococcus species are still the commonest pathogen in orofacial infections of odontogenic origin. Administration of amoxicillin clavulanic acid combination and metronidazole followed by surgical drainage of abscess and extraction of infected teeth, yielded satisfactory resolution of infection.

Keywords Odontogenic infection · Submandibular space · Streptococcus · Orofacial infections

Introduction

Dental infection has plagued humankind for as long as our civilization is. Hardly any imagination is required to picture a primitive man suffering pain and swelling of the face due to an abscess tooth, the cause of which could be due to periodontal trauma or dental caries. Facial space infection has been recognized and described since the time of Galen in the second century. He has outlined a deep cervical space in his studies.

A fight against microorganism by man dates back to ancient civilization. Despite all these, even after centuries and endless research, mankind has not been successful in eradicating microbial infections in total. Much water has flown since the discovery of penicillin and the researchers talk in terms of conquering organisms that are penicillin resistant betalactams and newer highly effective cephalosporins as well as quinolones. Although penicillin was considered the long awaiting panacea for dental infection the bacteriological spectrum of the oral flora and the understanding of its complexities have undergone rapid evolution since penicillin was introduced, microorganisms are still a step ahead. The newer and more potent antibiotics too have faced a stiff resistance.

N. S. Fating · D. Saikrishna (✉) · S. K. Shetty
Department of Oral and Maxillofacial Surgery,
JSS Dental College and Hospital, Mysore, India
e-mail: degalasaikrishna@gmail.com

N. S. Fating
e-mail: coolnitin15@gmail.com

G. S. Vijay Kumar · M. Raghavendra Rao
Department of Microbiology, JSS Medical College,
Mysore, India

Treatment of localized infection was probably the first primitive surgical procedure performed, and it most likely involved the opening of bulging abscesses with sharp stones or pointed sticks. Even today the principle remains the same though the technique has improved.

An understanding of the pathogenic and proper management of oral infections is of critical importance to the dental practitioner. Since microorganisms vary from region to region as do their susceptibilities, it is of vital importance that such studies are to be done. This will help in monitoring the continuous evolution of microorganisms and their susceptibility to commonly used drugs.

There have been claims in the literature that changes have occurred in the causative organisms of maxillofacial infections. Therefore, a prospective clinical and microbiological study was designed to check the validity of such claims.

Patients and Methods

Twenty-six patients diagnosed for having orofacial infections of odontogenic origin were selected randomly from Department of Oral and Maxillofacial Surgery, in our institute after obtaining ethical clearance. Patients with isolated or multiple orofacial infections of all age groups and gender were included in the study. Whereas patients already started with antibiotics and/or underwent incision and drainage were excluded from the study. After taking detailed history each patient was thoroughly examined. The pus was collected from the involved space.

Specimen Collection

Extra oral approach was preferred to eliminate contamination with oral flora. But in cases where extra oral approach was not possible, pus sample was collected intra orally after proper preparation of site. The extra oral sites were prepared with spirit or povidone iodine or a combination of these. Intra oral sites were prepared with chlorhexidine. Disposable syringes (5 ml) with disposable needle of 18 gauges were used to aspirate the pus from the abscess. The aspirated pus was immediately injected in thyoglycollate broth and transported to Department of Microbiology. Pus samples were processed by Smear studies of gram staining, Aerobic culture, Anaerobic culture.

Gram Staining

Pus sample was subjected to gram staining and different morphological forms of bacteria were noted.

Aerobic Culture

For aerobic culture the samples were inoculated on MacConkeys agar and blood agar and incubated at 37 °C for 18–24 h. After 24 h, the plates were observed for growth of organisms. The organisms were identified by gram's staining and standard biochemical tests.

Anaerobic Culture

For anaerobic culture, sample was inoculated into plain blood agar and incubated anaerobically at 37 °C, using gas pack, in anaerobic jar for 48–72 h. Strains of pseudomonas were kept in anaerobic jar as a control. The plates were observed for growth of organisms. The organisms were identified by gram's stain and morphology.

Antibiotic Sensitivity Test

Antibiotic sensitivity was done for aerobic organisms only by disk diffusion method for antibiotics listed in Table 1.

All patients were started with empirical antibiotics amoxicillin + clavulanic acid and metronidazole. Doses were adjusted according to patient's age and body weight. Causative teeth were identified by clinical, radiological examination and were immediately extracted. Incision and drainage was carried out under aseptic conditions. After culture and sensitivity, depending on the clinical course of the disease appropriate antibiotics were given.

Table 1 Drugs used and their strength

Sl. no.	Antibiotic	Strength
1	Cefazolin	30 mcg
2	Cefuroxime	30 mcg
3	Cefotaxim	30 mcg
4	Ceftriaxone	30 mcg
5	Cefixime	5 mcg
6	Cefoperazone	75 mcg
7	Penicillin G	10 U
8	Amoxicillin CV	50 + 10 mcg
9	Erythromycin	15 mcg
10	Gentamycin	10 mcg
11	Amoxicillin	25 mcg
12	Vancomycin	30 mcg
13	Ciprofloxacin	5 mcg
14	Clindamycin	2 mcg
15	Imipenem	10 mcg
16	Doxycycline	30 mcg
17	Amikacin	30 mcg
18	Linezolid	30 mcg

Results

Among the 26 patients 14 were males (53.8 %) and 12 females (46.2 %). The mean age of patient was 35.76 years with standard deviation of 14.31. The minimum age of male patients was 9 years and maximum age was 69 years. The minimum age of female patients was 19 years and maximum age was 55 years. There was no significant difference found in occurrence of odontogenic infections in males and females. (P value is 0.695, which is not significant). In our study the most frequent source of infection was infected mandibular third molar (31.70 %) followed by mandibular second molar (17.07 %), and mandibular first molar (12.21 %) (Fig. 1). Submandibular space was most commonly involved in 38 % of cases followed by sub mental space in 16 % of cases (Fig. 2).

Microbiological Evaluation

Gram positive cocci were found most frequently (74.13 %) followed by gram negative bacilli (18.97 %) (Fig. 3). Out of 26 patients considered in this study the pus sample from 18 patients showed mixed aerobic and anaerobic growth of

Fig. 1 Frequency of causative teeth

Fig. 2 Site distribution of orofacial space infections

Fig. 3 Type of bacteria in gram stain smear study

Fig. 4 Type of isolates

microorganisms (69.23 %), whereas pus sample from 8 patients showed purely anaerobic growth (30.77 %). None of the 26 samples tested showed purely aerobic growth. Anaerobic growth was found in all 26 cases (Fig. 4). In our study totally 20 strains of aerobic bacteria were isolated. Out of these 14 strains of Alpha hemolytic streptococci (70 %), 2 strains of Beta hemolytic streptococci (10 %), 2 strains of *Staphylococcus aureus* (10 %), 1 strain of *Acinetobacter* (5 %), and 1 strain of *Klebsiella* species (5 %) were found. Totally 38 strains of anaerobic bacteria were isolated out of which 24 were anaerobic streptococci (63.15 %), 5 strains of *Bacteroides* (13.16 %), 3 strains of *Fusobacterium* (7.89 %) and 1 strain each of *Porphyromonas gingivalis* (2.63 %), Anaerobic staphylococci (2.63 %), *Veillonella* (2.63 %) and *Prevotella melaninogenica* (2.63 %) respectively were found. Among 58 strains isolated in this study 34.45 % were aerobic, the most common being Alpha hemolytic streptococci which is 24.14 % of the total number of isolates and 65.55 % were anaerobic, the most common being anaerobic streptococci which is 41.37 % followed by *Bacteroides* which is 8.63 % of the total number of isolates (Figs. 5, 6).

Fig. 5 Percentage of aerobic bacteria among total number of isolates

Fig. 6 Percentage of anaerobic bacteria among total number of isolates

Antibiotic Sensitivity Pattern for Aerobic Strains

All the isolated aerobic strains (100 %) were sensitive to Gentamycin, Vancomycin, Imipenam and Linezolid. Whereas 80 % strains were sensitive to penicillin G, amoxicillin and Amox CV. 20 % of the isolated strains were resistant to Penicillin G, Amox CV, and Amoxicillin. 10 % of them were resistant to doxycycline, cefixime (Table 2).

Discussion

The microbial residents of the oral cavity constitute one of the most varied floras in human body. The variation relates to the many microenvironments in the oral cavity, such as

the various surfaces of the teeth, gingival sulcus, tongue, and buccal mucosa. Each organism has a unique set of conditions that permits the organisms to establish residency and thrive, including receptors for selective adherence, appropriate nutrients and oxygen tension or simply physical protection from unfavorable conditions. For these reasons an understanding of the nature of oral flora and its dynamics is important in Orofacial infections (Figs. 7–14).

In our study 26 patients with orofacial space infections of odontogenic origin were considered.

The age group most commonly involved is in the third and fourth decades of life. The mean age was 34.13 years. This finding is comparable to the age distribution reported in various studies [1–4].

Male patients were more commonly involved than female patients. Male to female ratio was 1.17:1. This finding can be compared to the gender distribution given by other studies [2, 4–7].

Mandibular third molar (31.7 %) was the most common causative tooth followed by mandibular second molar (17.7 %) and mandibular first molar (12.2 %). These findings are similar with the studies performed previously [4–7], where third molar was the commonest tooth followed by second and first molar and are comparable with few other studies [8–10], whereas mandibular first molar was most commonly involved in other studies also [4, 11, 12].

The most commonly involved space was submandibular (38 %) followed by submental space (16 %). This finding is similar to the findings of other studies [8, 11, 13–16], whereas most frequent involvement of buccal space was reported in a study [4] and frequent involvement of den-toalveolar space was documented in another study by [7].

Microorganisms

The concept of a mixed aerobic anaerobic infection is an important one relative to odontogenic infections. Odontogenic infection is due to the interdependent and synergistic metabolism of a variety of microorganisms. The role played by different microorganisms in the group may be difficult to establish but at least in some cases, it has been demonstrated that individual members of the group produce metabolites that are essential for the growth of other microorganisms in the group. They produce substances that create a favorable pH in the environment, or they consume oxygen and facilitate anaerobic growth.

Gram positive cocci were observed in 74.13 % of the gram stain smear study, gram negative bacilli in 18.97 %, gram negative cocci in 3.45 % and gram positive bacilli in 3.45 % of the cases. These findings were almost similar to findings of other studies [3, 4, 6–25].

Organisms grown were aerobic and anaerobic in 69.23 % of cases and anaerobic only in 30.77 %. There

Table 2 Antibiotic sensitivity pattern for aerobic strains

Sl. no.	Antibiotic	Sensitivity	%	Moderate sensitivity	%	Resistant	%
1	Cefazolic	19	95.0	0	0.0	1	5.0
2	Cefuroxime	19	95.0	0	0.0	1	5.0
3	Cefotaxim	19	95.0	0	0.0	1	5.0
4	Ceftriaxone	19	95.0	0	0.0	1	5.0
5	Cefixime	18	90.0	0	0.0	2	10.0
6	Cefoperazone	19	95.0	0	0.0	1	5.0
7	Penicillin G	16	80.0	0	0.0	4	20.0
8	Amoxicillin CV	16	80.0	0	0.0	4	20.0
9	Erythromycin	19	95.5	0	0.0	1	5.0
10	Gentamycin	20	100.0	0	0.0	0	0.0
11	Amoxicillin	16	80.0	0	0.0	4	20.0
12	Vancomycin	20	100.0	0	0.0	0	0.0
13	Ciprofloxacin	19	95.0	1	5.0	0	0.0
14	Clindamycin	19	95.0	0	0.0	1	5.0
15	Imipenem	20	100.0	0	0.0	0	0.0
16	Doxycycline	18	90.0	2	10.0	0	0.0
17	Amikacin	17	85.0	3	15.0	0	0.0
18	Linezolid	20	100.0	0	0.0	0	0.0

Fig. 7 Aerobic streptococci growth **Fig. 8** Anaerobic streptococci growth

was no case where only aerobic organisms were grown. Anaerobic growth was seen in all 26 cases. These findings are comparable to studies conducted in past [2, 3, 7, 9, 16, 17, 19], whereas a study [4] showed increased pure aerobic isolates compared to anaerobic isolates.

Alpha hemolytic streptococci were found in 70 % among aerobes, a finding similar to that of previous studies [9, 14, 16]. Beta hemolytic streptococci were present in 10 % of the aerobes isolated. Streptococci were the most

common aerobes isolated. This finding was similar to other studies [4, 7, 19, 25]. But staphylococci were most commonly isolated as per other authors [1, 2, 5].

Anaerobic bacteria were present in 65.5 % among the total number of isolates, a finding similar to other studies [2, 18, 19]. Most common anaerobe isolated was anaerobic streptococci (41.37 %), among total isolates, followed by bacteroids (8.63 %), which is comparable to other studies [7, 11, 14].

Fig. 9 Growth of multiple organisms grown in anaerobic condition

Fig. 10 Sensitivity test using antibiotic disc

Fig. 11 Pus aspirate

Antibiotic Sensitivity

Various literature reports that many species of organisms were resistant to penicillin. However most patients were clinically well by the time penicillin resistance was discovered. There are three likely explanations [26] for this:

- (1) In vitro resistance does not necessarily imply in vivo resistance, particularly in mixed infections.

Fig. 12 Pre operative submandibular space infection

Fig. 13 Placement of corrugated rubber drain

- (2) The source of infection had usually been removed and / or surgical incision and drainage accomplished which even without antibiotics can effect a cure in many cases.
- (3) The possibility that in interdependent, synergistic, mixed infections, as long as one bacterial species is sensitive to penicillin, the entire pathogenic complex may be rendered nonpathogenic.

In the present study Penicillin G, Amoxicillin and Amox CV resistance was seen in 20 % of aerobes, which is comparable to a study [3], whereas Cefixime resistance was seen in 10 % of aerobes. All the organisms were found sensitive

Fig. 14 Post operative seventh day

to Clindamycin, Gentamycin and Linezolid. Organisms were sensitive to Cephalosporins to a higher degree than to Penicillin. Most of the strains of aerobic organisms in this study were sensitive to Penicillin—a finding in agreement with many studies [4, 9, 16, 21], whereas in other studies Penicillin resistance was high [5, 7, 20].

Antibiotic resistance is an important consideration in the management of orofacial odontogenic infections. Although it is not considered a virulence factor per se, β lactamase activity among gram negative anaerobic rods may be responsible for clinical failures with Penicillin treatment. Despite this, Penicillin remains the recommended agent for the non allergic patient with mild to moderate infection. However, in more severe cases where there is a narrow margin of acceptance of possible therapy failure it is recommended to resort to agents with adequate anaerobic spectrum such as Clindamycin or combinations of an Aminopenicillin with β -lactamase inhibitor [23].

First, successful treatment of these infections depends heavily upon changing the environment through debridement and/or incision and drainage. Doing so alters the ability to sustain anaerobic growth by removing hypoxic tissue. Without the anaerobes the aerobic component of the infection may be rendered nonpathogenic [22].

Various methods have been introduced for species definition based on phenotypic features such as 16S rRNA [24] polymerase chain reaction/restriction fragment length polymorphism profile determinations [24], which can help distinguish various species that are otherwise difficult to differentiate. However, owing to a lack of facilities and the heavy price tag attached to these procedures, we were

unable to compare the present results with molecular methods. This may be a limitation of this study. A large multicentric clinical study using molecular analysis of genetic materials may rectify the geographic and technical shortfalls of the present study.

Conclusion

Streptococcus species is still the most common causative pathogen in orofacial infections of odontogenic origin. The microbiological flora is usually multi microbial having mixture of both aerobic and anaerobic organisms. The same empirical antibiotic therapy of amoxicillin plus clavulanic acid combined with metronidazole along with surgical drainage of infection and extraction of causative teeth, yielded satisfactory resolution of infection.

References

1. Virolainen E, Haapaniemi J, Aitasalo K, Suonpaa J (1979) Deep neck infections. *Int J Oral Surg* 8:407–411
2. Kannangara DW, Thadepalli H, McQuirter JL (1980) Bacteriology and treatment of dental infections. *Oral Surg Oral Med Oral Pathol* 50:103–109
3. Hunt DE, Meyer RE (1983) Continued evolution of the microbiology of oral infections. *JADA* 107:1983
4. Yuvaraj V, Alexander M (2012) Microflora in maxillofacial infections—a changing scenario? *J Oral Maxillofac Surg* 70: 119–125
5. Goldberg MH (1970) The changing biologic nature of acute dental infection. *JADA* 80:1048–1051
6. Dodson TB, Perrot DH, Kaban LB (1989) Pediatric maxillofacial infections: a retrospective study of 113 patients. *J Oral Maxillofac Surg* 47:327–330
7. Kohli M, Mathur A (2009) In vitro evaluation of microbiological flora of orofacial infections. *J Maxillofac Oral Surg* 8:329–333
8. Indresano AT, Haug RH, Hoffman MJ (1992) Third molar as a cause of deep space infections. *J Oral Maxillofac Surg* 50:33–35
9. Kuriyama T, Karasawa T, Nakagawa K, Saiki Y, Yamamoto E, Nakamura S (2000) Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 90:600–608
10. Tung-Yiu W, Jehn-Shyun H (2000) Cervical necrotizing fasciitis of odontogenic origin. A report of 11 cases. *J Oral Maxillofac Surg* 58:1347–1352
11. Storoe W, Haug RH, Lillich TT (2001) The changing face of odontogenic infections. *J Oral Maxillofac Surg* 59:739–748
12. Wang J, Ahani A, Pogrel MA (2005) A five-year retrospective study of odontogenic maxillofacial infections in a large urban public hospital. *Int J Oral Maxillofac Surg* 34:646–649
13. Adekeye EO, Adekeye JO (1982) The pathogenesis and microbiology of idiopathic cervicofacial abscesses. *J Oral Maxillofac Surg* 40:100–106
14. Labriola JD, Mascaro J, Alpert B (1983) The microbiologic flora of orofacial abscesses. *J Oral Maxillofac Surg* 41:711–714
15. Flynn TR, Shanti RM (2006) Severe odontogenic infections, part 1: prospective report. *J Oral Maxillofac Surg* 64:1093–1103

16. Rao DD, Desai A (2010) Comparison of maxillofacial space infection in diabetic and nondiabetic patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 110:e7–e12
17. Gill Y, Scully C (1990) Orofacial odontogenic infections: review of microbiology and current treatment. *Oral Surg Oral Med Oral Pathol* 70:155–158
18. Konow LV, Nord CE, Nordenrarn A (1981) Anaerobic bacteria in dentoalveolar infections. *Int J Oral Surg* 10:313–322
19. Brook I, Grimm S, Kielich RB (1981) Bacteriology of acute periapical abscess in children. *J Endod* 7:378–380
20. Quayle AA, Russel C, Hearn B (1987) Organisms isolated from severe odontogenic soft tissue infections: their sensitivities to cefoietan and seven other antibiotics, and implications for therapy and prophylaxis. *Br J Oral Maxillofac Surg* 25:34–44
21. Gilmore WC, Jacobus NV, Gorbach SL, Doku HC, Tally FP (1988) A prospective double-blind evaluation of penicillin versus clindamycin in the treatment of odontogenic infections. *J Oral Maxillofac Surg* 46:1065–1070
22. Moenning JE, Nelson CL, Kohler RB (1989) The microbiology and chemotherapy of odontogenic infections. *J Oral Maxillofac Surg* 47:976–985
23. Stefanopoulos PK, Kolokotronis AE (2004) The clinical significance of anaerobic bacteria in acute orofacial odontogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 98:398–408
24. Riggio MP, Aga H (2007) Identification of bacteria associated with spreading odontogenic infections by 16S rRNA gene sequencing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 103:610–617
25. Warnke PH, Becker ST (2008) Penicillin compared with other advanced broad spectrum antibiotics regarding antibacterial activity against oral pathogens isolated from odontogenic abscesses. *J Cranio-Maxillofac Surg* 36:462–467
26. Lewis MA, Mac Farlane TW, McGavan DA (1986) Quantitative bacteriology of acute dentoalveolar abscess. *J Med Microbiol* 21:101