J Maxillofac Oral Surg 8(4):329–333

PROSPECTIVE STUDY

In vitro evaluation of microbiological flora of orofacial infections

Received: 29 April 2008 / Accepted: 2 December 2009

Munish Kohli[1] ✉ · **Asha Mathur**[2] · **Monica Kohli**[3] · **Saif Rauf Siddiqui**[4]

[1] HOD, Dept. of Oral and Maxillofacial Surgery
[2] HOD, Dept. of Pathology and Microbiology
[4] Postgraduate Student, Dept. of Oral and Maxillofacial Surgery

Saraswathi Dental College and Hospital, Lucknow

[3] Associate Professor, Dept. of Anesthesiology, King George Medical College, Lucknow

Abstract
Objective To assess the most common micro-organisms causing odontogenic infections and their antimicrobial susceptibility.
Methods The study was conducted in 80 patients with orofacial infection. The pus sample was collected, cultured (aerobically and anaerobically) and stained for morphological study of the isolates. Antibiotic sensitivity test for the isolates were performed.
Results A total of 109 micro-organisms were isolated, no pathogenic organism were isolated in 3 cases. Out of 109 micro-organism isolated, 107 bacteria and 2 fungi were identified. 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. Among the entire pure gram positive isolates, ofloxacin was the most sensitive drug 83.33% followed by ciprofloxacin 76.2% and sparfloxacin 76.2%. The most resistant drugs were amoxicillin (92.85%) and ampicillin (92.85%). Cefotaxime was found sensitive in 75% of pure gram negative isolates.
Conclusion Ofloxacin was the most sensitive drug followed by ciprofloxacin and sparfloxacin for pure gram positive isolates. The most resistant drugs were amoxicillin and ampicillin. The gram negative colonies were sensitive to Cefotaxime.

Keywords Microbiological flora · Dentoalveolar abscess · Antibiotic sensitivity

Address for correspondence:

Munish Kohli
26, Napier Road Colony
Part 1 Thakurganj, Lucknow
Uttarpradesh, India
Ph: +919415764250
E-mail: munishkohli2007@rediffmail.com

Introduction

Most odontogenic infections arise as a sequel to pulpal necrosis caused by caries, trauma, periodontitis etc. They ranges from periapical abscesses to superficial and deep infections in the neck [1]. Some resolve with little consequences some lead to severe infections of head and neck region. Complications such as osteomyelitis, airway obstruction, infections of the carotid sheath, sinusitis, septicemia, meningitis, brain abscess, cavernous sinus thrombosis, mediastinitis and distant metastatic foci of infections have also been reported. This necessitates deliberate and timely efforts to establish mechanical debridement and drainage as well as appropriate antibiotic therapy.

Selection, collection and transportation of specimens are important in determining the usefulness of the laboratory results [2]. This study has been designed to assess the most common micro-orgranism causing odontogenic infection and their antimicrobial susceptibility in our population.

The present study was undertaken as an endeavor to elicit information about the type of microorganisms causing orofacial infections in Indian population and their antimicrobial susceptibilities.

There are several studies in the literature concerning the epidemiology of odontogenic infections. Our study was an effort to make a comparison with other studies in the context of:

1. Source of infection
2. Age
3. Gender
4. Site involved
5. Distribution of odontogenic infection
6. Microbiological study
7. Antibiotic susceptibilities

Material and method

The study was conducted in total of 80 patients who reported to the department of Oral and Maxillofacial Surgery, Saraswati Dental College and Hospital, Lucknow, with moderate to severe oral infections with abscess in orofacial region. This study was conducted between October 2005 to June 2007. The patients were mainly from the rural areas, nearby villages, and the neighboring district of Lucknow (Barabanki).

Proper history, clinical signs and symptoms and prior use of antibiotic were recorded before specimen collection. Majority of the patients were not receiving antibiotic therapy prior to study. Collection of the pus sample from the patient presenting with dento-alveolar abscess, buccal space abscess, postoperative infection, candidiasis, submandibular,

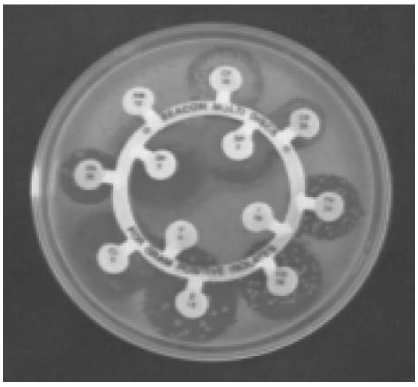

Fig. 1 Antibiotic sensitivity test showing sensitivity of many drugs

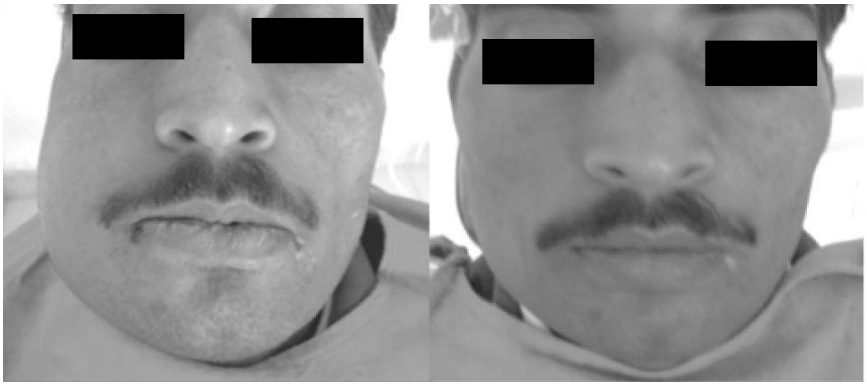

Fig. 2 Pre and postoperative pictures of a patient with buccal space infection

sublingual, canine space infection, tubercular osteomyelitis and infective dentigerous cyst were selected. The pus samples were collected aseptically by aspirating the abscess using sterile 18/22 gauge needle with a 2ml syringe introduced intraorally or extraorally. Any free air was discharged from the syringe and the needle was capped immediately.

After aspirating, the specimen was immediately inoculated in sterile Robertson cooked meat broth (RCM) [2] for transportation of anaerobic organisms.

Specimen culture

The specimens were inoculated on to blood agar, and McConkey' agar and incubated aerobically and anaerobically at 37°C for 18 to 24 hours.

Study of culture characteristics

- The developed colonies were observed and smears were made. The smears are stained by Gram's method for the morphological study of the isolates. Those cultures where no growth was observed were further incubated at 37°C for the development of bacteria with slow growth rates if any and identified.
- For anaerobic culturing, aspirated material were inoculated to blood agar plate and robertson cooked meat broth.
- These plates were kept inside anaerobic jar and incubated at 37°C for 48 hours. After 48 hours the development of colonies on blood agar was observed. The smears were made from the colonies and stained by grams method for the morphological characterization of isolates.
- The specimens were sub cultured to two blood agar plates and one was incubated at 37°C aerobically and other one anaerobically. This is to rule out the facultative anaerobes, if any, present in the clinical specimen.
- For the identification of the micro-organisms, various biochemical tests (catalase, oxidase, coagulase test) were performed. These tests were used in the preliminary identification of bacteria.
- Selective media were employed to grow the isolates for antibiotic sensitivity assay [2].
- The samples were also subcultured on Sabourad's dextrose agar, while in one case the culture was done on Lowenstein-Jensen media. After first 24 hours the development of colonies was observed.

Antibiotic sensitivity tests

Antibiotic sensitivity tests for the isolates were performed in nutrient agar by disc diffusion method of Kirby-Bauer. It involves spreading a suspension of bacteria over adjacent part of nutrient agar plate. The ring of antibiotic disc is placed (Fig. 1). Discs are incubated overnight at 37°C. Antibiotics diffuse into the agar and inhibit the growth of sensitive bacteria in a semicircular zone around the disc. When resistance of a given antibiotic is present the zone radius will be reduced or there will be no zone at all. The zone of inhibition were measured and recorded as sensitive / moderately susceptible or resistant as indicated in the Kirby-Bauer methods.

Result

Total of 80 pus samples were collected and analysed. 3 cases (3.75%) did not show any pathogenic micro-organism where as 75 cases (93.75%) showed bacterial growth and 2 cases (2.5%) of fungal growth were obtained.

The age of the study group ranged between 10 years to 70 years. 26 cases (32.5%) were in 21–30 year age group and 23 cases (28.75%) in 31–40 year age group.

Of the 80 patients, 56 patients (70%) were male and 24 patients (30%) were female. The male:female ratio was 2:1.

In the study group it was observed that, 92 teeth were involved. Majority of the infections were associated with mandibular teeth (57.60%).

The mandibular first molar (26%) was most commonly involved tooth followed by maxillary first molar (18.47%) and mandibular second molar (15.2%).

The most common odontogenic infection observed were dentoalveolar abscess (70%) and buccal space abscess (13.75%) (Table 1). 2 cases each of candidiasis and submandibular space infection (2.5%) were reported.

We isolated 109 micro-organisms, of which 107 were bacteria and the rest were yeast. Of the bacteria (70)64.22% were gram positive cocci and (28)25.69% were gram negative bacilli. Gram negative cocci were few (2)1.83%.

Strict aerobes were found in 69 (63.30%) isolates and strict anaerobes in 40(36.70%) isolates. Pure aerobic isolates were identified in 28(35%) of specimens, while the pure anaerobes were isolated in 18(22.5%) instances. Mixed anaerobes and aerobes in 10(12.5%) were observed. Mixed anaerobes were reported in 6(7.5%) instances while mixed aerobes were isolated in 15(18.75%) instances.

In 3 cases the sample was found sterile with no organism isolated. Fungus (candida albicans) was identified in 2 cases (2.5%).

Among the total 109 micro-organisms isolated anaerobic streptococci were seen in 33(29.46 %) samples. They were the most common. Staphylococcus aureus was

isolated in 24(21.43 %), Pseudomonas aeuroginosa in 13(11.60 %), Streptococcus pyogens 12(10.72%), Lactobaccilus 6 (5.35%), Bacteroids 5(4.46 %), Klebsiella 3(2.67%), E.coli 1(0.89%) Neisseria meningitides 1(0.89%), Mycobacterium tuberculosis 1(0.89%), Proteus vulgaris 3 (2.67%), Veilonella 1(0.89%), Candida albicans in 2 cases (1.78%) were isolated (Table 2).

For pure gram positive

Staphyloccous aureus was isolated in 16 instances in pure form which was resistant to amoxicillin (15) and ampicillin (15)93.75%.

Ciprofloxacin was found 100% effective against all 16 isolates while ofloxacin (14)87.5%, sparfloxacin (13)81.25% roxithromycin (10)62.5% effective. Vancomycin (9) and cefazolin (9) were found to be effective 56.25% (Table 12).

Streptococcus pyogens was sensitive to ciprofloxacin, ofloxacin, sparfloxacin and erythromycin, 85.71% (6 out of 7) of the organism. Amoxicillin was found to be resistant in most of the isolates (85.71%) of streptococcus pyogens.

Lactobacillus isolated in 1 instance was found to be sensitive to ofloxacin, sparfloxacin ciprofloxacin and roxithromycin.

Anaerobic streptococci was resistant to amoxicillin (16)94.1% and ampicillin (15) in 88.2% of isolates.

Ofloxacin (14)82.35% and vancomycin (13)76.47% were found senstive against all the isolates.

Mycobacterium tuberculosis was isolated in 1 instance where no antibiotic sensitivity was checked. Patient was started with the anti tubercular treatment.

Among the entire pure gram positive isolates, ofloxacin was the most sensitive drug 83.33% followed by ciprofloxacin 76.2% and sparfloxacin 76.2%.

The most resistant drugs were amoxicillin (92.85%) and ampicillin (92.85%).

For pure gram negative

Out of 4 pure gram negative isolates (2 Pseudomonas, 1 Neisseria Meningitides and 1 E.Coli) were subcultured. The colonies were sensitive to Cefotaxime in 3 cases (75%). Amikacin, ofloxacin, ciprofloxacin and amoxicillin were effective in 2 cases each (50%). The organisms were resistant to other drugs.

Discussion

Most odontogenic infections arise as a sequel to pulp necrosis caused by caries or trauma. Periodontal infections, pericoronitis, trauma and surgery are other sources responsible for orofacial infections. Most of the odontogenic infections resolve with little consequences although, occasionally complications may lead to more severe infection of the head and neck, particularly in immuno-compromised or debilitated patients (Gill et al. 1990) [3].

Studies have described the development of odontogenic infections in varying age groups ranging from 6 to 79 years (Kannangara et al. 1980) [4]. Bartlett and O'Keefe (1979) [5] reported an age range of 23 to 70 years, with a mean of 43 years involving 20 patients. In our study out of the total of 80 cases 26(32.5%) were between age groups of (21–30) years.

Kannangara et al. (1980) [4] reported a male predominance in their study, finding 40(66%) males and 21(34%) females. In 1993 Krishnan et al. [6] have also noted male predominance with 32 males and 18 females. In contrast Hunt et al. (1978) [7] noted a female predominance, 43 females (59%) and 30 males (41%). The present study of odontogenic infections identified a slight male predominance, with 56 males (70%) and 24 females (30%).

In this study the majority of the infections are seen to involve the mandibular teeth (57.60%) than the maxillary teeth (42.39%). The mandibular first molar (26%) was the most frequently involved tooth. Wang et al. (2005) [8] and Krishnan et al. (1993) [6] have also found that mandibular teeth were more involved in odontogenic infections. Obayashi et al. [9] state that among the maxillary teeth, maxillary first molar was the most common cause tooth of odontogenic infection. In our study, the maxillary first molar was involved in 18.47% of the cases only. The present study shows involvement of the mandibular third molar in 10% cases which corroborates with the finding of Parker et al. (2001) [10] while according to Storoe W et al. (2001) [11], it was the most frequently involved tooth.

Dentoalveolar abscess (70%) was the most common abscess in our study. The common pathogenic sequence is a necrotic pulpal inflammation extending into periapical area which, if untreated may penetrate through the cortical bone to involve the potential spaces [12]. One of the most common odontogenic infections is the acute dentoalveolar abscess also reported by Gill et al. (1988) [13].

Out of the 80 cases, 11 cases of buccal space infections (13.75%) were reported which is second most common involved site. Few authors reported the buccal space infection as the third most common facial space involved. Storoe et al. (2001) [11] reported 11.4% and Parker et al. (2001) [10] as 9.4% of the buccal space infection in their study. Krishnan et al. (1993) [6] in his study reported that, out of 50 patients 11 cases were of buccal space infection.

In our study 2 cases (2.5%) of submandibular space infection were reported individually and 2 cases along with other facial spaces. Chow et al. (1978) [14], Krishnan et al. (1993) [6], Storoe et al. (2001) [11] reported that the submandibular space infections are frequently involved in mandibular odontogenic infections.

A case of tubercular osteomylitis was encountered in this study which was also reported by Khosla VH (1970) [15].

The pathogenic microbiota of the oral cavity are complex and, fluctuate with age, diseases, conditions and site of resistance. Studies indicate that the majority of infections consist of mixed aerobic and anaerobic flora (65% to 70%), or are exclusively anaerobic (25% to 30%), whereas only 5% are exclusively aerobic. Most frequently and consistently isolated organisms are aerobic streptococci, anaerobic streptococci, bacteroides. Other micro-organisms like fungi, virus as causative for abscesses are rarely reported in literature (McManners et al. 1990) [16].

Bacterial isolations

In this study pure anaerobic bacteria were isolated in 36.70% and aerobic bacteria were isolated in 63.30% of cases. The percentage of anaerobic bacteria was less as compared to other studies, but it is similar to the study by Hunt et al. (1978) [7] and Kannangara et al. (1980) [4].

The anaerobic flora isolated was predominantly of anaerobic streptococci in 33 cases (29.46%) along with 5 cases of Bacteriodes (4.46%), Lactobaccilus in 6 instances (5.35%) and Vellionella in one instance (0.89%). Earlier studies (Labriola et al. (1983) [17], Kuriyama et al. (2000) [18], Gill and Scully C et al. (1988) [13] have reported about mixed anaerobic flora

in orofacial infection. The pure anaerobic organisms are produced in the late stage of abscess formation, through overgrowth of anaerobes (Lacey, 1984) [19].

Staphylococcus aureus was isolated in our study in total 24 cases (21.43%). The frequent isolation of staphylococci in pus samples from odontogenic infections have been reported in previous studies (Storoe et al. 2001[11] and Kannangara, 1980[4]).

The isolation of pseudomonas in 13 cases (11.60%) is high when compared to other studies (Kuriyama et al. 2000 [20], Gill and Scully et al. 1990 [3]). The injudicious use of antibiotics in dentistry may be the reason for isolation of more percentage of drug resistant pathogen like pseudomonas in this study.

Streptococcus pyogenes have been isolated in 10.72% of the cases in our study. Sakaguchi et al. (1997) [21] reported 13.8% of streptococcus pyogenes in his study.

Other gram-negative bacilli like klebsiella, proteus vulgaris were also isolated in this study and were similar to earlier studies. Bartlett and O'Keefe (1979) [5], Chow et al. (1978) [14]. According to Walton (1999) gram-negative bacilli isolated in orofacial infections are likely key players in synergism with other bacterial species.

Fungal isolation

The Candida albicans was isolated in two cases in this study. The search of literature revealed occurrence of Candida in pus sample in two instances as reported by McManners et a1. (1990) [16]. The identification of Candida in pus shows that it causes superficial infection as well as deep infections also.

Antibiotic sensitivity

When antibiotics are prescribed for the treatment of orofacial infections, few important factors should be considered, like severity of infection, common pathogens encountered, antimicrobial susceptibility and resistance status, patient's age, health, allergies etc. Most of the odontogenic infections are successfully managed by incision and drainage, together with extraction/root canal therapy of the affected tooth. Sometimes infections from these abscesses may spread, leading to life threatening conditions. Timely and deliberate efforts to establish debridement and drainage as well as appropriate antibiotic therapy should be selected by clinician (Krishnan et al. 1993) [6].

Table 1 Distribution of odontogenic infection

Odontogenic Infections	Frequency	Percentage (%)
a. Dento alveolar abscess	56	70
b. Buccal space abscess	11	13.75
c. Postoperative infection	3	3.75
d. Candidiasis	2	2.5
e. Submandible space infection	2	2.5
f. Submandibular space infection and buccal space abscess	1	1.25
g. Buccal space abscess and canine space infection	1	1.25
h. Sublingual, buccal space and submandibular space infection	1	1.25
i. Parotid abscess	1	1.25
j. Dentigerous cyst	1	1.25
k. Tubercular osteomyelitis	1	1.25
Total	80	

Table 2 Number and types of micro-organisms isolated

S. No.	Micro-organisims isolated	Frequency	%
1.	Anaerobic streptococci	33	29.46
2.	Staphylococcus	24	21.43
3.	Pseudomonas aeuroginosa	13	11.60
4.	Streptococcus pyogens	12	10.72
5.	Streptococcus viridans	1	0.89
6.	Lactobacillus	6	5.35
7.	Klebsiella	6	5.35
8.	Bactroides	5	4.46
9.	Proteus vulgaris	3	2.67
10.	Mycobacterium tuberculosis	1	0.89
11.	Neisseria meningitides	1	0.89
12.	Veilonella	1	0.89
13.	E-coli	1	0.89
14.	Candida albicans	2	1.78
15.	Sterile/No pathogenic organism isolated	3	2.67
	Total	112	100

Out of 17 isolates of anaerobic streptococci, 94.1% were amoxicillin resistant and 88.2% were ampicillin resistant. The penicillin group resistant bacteroids were isolated in earlier studies by Labriola et al. (1983) [17]; Kuriyama et al. (2000) [20]. One study by Drucker et al. (1971) [22] reported 85% microorganisms resistant to penicillin. Ofloxacin (82.35%) and vancomycin (76.47%) were found sensitive against anaerobic streptococci.

Staphylococcus aureus was isolated in 16 instances in pure form, which were resistant to amoxicillin and ampicillin (93.75%).

First drug of choice for Streptococcus pyogens were ciprofloxacin, ofloxacin, sparfloxacin and erythromycin, as they were found effective against 85.71% (6 out of 7) of the organisms, while amoxicillin was found to be resistant in most of the isolates (85.71%). Ciprofloxacin was found 100% effective while ofloxacin (87.5%), sparfloxacin (81.25%) roxithromycin

(62.5%) were also effective. Erythromycin has essentially the same antimicrobial spectrum as penicillin and is useful when there is hypersensitivity to penicillin. Vancomycin and cefazolin were found to be effective only in 56.25% of the cases. Lactobacillus showed good sensitivity against ofloxacin, sparfloxacin ciprofloxacin and roxithromycin.

Majority of the gram-negative isolates in this study showed Cefotaxime as most sensitive (75%), while amikacin, ofloxacin, ciprofloxacin and amoxicillin were sensitive in 50% cases only. The cefotaxime is a third generation of cephalosporin has wide spectrum of action. It is active against oral anaerobes and gram negative.

Conclusion

The study was primarily aimed at investigating the type of bacteria or micro-organism causing odontogenic infections, the antibiotic susceptibility of the micro-organisms causing orofacial abscess and antibiotic resistance status of micro organisms.

Based on the finding of our study, the following conclusions were derived:

1. Majority of the odontogenic infections were seen to involve mandibular teeth (57.60%). Mandible first molar was found to be more affected (26%) followed by maxillary first molar (18.47%).
2. The most common cause of odontogenic infection was found to be dentoalveolar abscess (70%).
3. Our study showed that the microbiological flora of orofacial abscess consists of complex mixture of aerobic and anaerobic bacteria.
4. The micro-organisms isolated ranges from anaerobic streptococci, staphylococcus, negative bacilli, bacteroids, streptococcus pyogens, mycobacterium tuberculosis and fungi, candida albicans.
5. For the gram-positive isolates, ofloxacin was found to be the most sensitive drug 83.33%, followed by ciprofloxacin 76.2% and sparfloxacin 76.2%.
6. Cefotaxime was found to be most sensitive drug for majority of gram-negative isolates (75%).

The culture and sensitivity determinations provide definitive information about the causative organisms and their antibiotic susceptibilities that assist the clinician to prescribe an effective antibiotic.

References

1. Simpson AJH (2002) Rational antibiotic therapy. Surgery 20(8): 177–179
2. Helstad AG, Kimball JL, Maki DG (1977) Recovery of anaerobic, facultative and aerobic bacteria from clinical specimens in three anaerobic transport system. J Clin Microbiol 5(6): 564–569
3. Gill Y, Scully C (1990) Orofacial odontogenic infections : Review of microbiology and current treatment. Oral Surg Oral Med Oral Pathol 70(2): 155–158
4. Kannangara DW, Thadepalli H, McQuirter JL (1980) Bacteriology and treatment of dental infections. Oral Surg Oral Med Oral Pathol 50(2): 103–109
5. Bartlett JG, O'Keefe P (1979) The bacteriology of perimandibular space infections. J Oral Surg 37(6): 407–409
6. Krishnan V, Johnson JV, Helfrick JF (1993) Management of Maxillofacial infections: a review of 50 cases. J Oral Maxillofac Surg 51(8): 868–873
7. Hunt DE, King TJ, Fuller GE (1978) Antibiotic susceptibility of bacteria isolated from oral infections. J Oral Surg 36(7): 527–529
8. 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(6): 646–649
9. Obayashi N, Ariji Y, Goto M et al. (2004) Spread of odontogenic infection originating in the maxillary teeth : computerized tomographic assessment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 98(2): 223–231
10. Parker MI, Khateery SM (2001) A retrospective analysis of orofacial infection requiring hospitalization in Al-Madinah, Saudi Arabia. Saudi Dent J 13(2): 96–100
11. Storoe W, Haug RH, Lillich TT (2001) The changing face of odontogenic infections. J Oral Maxillofac Surg 59(7): 739–748
12. 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(4): 398–408
13. Gill Y, Scully C (1988) The microbiology and management of acute dentoalveolar abscess : views of British Oral and Maxillofacial Surgeons. Br J Oral Maxillofac Surg 26(6): 452–457
14. Chow AW, Roser SM, Brady FA (1978) Orofacial odontogenic infections. Ann Intern Med 88(3): 392–402
15. Khosla VH (1970) Tuberculous osteomyelitis of the mandible: report of case. J Oral Surg 28(11): 848–853
16. McManners J, Samaranayake LP (1990) Suppurative oral candidiasis. Review of literature and report of a case. Int J Oral Maxillofac Surg 19(5): 257–259
17. Labriola JD, Mascaro J, Alpert B (1983) The microbiologic flora of Orofacial abscesses. J Oral Maxillofac Surg 41(11): 711–714
18. Kuriyama T, Nakagawa K, Karasawa T, 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(5): 600–608
19. Peterson: Contemporary Oral and Maxillofacial Surgery. 4th ed. Chapt. 15; 344–355
20. Kuriyama T, Nakagawa K, Karasawa T, Saiki Y, Yamamoto E, Nakamura S (2000) Past administration of beta-lactam antibiotics and emergence of beta lactamase producing bacteria in patients with orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 89(2): 186– 192
21. Sakaguchi M, Sato S, Ishiyama T, Katsuno S, Taguchi K (1997) Characterization and management of deep neck infections. Int J Oral Maxillofac Surg 26(2): 131–134
22. Drucker DB, Jolly M (1971) Sensitivity of oral micro-organisms to antibiotics. Br Dent J 131(10): 442–444

Source of Support: Nil, **Conflict of interest:** None declared.