ORIGINAL ARTICLE

Microbial Flora and Their Antibiotic Susceptibility in Oral and Maxillofacial Infections at BPKIHS: A Prospective Observational Study

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Abstract

Background: We aimed to find out the causative bacteria involved in oral and maxillofacial infections, and to evaluate the antibiotic susceptibility by pus culture and sensitivity test.

Methods: This prospective observational study conducted over a one-year period enrolled all 41 consecutive patients with features of oral and maxillofacial infections visiting the oral and maxillofacial surgery clinic at the university hospital of B. P. Koirala Institute of Health Sciences. Pus was collected on a sterile syringe and sent to the microbiology laboratory. Pus samples were inoculated in 5% sheep blood agar and MacConkey agar and incubated at 37°C for 48 hours. Identification of the bacterial colony was done by gram staining and different biochemical tests. Antibiotic susceptibility tests were done by disc diffusion method on Mueller-Hinton agar as per CLSI guidelines.

Results: Out of 41 patients, 22 were male and 19 were female patients. The average age of the patients was 41.63 years. Odontogenic infection (17, 41.46%) was found to be the most common. Submandibular space (15, 36.59%) was the most common fascial space involved. The most common organism cultured was *Enterococcus faecalis* (13, 31.71%). The antibiotic susceptibility pattern of the isolates showed that 50% of the cultured organisms (n = 8) were resistant to Penicillin; five of them were *Enterococcus faecalis* and three were *Staphylococcus aureus*.

Conclusion: We found that *Enterococcus faecalis* as the common organism causing oral and maxillofacial infections with high resistance to Penicillin.

Keywords: Antibiotic susceptibility, *Enterococcus*, Infection, Oral and Maxillofacial

Declarations

Ethical approval and consent to participate: This study was conducted with prior ethical approval from Ethical Review Board of BPKIHS (Ref No. 175/073/074 – IRC) and informed consent has been obtained from participants prior to the enrollment.

Consent for publication: Informed consent was obtained from the patient for the publication of identifying features along with the manuscript.

Availability of data and materials: The full data set supporting this research is submitted to the journal.

Competing interest: None

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Oral and maxillofacial infections are common problems in dental practice. Most of these infections are odontogenic in origin [1]. Different fascial spaces are involved in maxillofacial infections and can spread rapidly from one space to another and can prove to be fatal with airway problems and septicemia. Management of these infections include removing the source of infection by extraction or endodontic therapy of the offending tooth, incision and drainage followed by proper selection of the antibiotics [2].

The culture and sensitivity test of the pus drained from the infected site yields a good knowledge of the organism and its susceptibility to antibiotics and thus can guide in the proper selection of antibiotics and render proper care and treatment. Among the commonly used antibiotics, Penicillin still remains the drug of choice because of its safe use, minimal side effects and broad spectrum, especially in combination with beta-lactamase inhibitors. Nevertheless, the occurrence of penicillin resistance has become a challenge in clinical practice [3].

Information is available in the literature concerning the changes in the occurrence of the causative microorganisms in oral and maxillofacial infections. Also, there are reports of resistance being developed against the antibiotics [4]. However, no such data is available in our context. Thus, this study was aimed to evaluate the microbial flora and their antibiotic susceptibility in oral and maxillofacial infections.

METHODS

This prospective observational study which was conducted for a one year period from April 2017 to March 2018 included successive patients visiting the Department of Oral and Maxillofacial Surgery of B. P. Koirala Institute of Health Sciences, Dharan with features of oral and maxillofacial infections and yielding pus. Under the aseptic condition, pus samples were collected on a sterile syringe and transported to the microbiology laboratory for culture and sensitivity testing. Pus samples were inoculated in 5% sheep blood agar and MacConkey agar and incubated at 37°C for 48 hours. Further identification of the bacterial colony was done by gram staining and different biochemical tests as per standard microbiological tests in order to confirm bacterial etiology. Antibiotic susceptibility tests were done by disc diffusion method on Mueller-Hinton agar as per Clinical & Laboratory Standards Institute (CLSI) guidelines [5].

The study protocol was approved by Institutional Review Committee of BPKIHS and the study procedures were performed in accordance with the tenets of the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practices, and relevant local laws and regulations. All participants provided voluntary signed informed consent and demonstrated the ability to understand and comply with study protocols before the study started.

RESULTS

The study involved 22 (53.66%) male and 19 (46.34%) female patients. There was no missing data. The mean age of the patients was 41.63 ± 19.07 years with the range from 7 to 79 years. Four patients were diabetic, seven were hypertensive, one was Hepatitis B positive, and one was pregnant (2nd trimester). Odontogenic infection (41.46%) from mandibular molars was the most common cause of infection followed by post extraction infection (14.63%) **(Fig. 1)**. Submandibular space (39.02%) was the most common fascial space to be infected followed by subcutaneous and submental space, each 17.03%. **(Fig. 2)**.

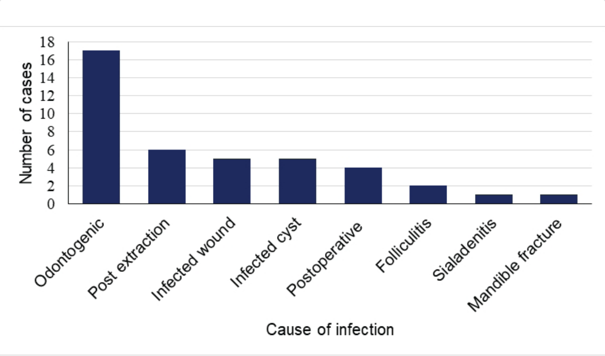

Figure 1: Frequency distribution of cause of infection.

Nine different bacteria were isolated **(Fig. 3)**. Out of the 41 samples of pus, single bacteria was cultured in 39 samples whereas two of the samples had 2 bacteria cultured in each. Twenty-three were gram positive cocci and 20 were gram negative bacilli. The most common organism cultured was *Enterococcus faecalis* (31.71%) followed by *Staphylococcus aureus* (14.63%), *Acinetobacter anitratus* and *Klebsiella pneumoniae* each 12.19%. Anaerobic culture and sensitivity test was not performed as the services are not available at the institute.

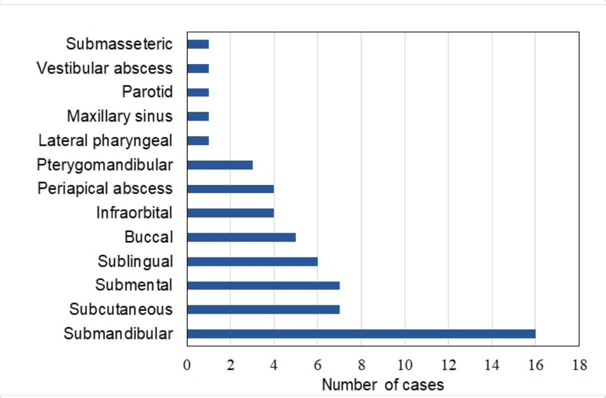

Figure 2: Frequency distribution of infected spaces.

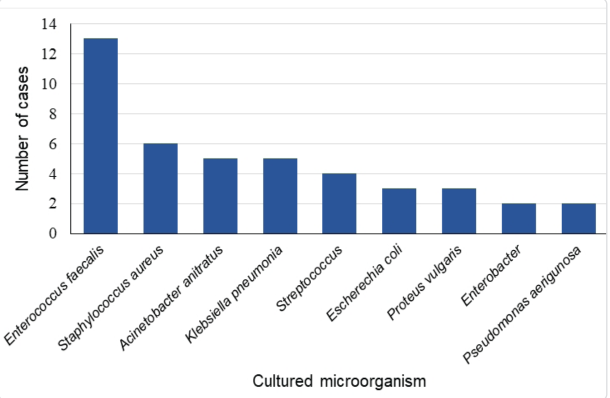

Figure 3: Frequency distribution of cultured microorganisms.

Antibiotic susceptibility pattern of the isolates showed that 50% of the cultured organisms (n = 8) were resistant to Penicillin- five were *E. faecalis*, and three *S. aureus*. Among the 15 cultured organisms tested for Ampicillin 80% (n = 12) were resistant, mainly the gram negative ones. Similarly, 55.5% (n = 5) were resistant to Amoxicillin Clavulanate, three *S. aureus* while *E. faecalis* and *Enterobacter* were found to be one each. Among the organisms tested for Cephalosporin group of drugs, 28.13% were resistant with only 22.7% of strains resistant to 3rd generation and no resistance to 4th generation Cephalosporins. Resistance against Quinolones was observed in 26.67% (n = 16) strains with most of the strains sensitive to Ofloxacin. Similarly, most of the organisms were sensitive to Amikacin, with three strains of *E. faecalis* and one *K. pneumoniae* reported resistance. E. faecalis was found to be 100% sensitive to Gentamycin and Azithromycin. Similarly, *E. faecalis* (n = 13), *Streptococcus* (n = 3), *S. aureus* (n = 5) were sensitive to Vancomycin without any resistance. **(Table 1)**

High resistance to the Penicillin group of drugs was observed while third generation Cephalosporins, Ofloxacin, and Amikacin were found to be sensitive to both gram positive and negative organisms in this study. Drugs effective against *E. faecalis* were Vancomycin, Gentamycin, Azithromycin. **(Table 1)**

DISCUSSION

This study was conducted to evaluate microbial flora and antibiotic susceptibility in oral and maxillofacial infections. The most common organism cultured was *Enterococcus faecalis* and high resistance to Penicillin was observed.

The age of the patients presenting with oral and maxillofacial infections was from 7 to 79 years with more patients in the third and fourth decades. This finding is similar to other studies found in the literature [6, 7]. Gender distribution was comparable to other studies [6, 8]. Odontogenic infection from mandibular molars was the most common cause of infection similar to other studies [9, 10]. Submandibular space was the most common fascial space to be infected because of the involvement of the mandibular molars as the sources of infection which was similar to other studies [4, 6, 11].

In this study, *Enterococcus faecalis* was the predominant bacteria isolated which is contradictory to most of the studies which suggested *Streptococcus* [6, 8, 11, 12] as the most common isolate. *E. faecalis* is a gram positive, nonmotile bacteria found in the gastrointestinal tract of humans [13]. It can invade dentinal tubules and colonize the root canal space [14]. So it is responsible for most of the endodontic infections [15]. The reason for the high occurrence of *Enterococcus* could be due to the fact that it is commonly found in the root canals of the teeth [13, 14] which in turn is the pathway for fascial space infection of odontogenic origin. This organism is one of the pathogens causing nosocomial infections [16]. However, the possibility of contamination from the dental operatory surfaces during the collection of the specimen is unlikely as the specimens were collected under the aseptic condition with rigorous antimicrobial preparation. And the potential for nosocomial transmission of *Enterococci* from clinical contact surfaces of dental operatory appears to be very small [17].

Another common aerobic bacteria isolated was *Staphylococcus aureus* as found in other studies [6, 8]. This study involved patients with wound infection in the face (subcutaneous abscess) which could be the

Table 1: Frequency distribution of antibiotics susceptibility to microorganisms

Antibiotics	**Frequency**	***P vulgaris***	***A anitratus***	***E faecalis***	***K pneumoniae***	***Streptococcus sp***	***Enterobacter sp***	***S aureus***	***E coli***	***P aeruginosa***
Penicillin (n = 16)	S (n = 8)			5		3				
	R (n = 8)			5				3		
Ampicillin (n = 15)	S (n = 3)		1	2						
	R (n = 12)	2	2	1	4			1	2	
Piperacillin (n = 3)	S (n = 2)						1			1
	R (n = 1)						1			
Other ß Lactams (n = 5)	S (n = 3)			1 sulbactam		1 sulbacatm				1 carbenicillin
	R (n = 2)						1 aztreonam	1 cloxaciliin		
Amoxiclav (n = 9)	S (n = 4)			2		2				
	R (n = 5)			1			1	3		
P-tazobactum (n = 6)	S (n = 6)	1	1		3				1	
	R (n = 0)									
1st generation Cephalosporins (n = 3)	S (n = 0)									
	R (n = 3)			1				1	1	
2nd generation Cephalosporins (n = 4)	S (n = 3)							3		
	R (n = 1)							1		
3rd generation Cephalosporins (n = 22)	S (n = 17)	2	4	1	3		1	3	1	2
	R (n = 5)		1		1	1		2		
4th generation Cephalosporins (n = 3)	S (n = 3)			2				1		
	R (n = 0)									
Meropenem (n = 13)	S (n = 12)	2	3		3		1		2	1
	R (n = 1)								1	
Imipenem (n = 5)	S (n = 5)	1	2		1					1
	R (n = 0)									
Ofloxacin (n = 27)	S (n = 22)	3	3	7	3	2	1	2		1
	R (n = 5)			2				2	1	
Ciprofloxacin (n = 16)	S (n = 10)	1	3	2				3		1
	R (n = 5)			1	1			1	2	
	P (n = 1)			1						
Levofloxacin (n = 17)	S (n = 11)	2	1	3	4					1
	R (n = 6)			2				1	3	
Vancomycin (n = 21)	S (n = 21)			13		3		5		
	R (n = 0)									
Azithromycin (n = 13)	S (n = 12)			8		2		2		
	R (n = 1)							1		
Amikacin (n = 28)	S (n = 24)	2	3	7	3	2		2	3	2
	R (n = 4)			3	1					
Gentamycin (n = 15)	S (n = 13)		1	10		1		1		
	R (n = 2)				1			1		
Tigecycline (n = 19)	S (n = 12)	1	4	2	2	1	1	1		
	R (n = 6)	2			2				1	1
	P (n = 1)				1					

S: sensitive, R: Resistant, P: Partial resistant contributing factor to the prevalence of *Staphylococcus aureus*. The involvement of gram negative organisms in maxillofacial infections has been reported in the literature [4, 8]. Gram negative organisms like *Klebsiella* and *Acinetobacter* showed increased occurrence in this study. *Klebsiella* has been found to be more common isolate in maxillofacial infections in Asian countries which could be related to the higher prevalence of diabetes in the Asian societies [1, 18, 19]. *Acinetobacter* has also been commonly involved in this study contrary to other reports in the literature. It is associated with nosocomial infection and the isolates are from skin, oropharynx and digestive tract of hospitalized patients most of the time [20]. Similarly, it is also reported to be involved with community-acquired pneumonia occurring during the rainy season among people with a history of alcohol abuse in tropical regions of Australia and Asia. The reason for the higher prevalence of *Acinetobacter* infections in certain geographic areas may be due to warm humid air that favors the growth of bacteria [21]. Further studies are required to evaluate the relation between environment/ geographic variations and virulence of *Acinetobacter* in maxillofacial infections.

High resistance to the Penicillin group of drugs was observed in our study contradictory to other studies [6, 9, 11, 22]. Penicillin resistance was observed mostly in *E. faecalis, K. pneumoniae* and *S. aureus*. The reason for such a finding could be because of the predominance of cultured bacteria like *E. faecalis, K. pneumoniae* in this study. Also, the irrational use of higher antibiotics in the country may have resulted in the evolution of resistant strains. Despite such high resistance to Penicillin in this study the clinical course of the patients did not show deterioration which could be due to the fact that infections were treated surgically and supported medically. Once the source of infection is removed and drainage established, infections may subside even without the use of antibiotics. Similarly, in vitro resistance may not necessarily infer in vivo resistance [9]. But in the context of *E. faecalis* infections if the patients do not respond to Penicillin therapy then Azithromycin, Gentamycin, and Vancomycin may be recommended considering their susceptibility as per this study. Vancomycin was found to be sensitive to all the gram positive organisms cultured without any evidence of resistance. So it can be used as an antibiotic of last resort [10]. Similarly, because of high occurrence of gram negative organisms third generation Cephalosporins, Ofloxacin, and Amikacin can be used for the broad spectrum coverage for mixed infections as per this study.

The study is conducted in a narrow geographical location and for the confirmation of the findings large-scale, multicentric studies using molecular analysis is recommended. The lack of anaerobic culture reports in the study can be considered a limitation of this study as anaerobic culture and sensitivity services are not available at the institute. Also, this study involved overall oral and maxillofacial infections presented to the clinic which might have led to the diversity in the result.

CONCLUSION

Orofacial infections are mainly of odontogenic origin and the occurrence of *Enterococcus faecalis* as the microorganism in odontogenic infections showing high resistance to Penicillin has been observed in this study.

References

1. Huang TT, Tseng FY, Yeh TH, Hsu CJ, Chen YS. Factors affecting the bacteriology of deep neck infection: a retrospective study of 128 patients. Acta Otolaryngol. 2006;126(4):396-401. DOI:10.1080/00016480500395195
2. Peterson LJ. Contemporary management of deep infections of the neck. J Oral Maxillofac Surg. 1993;51(3):226-231. DOI:10.1016/s0278-2391(10)80162-4
3. Poeschl PW, Spusta L, Russmueller G, Seemannet 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(2):151-156. DOI:10.1016/j.tripleo.2009.12.039
4. Storoe W, Haug RH, Lillich TT. The changing face of odontogenic infections. J Oral Maxillofac Surg. 2001;59(7):739-749. DOI:10.1053/joms.2001.24285
5. Vandepitte J, Verhaegen J, Engbaek K, Piot P, Heuck CC, Rohner P, et al. Basic laboratory procedures in clinical bacteriology. 2nd ed. Geneva: World Health Organization; 2003.
6. Bahl R, Sandhu S, Singh K, Sahai N, Gupta M. Odontogenic infections: microbiology and management. Contemp Clin Dent. 2014;5(3):307-11. DOI:10.4103/0976-237X.137921
7. Kannangara DW, Thadepalli H, McQuirter JL. Bacteriology and treatment of dental infections. Oral Surg Oral Med Oral Pathol. 1980;50(2):103-109. DOI:10.1016/0030-4220(80)90194-2
8. Rega AJ, Aziz SR, Ziccardi VB. Microbiology and antibiotic sensitivities of head and neck space infections of odontogenic origin. J Oral Maxillofac Surg. 2006;64(9):1377-80. DOI:10.1016/j.joms.2006.05.023
9. Yuvaraj V, Alexander M, Pasupathy S. Microflora in maxillofacial infections--a changing scenario?. J Oral Maxillofac Surg. 2012;70(1):119-125. DOI:10.1016/j.joms.2011.02.006

10. Farmahan S, Tuopar D, Ameerally PJ, Kotecha R, Sisodia B. Microbiological examination and antibiotic sensitivity of infections in the head and neck. Has anything changed?. Br J Oral Maxillofac Surg. 2014;52(7):632-635. DOI:10.1016/j.bjoms.2014.02.028
11. 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. DOI:10.4103/2231-0746.95318
12. Sanchez R, Mirada E, Arias J, Pano JR, Burgueño M. Severe odontogenic infections: epidemiological, microbiological and therapeutic factors. Med Oral Patol Oral Cir Bucal. 2011;16(5):e670-e676. DOI:10.4317/medoral.16995
13. Flanagan D. Enterococcus faecalis and Dental Implants. J Oral Implantol. 2017;43(1):8-11. DOI:10.1563/aaid-joi-D-16-00069
14. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93-98. DOI:10.1016/j.joen.2005.10.049
15. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology (Reading). 2009;155(Pt 6):1749-1757. DOI:10.1099/mic.0.026385-0
16. Ryan KJ, Ray CG, editors. Sherris medical microbiology, 4th ed. New York: McGraw-Hill; 2004.
17. Vidana R, Sillerstrom E, Ahlquist M, Lund B. Potential for nosocomial transmission of Enterococcus faecalis from surfaces in dental operatories. Int Endod J. 2015;48(6):518-27. DOI:10.1111/iej.12342
18. Lee YQ, Kanagalingam J. Bacteriology of deep neck abscesses: a retrospective review of 96 consecutive cases. Singapore Med J. 2011;52(5):351-5. PMID: 21633769
19. Chang CM, Lu FH, Guo HR, Ko WC. Klebsiella pneumoniae fascial space infections of the head and neck in Taiwan: emphasis on diabetic patients and repetitive infections [published correction appears in J Infect. 2006 Sep;53(3):215]. J Infect. 2005;50(1):34-40. DOI:10.1016/j.jinf.2003.11.014
20. Jung J, Park W. Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. Appl Microbiol Biotechnol. 2015;99(6):2533-48. DOI:10.1007/s00253-015-6439-y
21. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med. 2008;358(12):1271-81. DOI:10.1056/NEJMra070741
22. Kuriyama T, Karasawa T, Nakagawa K, Saiki Y, Yamamoto E, Nakamura S. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90(5):600-8. DOI:10.1067/moe.2000.109639