Documentation of the Spectrum of Microbiological Flora and their Antibiotic Sensitivity in oral Infections in the Local Population in Pune, India

Lade Asmita Y.*[1], Waknis Pushkar[2], Shah Seemit[1], Dadhich Anuj[1], Saluja Harish[1], Sachdev Shivani[1]
[1]*Oral and maxillofacial surgery, Pravara Institute of Medical sciences, Dental college, Loni, Maharashtra, India*
[2]*Department, Oral maxillofacial surgery, DY Patil dental college, Pune*

***Corresponding Author**
Asmita Lade
E-mail ID: asmitaconnects@yahoo.co.uk

Abstract:

Aims & Objective: To assess the spectrum of microbiological flora and its antibiotic sensitivity in oral infections in local population.

Methodology: The institutional ethics committee approval was taken. Patients reporting with moderate to severe abscesses associated with facial space infection of odontogenic origin were selected for this study. The clinical history, signs, symptoms and routine diagnostic procedures were carried out. The surgical procedure was carried out following the standard protocols. Sample of pus was collected from the surgical site, in pre-produced media for culturing and subculturing. For microscopic examination gram staining was done to determine gross morphology of the bacteria and antibiotic sensitivity test was performed using different antibiotics against the bacteria isolated from the samples.

Results: Gram positive microorganisms were predominant. Aerobic bacteria were dominant (62.5%) than anaerobic bacteria (12.5%). Clindamycin was found to be more effective against most of the bacteria isolated than other antibiotics tested *

Keywords: Odontogenic infections, Periapical abscess, Facial infections, Microorganisms, Antibiotics

Introduction

Odontogenic infections encompass a spectrum ranging from basic periapical abscesses to more severe infections affecting both superficial and deep facial spaces in the neck. The micro-organisms have demonstrated adaptability and have exhibited a concerning tendency to resurface in ongoing cycles of disease in many different forms.[1]

The appropriate choice of empirical antibiotics is by information of frequently encountered pathogens and their resistance profiles which has been proof of a great help for the clinician[(2)]. Literature shows that very few research has been carried out to compile a list of prevalent pathogens responsible for orofacial infections especially in India. Hence, this study was conducted with a aim to gain information regarding the causative factors for odontogenic infections in the selected sample.

Methodology

The study samples were collected from patients of OMFS department of Dr .D.Y. Patil Dental College and Hospital Pune , India. Institutional ethics committee approval was taken prior to the commencement of the study. After explaining the aim and design of the study, 30 patients consented to participate in the study. The procedure such as needle aspiration, surgery, medications, and complication were informed along with written consent of the patient.

Patients reporting with moderate to severe abscesses associated with fascial space infection of odontogenic origin were selected. Patients willing to participate, of any age group and patients with moderate to severe abscesses associated with fascial space infections were included in this study. The patient who had antibiotic therapy more than five days before reporting were excluded from this study.

The clinical history, signs, symptoms, antibiotic and radiographic examination were done. The routine blood investigation, chest x-ray, and ECG were carried out as a part of the pre-anesthetic evaluation. Under general or local anesthesia surgical decompression or drainage were performed. The pre-operative clinical and intra-operative findings were noted as given in the Patient's data.

For the surgical procedure, the Armamentarium of surgery were Swab holder, Sterile drape, Towel clips, Mouth mirror, Probe, Tweezer, Disposable syringe (10cc and 5cc.), 18 gauge and 26 gauge needle., Periosteal elevator, Needle holder, Artery forceps, Cheek retractor, Suction tube cannula no.4, specific requirement - nutrient broth bottles. Cotton rolls were used to isolate the area intended for sampling and dried with sterile cotton swabs with 70% ethanol may be used to further disinfect and dry the area. General anesthesia or local anesthesia was administered depending on the clinical situation. The painting and draping were done in a usual manner. The skin or mucosa was decontaminated with an antiseptic solution (Betadine). Specimen (pus) was collected before surgical drainage, either intra- orally or extra-orally depending on the clinical situation by aspiration in the syringe with a large bore needle (18 gauge). The needle was capped promptly with a plastic cap. The part of this sample was injected into the bottle which contained nutrient broth. This sample was used to prepare a slide to examine under the microscope. After obtaining the sample usual drainage was

carried out. The wound was irrigated with betadine or saline. Drains were placed intra-orally. The sample was sent to the lab for microbiological study.[3]

The sample was collected in pre-produced media like Robertson's cooked meat medium(RCM), and thioglycollate medium and was transported to the laboratory immediately. It was then incubated for 48 hrs at 37 °C before subculturing it on anaerobic isolation media. RCM broth was prepared by using cooked meat particles, 500 gm of fresh bullock heart, 500 ml of water and 1.5 ml of NaOH 1 mol/liter.[3] Minced bullock heart was placed in alkaline boiling water for 20 minutes to neutralize the lactic acid. The liquid was drained off through the filter. The minced meat was pressed in a cloth and dried partially by spreading it on a cloth and filter. In this condition, it can be introduced in bottles without soiling them. Peptone infusion broth was prepared by using 500 ml of Liquid filtered from cooked meat, 2.5 gm of Peptone, and 1.25 gm of NaCI. Steamed at 100 °C for 20 min and added1 ml of pure HCL and filter, brought the reaction of the filtrate to 8.2, then steamed at 100°C for 30 min and adjusted reaction to pH 7.8. Complete media was prepared by placing the meat in to a depth of about 2.5 cm of 30 ml bottles and covered with 15 ml nutrient broth and then autoclaved at 121 °C for 30 min.[3]

Before the microscopic examination, gram staining was done to determine the gross morphology of the bacteria and to differentiate between gram-positive and gram-negative bacteria. Aerobic gram-positive cocci are Strep Aureus, Group A Streptococcus, Streptococcus Mutans, Streptosalivarius, Streptococcus Pyogens, and Streptococcus Pneumonia. Anaerobic Gram-positive cocci are Streptococci Peptostreptococci, Parvinomas Micro. Anaerobic Gram-positive rods: Lactobacillus, Actinotnycetes. Aerobic Gram-negative bacterias are Neisseria spp and Micrococcus Vellionella. Gram-negative rods are Proteus spp, E.Colli, Haemophilus Serratia spp. Klebsiella Spp, Pseudomonas Aeruginas G. Anaerobics are PorphyromonasGingivalis, Fusobacterium, Bacteroids Treponema Denticola.[3]

The culture was performed in the laboratory by closing windows and floor and restricting the movement of the people in the room. During inoculation, the culture medium was uncovered for only a few seconds. Inoculating the specimen on Blood agar and MacConkey agar by sector plate technique was done.[3]

For confirmation of the organisms different biochemical tests were performed. Inoculation of culture media was carried out by using an aseptic technique. Inoculated media was incubated as soon as possible to protect the viability of pathogens and to avoid dust contamination. It was kept away from sunlight. For most organisms, the optimum temperature used for incubation was 35- 37°C. A very dry atmosphere can impact the growth and survival of many pathogens such as Gonococci. Introduction of humidity in the form of a piece of damp blotting paper at the bottom of a candle jar is recommended for the culture of Gonococci.[3]

Culturing of anaerobes was done by adding sodium thioglycolate (reducing agent) and methylene blue (indicator). Anaerobic conditions were obtained by removing air from the anaerobic jar. It was replaced with hydrogen or nitrogen and hydrogen combination or with carbon dioxide in the presence of a catalyst such as Palladium, the hydrogen reacts with oxygen to form water. Steel wool coated with copper was also used to eliminate oxygen. Culture in carbon dioxide was also performed for the growth of micro-organisms such as Neisseria, and Gonorrhoeae. For Neisseria meningitis and Streptococcus pneumonia, an atmosphere enriched with carbon dioxide is necessary which can be provided by enclosing a lighted white smokeless candle in the air light jar. The oxygen contained is reduced as the candle burns by leaving carbon dioxide at 3-5 % concentration when the candle is extinguished. The plates after inoculation are placed immediately in the such jar.[3]

We then observed the colony characteristic and culture characteristics, resistance, Metabolism, biochemical properties, and motility. We observed the shape, surface, elevation, color, structure, consistency, degree of growth, and nature. In a fluid medium, we observed the degree of the growth, presence of deposit and its character, nature of surface growth, Ease of disintegration and odor.

We also performed the hanging drop method to test the motility test of the bacteria to get an idea of their shape, approximate size, general structure, and the differentiation between true motility.

After this, an antibacterial sensitivity test was performed. Organisms that grow up to the edge of the disc are considered resistant. Inoculation of the test plates has divided the plate into sections, according to the number of antibiotics (6 to 7 per plate)and incubated the plates overnight to 37cc-measure the diameters zone of inhibition of growth in mm.[3]

Results were observed divided into 3 categories- if the zone is less than 4 mm then it is resistant if the zone is 4-12 mm, then intermediate and if a zone is more than 12 mm then it is sensitive.[4]

Results

A total of 30 samples were studied for 30 patients. Out of 30 patients, 25 (83%) were males and 5 (17%) were females. The incidence of involvement was highest in buccal and submandibular space among the all cases. Buccal space (43%), submandibular space (37%), Palatal space and Ludwig's angina (7%), Temporal space, and osteomyelitis of the jaw (3%). This correlates with the maximum involvement of posterior teeth as a source of odontogenic infection.

Table 1: Sitewise distribution of micro-organisms

Site of infection	Aerobic organism	Anaerobic organism	No growth	Total
Buccal space infection	09	-	05	14
Submandibular space infection	10	-	02	12
Osteomyelitis	01	01	-	02
Ludwig's angina	-	01	01	02
Temporal space infection	-	01	-	01
Palatal swelling		01	-	01
Total	20 (62.5%)	04 (12.5%)	08 (25%)	32

Table 1 shows that the aerobic count (62.5%) of micro-organisms was higher than anaerobic micro-organisms (12.5%) in the sites. The incidence of aerobic micro-organisms as compared to anaerobic micro-organisms ison the higher side.

Table 2 : Staining and typing

	Buccal	Submandibular	Osteomyelitis	Ludwig's Angina	Temporal	Palatal swelling
Gram-negative	2	5	0	0		
Gram-positive	5	5	2	1	1	1

Table 2 shows, that the incidence of gram-positive micro-organisms in buccal and submandibular spaces was greater in number. At other sites involved in the study, only gram-positive micro-organisms were observed. This corrects the fact that gram-positive micro-organisms are involved more than gram-negative micro-organisms in orofacial infections. In this study, rods isolated were less than cocci .

The incidence of isolated MRSA (16.6%) and MSSA (I6.6%) was highest followed by Citrobacter Spp and streptococcus pyrogens. the other micro-organisms isolated were Actinomycetes Meveri Peptostreptococcus, Streptococcus fecalis, Propionibacter, Pseudomonas aenigthoso, and Acinetobacter in the minor count.

Table 3 shows the result of the antibiotic resistance test of different antibiotics with sensitivity and resistance rate.

Table 3: Antibiotic sensitivity test results

Antibiotics	Sensitivity test
Cefotaxime	S= 4 (19%) R= 3(14.2%)
Imipenem	S= 2 (9.5%) R=0
Ceftazidime	S= 3 (14.2%) R=1 (4.7%)
Chloramphenicol	S= 0 R= 1 (4.7%)
Clindamycin	S= 7 (33.3%) R= 2 (9.5%)
Cotrimoxazole	S= 4 (19%) R=9 (42.8%)
Gentamycin	S= 3 (14.2%) R=0
Oxacillin	S= 4 (19%) R= 3(14.2%)
Piperacillin	S=0 R=1 (4.7%)
Erythromycin	S= 8 (38%) R= 5 (23.8)
Cefuroxime	S= 7 (33.3%) R= 6 (28.5%)
Ciprofloxacin	S= 4 (19%) R= 1 (4.7%)
Amoxycillin	S=3 (14.2%) R=1 (4.7%)
Ofloxacin	S= 1 (4.7%) R= 2 (9.5%)
Amikacin	S= 8 (38%) R=0
Ampicillin	S= 2 (9.5%) R= 5 (23.8%)
Vancomycin	S= 3 (14.2%) R=0
Linezolid	S= 1 (4.7%) R=0
Tazobactum	S= 1 (4.7%) R=0
Cefoxitin	S=0 R=2 (9.5%)
PnG	S= 0 R=2 (9.5%)

Discussion

The most common pathology in the general population is oral infections. Most orofacial infections are odontogenic in origin.[4] Dental caries, caries involving pulp, periodontal abscess, and pericoronitis are the causative factors.[5] Odontogenic infections of Head and Neck region are usually managed by oral and Maxillofacial Surgeon. Overlooked odontogenic infections are life-threatening which are secondary to septicemia.

This study comprised 30 patients. Patients requiring immediate incision and drainage due to infections were identified. Among that 25 (83%) were male and 5 (17%) were females. Our reports were similar to Kannangara et al who reported a male predominance with 40 (66 %) males and 21 females (34%). In a study of 113 pediatric patients with odontogenic infections, a male predominance was observed, with 67 males (59%) and 46 females (41%).

The possible routes for the spread of infections and the typical involvement of mandibular molar teeth in odontogenic infections have been discussed. Other authors have also reported that mandibular molar teeth are most frequently involved teeth in odontogenic infection.[4,7,8] The involvement of mandibular first molar in our study, is in accordance with the previous studies.[4] There is no such explanation for this observation. Some studies suggest that among maxillary teeth, the maxillary first molar was the most common cause tooth of odontogenic infection.

Soft tissue infections of odontogenic origin typically propagate from the structures supporting the affected tooth, following the paths of least resistance to nearby fascial spaces.[4] In the review of the literature, single-space abscess had 3 very common findings, including submandibular space as the most predominant, followed by the buccal space, the canine space abscesses.[5,9,10] In this study predominant space involved was the buccal space followed by the submandibular space, Palatal space and Temporal space. This may be due to the anatomic relationship of odontogenic infections and close relationship to the affected space(s).

Isolated bacteria consisted both aerobic and anaerobic micro-organisms. Infections due to anaerobic and Gram-negative organisms have increased in comparison with past reports in the medical and dental literature. This may be related to improvements in isolating and culturing methods of anaerobic organisms.[11] But in this study, Gram-positive cocci were the predominant bacteria cultured from our specimens and Gram-negative rods were the second most common bacterial isolate. This is consistent with the results of other studies in the literature.[9]

The aerobic micro-organisms (62.5%) isolated in this study counted higher than anaerobic micro-organisms (12.5%). In this study anaerobic bacteria were minimum in percentage , than the observations made by Sabiston[12], Itzhak Brook[13] but it is same as Anthony J. Rega and coworkers[5], Kannangara et al.(1980)[6], Munish Kohli studies.[14] Isolation of Staphylococcus aureus were more, of which most were resistant to Methicillin (MRSA) (16.66 %) and also sensitive to Methicillin (MSSA) (16.66%). In 1998-2000 A J Smith, his Co-workers had carried studies, the result of this study was same as theirs.

In sample of pus of odontogenic infections, Staphylococci, a causative factor, were confirmed by studies.[6] Staph.aureus has clinical significance since it develops resistance to many known antibiotics. Our result of gram negative bacilli like Klebsiella (8.3 %) was isolated were same to previous studies. [9.11]

Pseudomonas isolated were 4.1 % only. The results were similar to the other studies.[16,17] In some studies isolation of Pseudomonas was 11.60 % little on the higher side than the results of the other studies. Pseudomonas , the drug resistant pathogen were more in number due to overuse of antibiotics in dentistry . There was no growth observed in 8 cases (25%) out of 30 cases in this study. In another study, there were 3.75 % cases in which no growth was observed.[14]

The drugs to be tested against each species of bacteria should be grouped in sets of six. The antibiotics on each set should be those most likely to be used for therapy of infections with a particular species. It is neither possible nor desirable to specify standard sets of drugs because of differences in local prescribing habits, in the resistance patterns of local pathogens.[1]

In this study, Staphylococcus showed susceptibility to Ciprofloxacin, Clindamycin, Cefuroxime, Erythromycin, Levofloxacin, and Vancomycin. A less percentage of isolated, Staphylococci showed poor susceptibility towards ampicillin and penicillin.[14]

The percentage of sensitivity to Clindamycin was more as compared to other antibiotics. This is similar to the findings of G.K.B.Sandor.[5] According to him Clindamycin has been

increasingly tested for use in a variety of dental infections. After the increased sensitivity to Clindamycin, it was followed by Amikacin, Gentamycin, and Erythromycin study.

Clindamycin was found effective as it is a unique antimicrobial that achieves high tissue concentrations, penetrates intracellularly, increases phagocytic activity, inhibits toxin production and has a post-antibiotic effect.[19]

In treating dentoalveolar abscesses, clindamycin demonstrates similar effectiveness to penicillin. Consequently, it should be regarded as a viable alternative for allergic patients or in cases where penicillin fails to produce results.[14] In our study, we found the most sensitive antibiotics were Cefuroxime and Clindamycin. For this study, limitations could be small sample size, error while performing laboratory testing, selection bias, etc.

A mixture of aerobic and anaerobic bacteria is involved in microbiological flora of odontogenic abscess can be confirmed by this study. A safe and ethical use of antibiotic and its its sensitivity should be done for treatment of odontogenic infections.

References

1. Gendron R, Grenier D, Maheu-Robert L. The oral cavity as a reservoir of bacterial pathogens for focal infections. Microbes Infect 2000;2(8):897-906.
2. Goldstein EJC, Clinical anaerobic infections, Anaerobe 1999; 5; 347-350.
3. Saini S, Gupta AN, Mahajan A, Arora DR. Microbial flora in orodental infections. Indian J Med Microbiol 2003;21(2):111-4. PMID: 17642993.
4. Chow AW, Roser SM, Brady FA. Orofacial odontogenic infections. Ann Intern Med. 1978;88(3):392-402.
5. Sandor GK, Low DE, Judd PL, Davidson RJ. Antimicrobial treatment options in the management of odontogenic infections J Can Dent Assoc 1998;64(7):508-14.
6. Kannangara DW, Thadepalli H, McQuirter JL. Bacteriology and treatment of dental infections. Oral Surg Oral Med Oral Pathol 1980;50(2):103-9.
7. Koneman's. Color Atlas and Textbook of Diagnostic Microbiology 2006; 6th edition; page:40.
8. Peterson LJ. Contemporary management of deep infections of the neck. J Oral Maxillofac Surg 1993;51(3):226-31.
9. Storoe W, Haug RH, Lillich TT. The changing face of odontogenic infections. J Oral Maxillofac Surg 2001;59(7):739-48; discussion 748-9.
10. Labriola JD, Mascaro J, Alpert B. The microbiologic flora of orofacial abscesses. J Oral Maxillofac Surg 1983;41(11):711-4.
11. Obayashi N, Ariji Y, Goto M, Izumi M, Naitoh M, Kurita K, Shimozato K, Ariji E. Spread of odontogenic infection originating in the maxillary teeth: computerized tomographic assessment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;8(2):223-31.
12. Sabiston CB Jr, Grigsby WR, Segerstrom N. Bacterial study of pyogenic infections of dental origin. Oral Surg Oral Med Oral Pathol 1976;41(4):430-5.
13. Brook I, Frazier EH, Marlin EG. Aerobic and Anaerobic microbiology of periapical abscess. Oral Microbiol and Immunol 1991;6(2):123-125.
14. Kohli M, Mathur A, Siddiqui SR. In vitro evaluation of microbiological flora of orofacial infections. J Maxillofac Oral Surg 2000;8(4):329-33.
15. Smith AJ, Roberyson D, Tang MK, Jackson MS, Mackenzie D, and Bagg J: Staphylococcus aureus in the oral cavity: A three-year retrospective analysis of clinical laboratory data: Brit Dent J 2003; 195:701-703.
16. Gill Y, Scully C. Orofacial odontogenic infections: review of microbiology and current treatment. Oral Surg Oral Med Oral Pathol Radiol Endod 1990;70(2):155-8.
17. Kuriyama T, Karasawa T, Nakagawa K, Nakamura S, Yamamoto E. Antimicrobial susceptibility of major pathogens of orofacial odontogenic infections to 11 beta-lactam antibiotics. Oral Microbiol Immunol 2002;17(5):285-9.

18. Collee JG, Fraser AG, Mannion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology 1996;4th edition; Page- 155.

19. Brook I, Lewis MA, Sándor GK, Jeffcoat M, Samaranayake LP, Vera Rojas J. Clindamycin in dentistry: more than just effective prophylaxis for endocarditis? Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(5):550-8.