



RESEARCH ARTICLE

EVALUATION OF ORAL PATHOGENIC MICROBES ASSOCIATED WITH PERICORONITIS AND THEIR SUSCEPTIBILITY TO DIFFERENT ANTIBIOTICS

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ABSTRACT

Background and Objective: The objective of the study was to identify the predominant oral pathogenic microbes associated with pericoronitis and antibiotic sensitivity of those predominant group of pathogens.

Materials and Methods: A total no of 40 patients were selected, subjected to Microbiological evaluation and after performing the antibiotic sensitivity test, proper antibiotic therapy was given. These subjects were divided into 2 groups –Pre antibiotic & Post antibiotic, each group constituted 20 patients. Swab was collected from the depth of the pericoronal pockets for microbiological evaluation and their Antibiotic sensitivity test.

Results: The results suggested that, both aerobic and anaerobic bacteria colonized in mixed infections of oral cavity as pericoronitis, but the distinguishing feature between these two groups that after proper antibiotic treatment the percentage of bacterial count (especially the anaerobes) markedly diminished.

Conclusion: It was concluded from the results that, Amoxicillin Clavulanate fully encompasses the aerobic microorganisms whereas Metronidazole was particularly effective against the anaerobes.

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INTRODUCTION

Pericoronitis is an inflammatory and infectious condition that accompany the clinical emergence of teeth (Winter, 1924). A healthy, fully erupted tooth is usually surrounded by the gingival tissue that typically extends not more than 2-3 mm coronally. However, crowns of the 3rd molar and occasionally other teeth may be covered to a considerably greater extent with gingiva and sometimes mucosa for a variety of anatomical causes such as partial eruption, malposition and immediate proximity of anterior border of ramus (Topazian Goldberg Hupp, 2002). Majority of cases of pericoronitis affects the young adults in the age of 20-29 years (Topazian Goldberg Hupp, 2002 and Kay, 1966). It typically begins with gingival tenderness and localized pain which sometimes radiates to face-ear-angle of mandible. Clinical examination reveals either an enlarged, tendered segments of soft tissue on the affected side covering one or more of the coronal surface including the occlusal surface of involved tooth or a little coverage of facial or lingual surface on mandibular 3rd molars and an inflamed pedicle like mass (operculum) projecting

from retromolar area to occlusal surface with exudation of pus just beneath the margin of pericoronal tissue (Topazian Goldberg Hupp, 2002; Kay, 1966; Jean-Lois sixou, 2003; Rajasuo, 1996; Justin Moloney, 2009). The oral cavity forms an indispensable part of human microbiome for its unique and diverse microflora distributed in various niches such as gingival crevice, periodontal pockets, dorsum of the tongue, buccal vestibule and other mucosal surfaces. Pericoronitis undoubtedly is an infectious process, where microflora are present in the distally located pseudopockets (Rajasuo, 1996; Van, 1997 and Peltroche, 2000). The microflora of mandibular third molar pericoronitis was previously considered to be composed of gram negative *fuso-spirochaetal* organisms and gram positive *Streptococci*. Most odontogenic infections are mixed anaerobic in origin. *Prevotella intermedia*, *Actinomyces israeliae*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Capnocytophaga spp*, *Actinobacillus actinomycetemcomitans*, and *Veillonella species*, –are the obligate anaerobes, which appears in pericoronal pocket (Konoen, 1999). Therapeutic management usually involves a local surgical procedure and prescription of antibiotics. Amoxicillin is most effective against the aerobic-flora, whereas Metronidazole alone or in combination with Spiramycin was most effective against obligate anaerobes

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(Konoen, 1999 and Peltroche, 2000). Pericoronal infection being the most common disease involving the mandibular third molar in teenager and young adults –even after its surgical management the complications may precipitate in the form of trismus, sensory nerve alteration, space infection, cellulitis associated with haemorrhage, dentoalveolar fracture and displacement of tooth. Most of these type of complications may arise as a result of antibiotic therapy which is unable to meet the microbial challenge under the pericoronal flap. Hence proper evaluation of the predominant oral pathogenic microbes associated with pericoronitis and their susceptibility to different antibiotics has been undertaken (Winter, 1924 and Kay, 1966). Keeping all these in mind, the study was carried out to know the status of oral microflora in normal healthy individuals, the predominant oral pathogenic microbes associated with pericoronitis, antibiotic sensitivity of the predominant groups of pathogenic microbes associated with pericoronitis and finally, to compare and corroborate all the aforesaid findings with a view to delineate proper antibiotic therapy for treatment and management of Pericoronitis.

MATERIALS AND METHODS

This explorative study was conducted in the department of Oral & Maxillofacial Pathology, GuruNanak Institute of Dental Sciences & Research (GNIDSR), Panihati, Kolkata in collaboration with Department of Microbiology, School of Tropical Medicine, Kolkata during the period of June 2015-July 2016. 40 patients were selected finally and subjected to microbiological evaluation and after performing the antibiotic sensitivity test, proper antibiotic therapy was instituted. These subjects were divided into 2 groups –Pre antibiotic & Post antibiotic, each group constituted 20 patients.

Selection of sample

Inclusion Criteria

- Patient selection was based on the clinical diagnosis of acute or subacute pericoronitis of the impacted (mandibular or maxillary) third molar, with pus detected by probing the periodontal pocket.
- The adults (18-34 years) were included in the study design for collection of sample.
- The patient had not received antibiotic treatment during the last 30 days before the study.
- Partially erupted molars (both clinically and radiographically evaluated) were included.

Exclusion Criteria

Patients who suffered from the systemic diseases like Diabetes mellitus, Congestive cardiac failure, hemorrhagic disorders, neurological problems, renal diseases along with mentally retarded patients were excluded in this study subject. The details of subjective and objective features of all finally selected study subjects were recorded in the clinical case sheet. Following this, written consents were received from all the selected patients in the specially prepared consent form.

Methods of Bacteriological Study

- >Before brushing of teeth sterile cotton swab was used to collect the specimen from the affected area of the patient's mouth.

- >A sterile swab stick was then inserted into the depth of the pericoronal and gingival pockets, after collection of the specimen, it was placed in a sterile container under all aseptic conditions and processed immediately.
- The swabs were directly inoculated onto blood agar.
- After isolation of bacteria in pure culture from a specimen, it has to be identified phenotypically by following studies -a) morphology of bacterial colony, b) staining (Gram's staining), c) motility test (hanging drop preparation), d) Biochemical test (Oxidase, Citrate, Indole, Urease, Triple sugar iron).
- Antibiotic sensitivity test (Kirby Bauer test) was performed to identify the predominant groups of microbial pathogen which are actively associated with the initiation and progression of the disease process
- After 7 days, the swabs were collected from same group of patients, the microbial culture was made, finally the antibiotic sensitivity test was again performed to compare and corroborate the changes in the distribution pattern of oral microflora in patients of pericoronitis and the result that we have obtained are presented here in a tabular form.

RESULTS

50 patients were selected for this study purpose, among them 10 were considered as Control (not suffering from pericoronitis, and their age group beyond 35) and 40 patients were registered as test sample (they were clinically screened, diagnosed and radiographically evaluated for the presence of impacted third molar and subsequently pericoronitis). At first, from the control groups swabs were collected from the different sides of oral cavity (gingival sulcus/saliva/buccal vestibule/lingual vestibule/dorsum of the tongue). These swabs then immediately inoculated in the Blood agar and kept it in the incubator at optimum temperature (37°C) for 24 hours, bacterial colony was obtained from each culture plate, they were identified by gram staining, and biochemical tests (Oxidase, citrate, Indole, TSI, Urease, Coagulase). The most commonly isolated microorganisms were the normal oropharyngeal commensals i.e. Streptococcus, Staphylococcus, Haemophilus, Enterococcus (gram +ve cocci), Lactobacillus (gram +bacilli), Veillonella, Neisseria (gram -ve cocci). Then, the swabs were collected from the patients who suffered from recurrent pericoronitis. The patients were all within the younger age group (18-34). These 40 patients were categorized into two groups -1st group (20) did not take any antibiotic therapy for the last 1 month during study period, and 2nd group (20) under the antibiotic treatment.

Sample collection from the 1st subgroup (10 patients) depends upon some criteria

- Partially erupted 3rd molar (no purulent exudates),
- The depth of the gingival sulcus < than 4mm,
- The 3rd molar soft tissue not traumatized by ipsilateral maxillary 3rd molar

The samples were inoculated into the culture plates (Blood/Nutrient/Mackony; s agar), and same procedures were repeated for bacterial inoculation. The most commonly isolated organisms were Aerobic –Staphylococcus [Fig. 1a] Haemophilus, Pseudomonas, [Fig 1b], Streptococcus [Fig 1c], Cornebacterium, Lactobacillus, Acinetobacter [Table 1] etc.

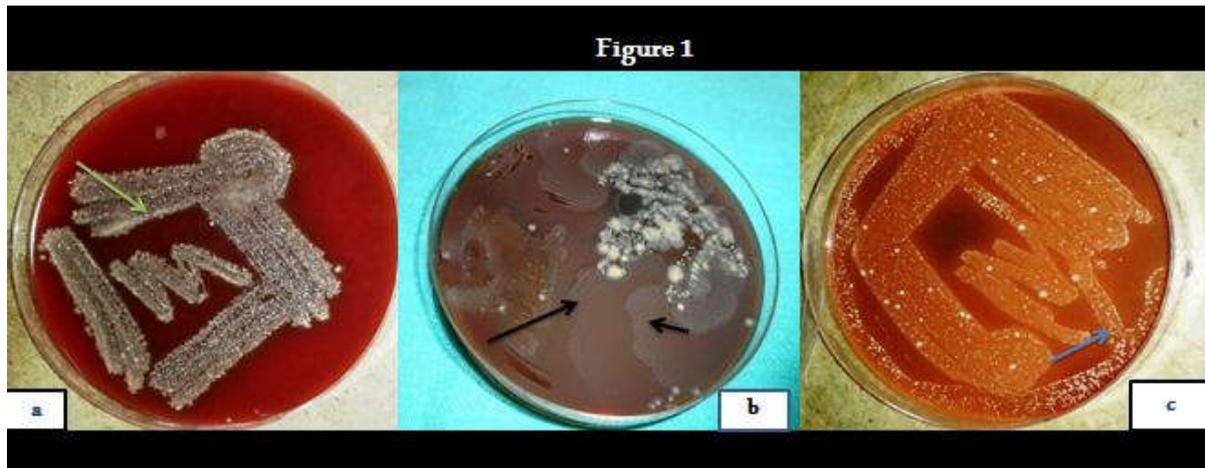


Figure 1. Culture plate containing Blood agar media depicts (a) smooth –creamy white-glistening colony of Staphylococcus (b) swarming colony of Pseudomonas (c) the growth of Streptococcus with hemolysis

Table 1. Aerobic bacteria isolated from 10 patients

Sites of sample collection(n=10)	Isolated aerobic microorganisms
1>pericoronal pocket of the partly erupted mandibular 3 rd molar	Streptococcus, B hemolytic streptococci, Enterococcus, Staphylococcus.
2>distal gingival pockets of adjacent mandibular 1 st & 2 nd molars	Non pathogenic Neisseria species, streptococcus, Pseudomonas, Corynebacterium, Micrococcus.
3>distal gingival pockets of the maxillary 1 st molar of the same site	Non specified gram negative rods, Acinetobacter, coagulase negative Staphylococcus, Lactobacillus.

Table 2. Anaerobic bacteria isolated from another 10 patients

Sites of sample collection(n=10)	Isolated anaerobic microorganisms
1>pericoronal pocket of the partly erupted mandibular 3 rd molar	Actinomycetes, Fusobacterium
2>distal gingival pockets of adjacent mandibular 1 st & 2 nd molars	Fusobacterium, Prevotella, Actinomycetes, Bacteriodes.
3> distal gingival pockets of the maxillary 1 st molar of the same site	Actinomycetes, Vincent bacilli.

Table 3. Comparison of aerobic bacterial % before and after antibiotic treatment

Aerobic organisms	% of Aerobes before Antibiotics	% of Aerobes after Antibiotics
1>Streptococcus sp	85-90%	< than 50%
2>Non pathogenic Neisseria sp	70%	<than 40%
3>Staphylococcus sp	75-80%	< than 60%
4>Acinetobacter sp	65-70%	< than 40%
5>Pseudomonas sp	75-80%	< than 50%
6>Gram negative rods	85-90%	< than 50%

Sample now collected from the next subgroup (10 patient) depends upon some criteria

- The partially erupted 3rd molars associated with purulent exudates, severe bleeding on slight provocation or spontaneously,
- Limited mouth opening,
- The depth of the gingival sulcus >than 4mm, poor oral hygiene,
- Limited mouth opening, submandibular lymphadenopathy,
- The 3rd molar soft tissue was traumatized by ipsilateral maxillary third molars.

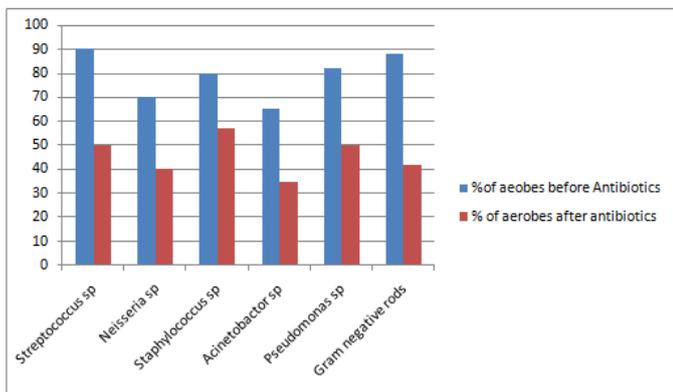
Samples were directly inoculated into fresh blood agar and immediately kept in anaerobic gas jar. Bacterial colony was obtained after 3 days. Here the most commonly isolated organisms were Anaerobic (facultative or obligate anaerobes) i.e. Actinomyces, Vincent bacilli [Table 2], Streptococcus. Other samples were collected from the 2nd group (patients were under the antibiotic regimen Amoxicillin Clavulanate along with Metronidazole), here the results of microbial culture

shows that the pericoronitis is a mixed infection of the oral cavity. Both aerobic and anaerobic bacteria were colonized, but the distinguishing feature between these two groups was that, after proper antibiotic treatment the % of bacterial count markedly diminished. The broad spectrum of Amoxicillin Clavulanate fully encompasses the microorganisms found in pericoronitis. Metronidazole is used particularly in infections due to polymicrobial flora, in which anaerobic microorganisms predominate. Its combination with a macrolide (spiramycin) extends the spectrum to certain non-obligately anaerobic bacteria, allowing its use in pericoronitis with a well documented mixed aerobic –anaerobic flora.

DISCUSSION

Pericoronitis is an inflammatory & infectious conditions that accompany the clinical emergence of teeth (Winter, 1924). A healthy and fully erupted tooth is surrounded by gingival tissue that typically extends not more than 2 to 3 mm coronally. Incomplete eruption of teeth provides a large stagnation area for accumulation of food debris under the gingival flap, this becomes infected easily and results in inflammation of the

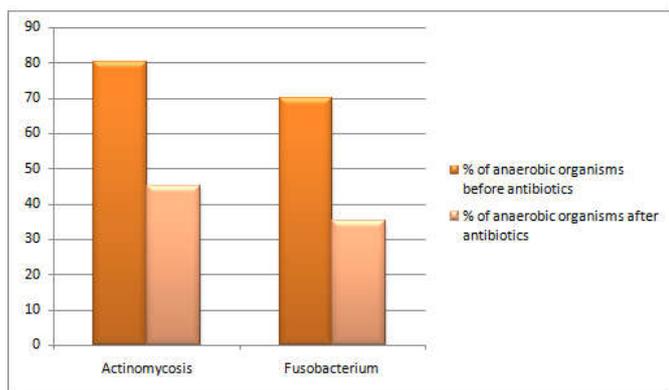
pericoronal tissue (Topazian Goldberg Hupp, 2002). Microbial flora develops in the distally located pseudo pocket –the major cause of pericoronitis. Therapeutic management usually involves a local surgical procedure and the prescription of antibiotics often of the β lactam family (Justin Moloney, 2009). In the present study, 50 patients were selected .Among them 10 patients were included in normal group having healthy fully erupted molars with no other oral diseases. Swabs were taken from buccal vestibule, floor of the mouth and labial mucosa. These swabs were cultured on blood agar plate and incubated at 37 °C for 24 hours. Following this, bacterial isolate was identified by gram staining and it showed the presence of normal flora of oral cavity such as –Streptococcus, Staphylococcus, Enterococcus, Neisseria, Vellionella (percentage of these commensals were very low).



Bar Diagram 1. Comparison of aerobic bacterial % before and after antibiotic treatment (according to Table 3)

Table 4. Comparison of anaerobic bacterial % before and after antibiotic treatment

Anaerobic organisms	% of Anaerobic organisms before Antibiotics	% of Anaerobic organisms after Antibiotics
Actinomycesis	75-80%	< than 50%
Fusobacterium	70%	< than 40%



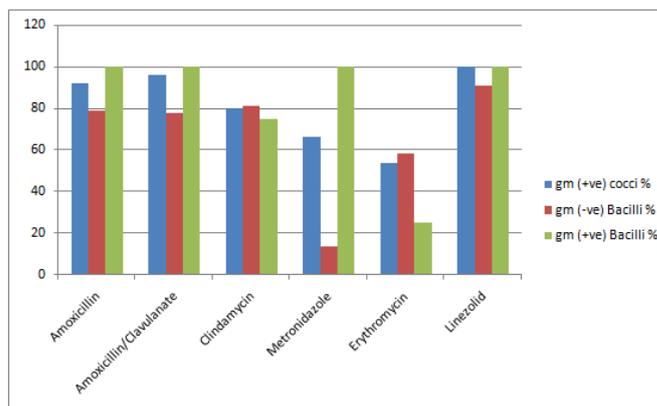
Bar Diagram 4.1. Comparison of anaerobic bacterial % before and after antibiotic treatment (according to Table 4)

Recent research has confirmed earlier studies that the resident microflora of animals and human play a positive role in the normal development of host and also having an active role in the maintenance of the healthy state by contributing to the host defences and preventing colonization by exogenous microorganisms. It has been estimated that the human body is made up of 37.2 trillion cells of which only 10% are mammalian. The remainder are the microorganisms that make

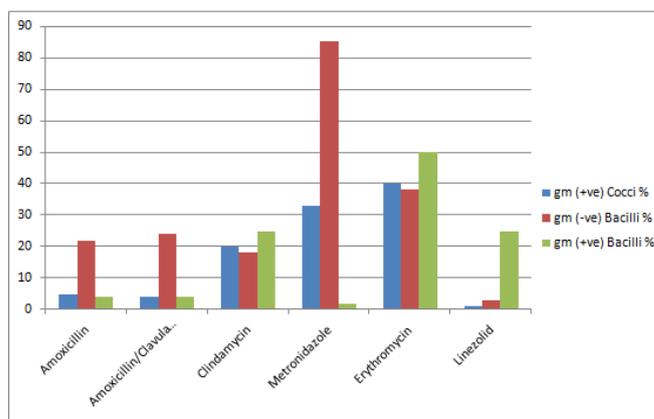
up the resident microflora of the host. Remaining 40 patients, in this study, were categorized into two groups-the 1st group (20 patients) suffered from recurrent pericoronitis associated with partially erupted third molar, mild tenderness without any antibiotic treatment in the last one month. The 2nd group (20 patient) under antibiotic treatment. These (20) 1st group consisted of two subgroups, each with 10 patient.

Table 5. Distribution of antibiotic susceptibility according to bacterial group (R=Resistant, S=Sensitive)

Antibiotics		Gram positive cocci (%)	Gram negative bacilli (%)	Gram positive bacilli (%)
Amoxicillin	R	5	22	4
	S	92	79	100
Amoxicillin/Clavulanate	R	4	24	4
	S	96	78	100
Clindamycin	R	20	18	25
	S	80	81	75
Metronidazole	R	33	85	2
	S	66	14	100
Erythromycin	R	40	38	50
	S	54	58	25
Linezolid	R	1	3	25
	S	97	91	50



Bar Diagram 6. Distribution of antibiotic sensitivity according to bacterial group according to Table 5



Bar Diagram 7. Distribution of antibiotic resistance according to bacterial group according to Table 5

The detectable microorganisms from the 1st subgroup were, Aerobic, Streptococcus, Haemophilus, Staphylococcus, Pseudomonas, Lactobacillus, Acinetobacter, Corynebacterium [Table 1] and these bacteriological findings were more or less supported by the other previous studies (Jean-Lois sixou, 2003; Justin Moloney, 2009; Van, 1997; Peltroche, 2000), except for Acinetobacter and Pseudomonas. These two microbes were

important observations of our research study, never being documented in previous studies. *Acinetobacter* and *Pseudomonas* were plays a significant role in pericoronitis, they colonized in the distally located pseudopockets. *Acinetobacter* were aerobic gram negative, encapsulated, pleomorphic bacilli and opportunistic pathogen. All strains of *Acinetobacter* were resistant to Penicillin and most strains were sensitive to broad spectrum antibiotics. *Pseudomonas* were slender, gram negative, non encapsulated, non sporing bacilli, strictly aerobic and on blood agar produces a typical swarming colony with some greenish hue which indicate hemolysis, also able to produce the pigments like Pyocyanin, Pyorubin, Pyomelanin. Here rest of the bacterial species that were isolated belongs to the normal oral flora.

However, in patients with oral infections, bacteria normally found in healthy mouths can also act as pathogens if predisposing factors or conditions prevail. Circumstances favouring infections include disturbance of the balance of the microbiota (e.g., by antimicrobial therapy) and disruption of the mucosal barriers by spontaneous or induced trauma (Jean-Lois sixou, 2003 and Justin Moloney, 2009). Unless care is taken to remove the indigenous bacteria, as in present study, the samples become contaminated and the "true pathogens" and their respective proportions may be masked. Dental plaque provides the medium for growth of bacteria, mostly gram positive organisms such as, *Streptococcus* and *Staphylococcus* species (Van, 1997 and Peltroche, 2000)

Next the samples collected from other subgroup 2 (10 patients). The samples were directly inoculated into the fresh blood agar and after proper maintenance of anaerobiasis, the bacterial colony were picked up from individual's culture plate. The most commonly isolated microorganisms were Anaerobic (facultative or obligate)- *Actinomyces*, Vincent bacilli, *Streptococcus* [Table-2] and these microbiological findings tentatively compared with previous studies (Jean-Lois sixou, 2003; Justin Moloney, 2009; Van, 1997; Peltroche, 2000). Most of the obligate anaerobes identified in this study may be found in the healthy oral cavity but also associated with oral diseases, numerous localized infections, particularly of the ear-nose-throat region and the respiratory system,^[4,8,9] usually in conjunction with infections associated with polymicrobial flora and systemic infections. The frequent presence of microaerophilic bacteria and facultative anaerobes that grow predominantly anaerobically (members of the genera *Streptococcus*, *Peptostreptococcus*, *Fusobacterium*) confirms the anaerobic shift of this flora. The members of the genus *Actinomycetes* were detected in the study-this *Actinomycetes* belongs to the normal flora of the oral cavity and may also be associated with dental caries and gingivitis (Jean-Lois sixou, 2003; Rajasuo, 1996; Justin Moloney, 2009 Leung, 1993). In pericoronitis, the Actinomycosis, one of the predominant cultivable microbial pathogens, present in the distally located pseudo pockets where the depth of gingival sulcus > than 4mm. Facultative anaerobic *Streptococcus*, the most frequent species on the buccal or palatal mucosa and crypts of the tongue (Jean-Lois sixou, 2003 and Justin Moloney, 2009). Gram positive species provide adhesion receptors for the attachment of gram negative species and in particular, obligately anaerobic bacteria (Jean-Lois sixou, 2003; Van, 1997; Peltroche, 2000). Their frequency in the oral cavity increases dramatically until tooth eruption. Most of the indigenous anaerobes were the part of the normal oral flora and act as a defending barrier against pathogens.

The obtained microbiological data reinforces the concept of the infection due to polymicrobial flora in case of pericoronitis and highlights the need for efficacy against aerobic and anaerobic flora when Antibiotic treatment was administered. Sample collection done from the next group (20 patients with recurrent pericoronitis, undergoing Antibiotic i.e. Amoxycillin Clavulanate along with Metronidazole combined treatment). Antibiotic susceptibility tests were performed and comparison of bacterial percentage before and after Antibiotics were established [Table 3,3.1,4,4.1]. Susceptibility to Amoxicillin, Amoxicillin/ Clavulanate, Metronidazole, Clindamycin, Erythromycin, Linezolid was evaluated [Table 5,6,7].

Here the results of microbial culture shows as a mixed infections of the oral cavity, both aerobic and anaerobic bacteria were colonized, but the distinguishing feature between these two groups that after proper antibiotic treatment the percentage of bacterial count (especially the anaerobes) markedly diminished. The broad spectrum antibiotics specially Amoxicillin-Clavulanate fully encompasses the microorganisms found in pericoronitis. Metronidazole, particularly useful in infections due to polymicrobial flora, in which anaerobic microorganisms predominate. Its combination with a macrolide (spiramycin) extended the spectrum to certain non-obligately anaerobic bacteria, allowing its use in pericoronitis with a well documented mixed aerobic-anaerobic flora (Jean-Lois sixou, 2003; Justin Moloney, 2009; Van, 1997; Konoen, 1999).

Conclusion

Assessment of the status of oral pathogenic microbes is an important parameter which will delineate the treatment guidelines during development and progression of Pericoronitis. It was established that, Amoxycillin Clavulanate fully encompasses the aerobic microorganisms while Metronidazole was particularly effective against the anaerobes. Accordingly, pre-operative, per-operative, post-operative uses of above combination of drugs will be of immense help to combat the postoperative infections or complications thereafter in surgical treatment and management. However, further detailed in depth studies are advocated in the line of present study.

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