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Metronidazole resistance in anaerobes isolated from patient with oral and maxillofacial infections attending muhimbili national hospital, dar-es-salaam, Tanzania

Abstract

Background: Oro-facial infections, mostly bacterial infections, are among the most commonly encountered problems in the oral and maxillofacial surgical practice world over. These infections if not properly diagnosed and treated usually result in significant morbidity and are often fatal. The infection is caused by both aerobic and anaerobic microorganism where as treatment is given using broad spectrum antibiotics mostly common combination of Amoxicillin and Metronidazole. Metronidazole is commonly targeted drug for anaerobes, however recently the anaerobes have shown resistance to this drug. The aim of this study was to isolate and identify anaerobes associated with oral and maxillofacial infection and their susceptibility pattern to Metronidazole in patients attending Muhimbili National Hospital, Dar-es-salaam, Tanzania.

Method: Seventy (70) samples were collected from patients with various forms of oral facial and infections that consented and were recruited for the study. The samples were processed for isolation and identification of bacteria as per standard microbiological techniques. The antimicrobial susceptibility was done by E-test (Biomerieux) methods as per CLSI guidelines.

Results: Anaerobes were isolated in 83% of oral and maxillofacial cases. Anaerobes were found to be over 99% sensitive to metronidazole while some isolated showed approximately 40% resistance to Metronidazole. Majority of isolated anaerobes display good sensitivity.

Conclusion: Resistance to Metronidazole observed in this study and also presence of anaerobes in these patients showed that there is a need to isolate anaerobes especially in patient with severe oral and maxillofacial infections and study their antimicrobial susceptibility pattern for better management.

Keywords: anaerobes, oral and maxillofacial infections, metronidazole

Sima E Rugarabamu

Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Tanzania

Correspondence: Sima E Rugarabamu, Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Tanzania, Email sima_luv@yahoo.com

Background

Oro-facial infections, mostly bacterial infections, are among the most commonly encountered problems in the oral and maxillofacial surgical practice world over. Infections involving the oral and maxillofacial region usually result in significant morbidity and are often fatal. These infections.[1] Purulent infections such as dental abscesses, Ludwing Angina are known to be caused by not only aerobes and facultative anaerobes but also by obligate anaerobes.[2] An increase in the role of these bacteria in clinical infections has been reported, however, exact incidence especially to anaerobes is unknown.[3] In Many Oral and maxillofacial clinics patients present with serious and often fatal infections that initially started as simple dental caries or periodontitis then progressed to periapical infection and spread to facial spaces/ In severe forms of orofacial infections like necrotizing fasciitis, deep space infection and osteomyelitis, culture studies involving both aerobic and anaerobic bacteriology are desirable to provide information on the likely pathogenic organisms causing the disease and their likely antibiotic sensitivity.[4] This is not done because isolation and sensitivity testing of anaerobes is very tedious, costly and time consuming. As a result treatment is given using broad spectrum antibiotics which may not be appropriate or may cause delay in healing and high morbidity.

Detection of bacteria along with their susceptibility pattern is important for better management of infections since some organism are now developing resistance to commonly used antimicrobial for infections.[5] Anaerobic bacteria are predominantly implicated in the pathogenesis of oral and maxillofacial infections hence their detection is important for better management of anaerobic infections. However, most laboratories do not attempt to isolate or do the susceptibility testing of anaerobes as it requires special expertise and expensive facilities.[6] Metronidazole is commonly targeted drug for anaerobes, however recently the anaerobes have shown resistance to this drug. It was therefore decided to study isolate and identify anaerobes associated with oral and maxillofacial infection and their susceptibility pattern to Metronidazole in patients attending Muhimbili National Hospital, Dar-es-salaam, Tanzania.

Material and methods

This was a descriptive cross-sectional hospital based study that took place between January 2014 to July 2014 at Muhimbili National Hospital (MNH) which is the largest referral, consulting and teaching hospital in Tanzania and was divided into two parts. This was conducted in the department of oral and maxillofacial surgery clinic in the school of dentistry. The clinic attends referral and non-referral patients from Dar-es-salaam and neighboring district and up country clinics. This was done in microbiology laboratory of Muhimbili University Dar -Es-Salaam.

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Study population

All patients who attended treatment and were diagnosed to have oral and maxillofacial infections at the Oral and Maxillofacial OPD and those admitted in oral and maxillofacial wards of MNH were eligible for the study. Enrollment was subject to obtaining an informed verbal consent.

Data collection

Interview was conducted was conducted using a structured standard questionnaire to obtain information regarding age, sex, the presenting symptoms, duration of the condition and medical history.

Clinical examination

General physical examination was carried out followed by extra oral and intraoral examination with the patient seated on a dental chair using natural or artificial light at the OPD. For Admitted patient's examination was carried out while lying on an examination bed. A special clinical form was used to record the following; Source of infection characteristic of the lesion, location, pain and tenderness, discharge and foul smell.

Isolation and identification of organism

Sample collection and transportation: Pus samples were collected aseptically by aspirating the abscesses using sterile syringe during the incision and drainage or wound dressing. After aspirating the specimen was immediately inoculated in two media, one special anaerobic transport media (BD curls anaerobic Transport) and the other universal container. Specimen was transferred to the laboratory within 20min processed for culture as early as possible within 2hrs.[7] The culture and sensitivity were conducted for the clinical specimens obtained from the patient before initiation of any antibiotic therapy. Sample not suitable for culture such as contaminated or those that did not meet the criteria such as exposure to antibiotic was discarded.

Specimen processing

Direct smear: This formed a crucial role in the processing of specimen by giving preliminary diagnosis of infection. Gram stain was done followed by examination under a microscope using oil immersion (100 × magnification), pus cells, bacteria cells and other characteristics such as fine slender, minute, pleomorphic features were appreciated. Samples from universal container were processed for aerobic culture to look for growth of aerobes and facultative anaerobes. After gram staining samples were inoculated on to (5%), sheep blood agar, chocolate agar and Mac Conkey agar. The plates were incubated aerobically at 370C (Mac Conkey) and under 5% CO_2 (Blood and Chocolate) and examined after 24h. After incubation predominant colonies were processed further and identified conventional methods. For anaerobic culture Blood agar containing kanamycin and Vancomycin (BD0403 CDC 5% sheep blood agar for anaerobes) was used. 5μg metronidazole and 10μg penicillin discs and 10μg Gentamycin was placed for presumptive recognition of anaerobes and the media was incubated in anaerobic jar in atmosphere generated using BD commercial gas generating kit in accordance with manufacturer's instructions. Anaerobic plates were examined after 48h Isolates were identified based on microscopic characteristics, colonial characteristic and biochemical test.[5]

Antimicrobial susceptibility testing

Antimicrobial susceptibility pattern of isolated aerobic bacterial pathogens was conducted by using E test (Biomerieux) anaerobic bacteria were determined by E test (Biomerieux, Sweden) according to CLSI guideline for anaerobic susceptibility testing.

Results

Isolated anaerobes

A total of 58 (83%) anaerobic organisms were isolated with the most common isolate being *Prevotella* 25(43%) followed by *Peptostreptococcus* 18(31%) Other isolates were, *Pophyromonas spp.* 3(5%), *Fusobacterium spp.* 2(3%) and *Bacteroides spp.* 5(9%) (Table 1 & 2).

Table 1 Isolation frequency of obligatory anaerobic organisms from oral facial infection

Bacteria no of isolate (%)		
Prevotella spp	25	43
Peptostreptococus	18	31
Bacteroides	5	9
Fusobacteria	2	3
Actinomyces	1	2
Pophyromonas	3	5
Non Identified Anaerobes	4	7
Total	58	100

Table 2 Sensitivity pattern of anaerobes isolated from oral facial infection

Isolate	Susceptibility (%)					
	Pen	Cefo	Mtr	Pen	Chlo	penem
Prevotella	65	95	60	90	95	95
P.streptococcus	88	94	88	94	NT	NT
Bacteroides	80	100	60	80	100	80
Pophyromonas	100	100	67	100	100	100
Fusobacteria	100	100	50	100	100	100

Mtr: Metronidazole; Chlo: Chloramphenicol; Tet: Tetracycline; Pen: Penicillin; Cefo: Am/Clavum= Clavulanic acid Amoxicilim; penem: Ibipenem.

Discussion

In this study a total of 70 samples were collected from different clinical conditions such as Dental abscesses, Ludwing's angina, Necrotising fasciitis and infected socket. Anaerobes were isolated in 83% which is in agreement with other studies that reported that infection due to anaerobes have increased in comparison to past reports[8] and also in this study the superior methods of collection of specimens and culture was applied hence could have attributed to improving their isolation. Regarding the bacterial species that were isolated, the findings of this study are in agreement with other researchers who also demonstrated that *Streptoccus species, Peptostreptococus, Prevotella/ Bacteroides, Fusobacteria* and others as frequently isolated organisms from oral and maxillofacial infections.[8–12] Metronidazole which is commonly used had a questionable activity in regarding to the similar to other studies that has recently reported resistance to anaerobes isolated from head and neck and other parts of the body.

Conclusion

Metronidazole is among the commonest drug used to treat oral facial and maxillofacial infections. However no much data is available on its sensitivity in anaerobes isolated in this region. Results of this study should therefore be an highlight on the need to isolate and study the antimicrobial susceptibility pattern of various drugs used to treat patients with oral and maxillofacial infections for better management of patients.

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3

Acknowledgements

Conflicts of interest

The authors declare that there is no conflict of interest.

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