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BACTERIOLOGY AND ANTIMICROBIAL SUCEPTIBILITY PROFILE OF AGENTS OF OROFACIAL INFECTIONS IN NIGERIANS

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A prospective study to determine the pattern of microorganisms seen in orofacial infections as well as investigating the antimicrobial susceptibility profile of the isolates was undertaken. Specimens were obtained aseptically from 25 patients presenting with orofacial infections at the Department of Oral Surgery and Pathology, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. The specimens were transported in an anaerobically pre-reduced transport medium for processing in the laboratory. Isolation and identification were done employing standard bacteriological techniques. Antimicrobial susceptibility testing was performed by the disc diffusion method. All the 25 clinical samples obtained yielded growth of bacteria. Anaerobes were cultured from 24 (96%) specimens while 1 specimen yielded only aerobic isolates. Altogether, 44 bacterial isolates were obtained and 40 (91%) were anaerobes. Most of these anaerobes were Gram-negative rods and Gram-positive cocci. About 75-100% of the anaerobes were susceptible to commonly available antibiotics. Strikingly, sulphonamides demonstrated the weakest *in-vitro* activity against all isolates. The study revealed again the polymicrobial nature of orofacial infections as well as the predominance of anaerobes in the aetiology of these infections. Erythromycin and penicillin should be considered as frontline drugs in the treatment of mild orofacial infections while drugs like ciprofloxacin and clindamycin can be reserved for more severe and resistant infections.

INTRODUCTION

Bacterial infections are among the most commonly encountered problems in the maxillofacial surgical practice and previous reports from Nigeria showed that orofacial infections remain a major problem. This problem persists in spite of the availability of broad spectrum of potentially useful antibiotics (1-6).

The microbiology of orofacial infections has been studied widely and various forms of aerobic and anaerobic microorganisms reflective of normal oral flora have been isolated. *Streptococcus* and *Staphylococcus* species as well as the Gram-negative anaerobic bacilli namely *Prevotella, Porphyromonas, Fusobacteria* species and anaerobic cocci are among the prevalent organisms isolated in most studies (7, 8).

Treatment of acute orofacial infections would require the use of empiric antibiotic prescriptions. A clinician can rely on the knowledge of the likely microorganisms that may cause an infection in a particular site of the body and the nature of the antibiotic susceptibility pattern in the local environment of his practice as a guide to the rational choice of antibiotic therapy. In severe or recalcitrant forms of orofacial infections like necrotizing fasciitis, deep space infections and chronic osteomyelitis, cultural studies involving both aerobic and anaerobic bacteriology are however desirable to provide information on likely pathogenic organisms causing the disease and their antibiotic susceptibility pattern. This piece of information would guide the clinician to select the most appropriate antibiotics available for the treatment of these infections.

Anaerobic bacteriology unfortunately is expensive and requires special facilities and expertise to perform. It is not readily available in many hospitals in

the developing countries even in the referral centers. In spite of the high prevalence of orofacial infections in Nigeria, only one report (7) incorporated anaerobic bacteriology in the study of dentoalveolar abscess. There is therefore a paucity of information about the identities of the anaerobic organisms associated with orofacial infections in this environment. The present study examined the microbiology of different types of orofacial infections with the aim of providing information on the prevalent microorganisms isolated in these diseases. Antibiotic sensitivity patterns of these organisms were determined in order to provide a guide to clinicians for making rational decisions over the choice of antibiotics in the management of these infections.

PATIENTS AND METHODS

A prospective study of 25 patients aged 17-65 years (17 males and 8 females) with various forms of orofacial infections was carried out (19 odontogenic and 6 non-odontogenic infection). Table 1 gives a breakdown of the types and sources of these infections; chronic suppurative osteomyelitis (5), acute dentoalveolar abscess (5), buccal space abscess (4), lateral pharyngeal abscess (1), submandibular space abscess (3), submandibular space cellulitis (1) and chronic suppurative maxillary sinusitis (3). All the patients were seen at the Department of Oral Surgery and Pathology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. Specimens for bacteriological investigation were obtained aseptically through intact mucosa or skin. Abscesses were either aspirated with sterile syringes or swabbed during incision and drainage while bone or granulation tissues were surgically obtained through an intraoral incision in patients with chronic osteomyelitis. Prior to these procedures, the skin or mucosa was cleaned with 70% alcohol and isolated with sterile gauze. Specimens were collected into an anaerobically pre-reduced transport medium (Bionor, Norway) and sent to the laboratory immediately.

Isolation and identification of organism

Specimens were collected into transport media (Bionor, Norway) and stored at 4°C and processed within 2 hours. Specimens were cultured on Nutrient agar (Oxoid, England) containing 6% whole blood incubated aerobically at 37°C, Cooked meat broth (Oxoid, England), Nutrient agar containing 6% lysed human time-expired blood and 0.5 mg/ml Vitamin K (Roche, Nigeria) and fastidious anaerobe agar (Techlab, USA), prepared according to the manufacturer's instructions and incubated anaerobically. Anaerobic incubation took place at 37°C in anaerobic jars in an atmosphere of 1% O_2/8%CO_2 generated using commercial gas-generating kits (BBL, Cockleysville, USA) in accordance with manufacturers' instructions. Plates were incubated at 37°C for 48-72 hours (for aerobic cultures) and 3-7 days (for anaerobic cultures). Colonies appearing on either plate were streaked unto fresh plates and incubated for 48 hours to 2 weeks. Gram-negative rods were identified using the API 20 E system (Biomerieux, France). All other isolates were identified by conventional biochemical tests (9). Isolates were maintained by cryopreservation using the medium of Gibson and Khoury (10).

Antibiotic sensitivity testing

Antibiotic sensitivity testing was conducted by the disc diffusion method (11). The test medium was Iso-sensitest agar (Oxoid, England) supplemented with whole blood for streptococci and lysed blood with vitamin K for anaerobes. Commercially available antibiotic disks were used and interpretation of inhibition zone was in accordance with manufactures instructions (AB Biodisk, Sweden). *Staphylococcus aureus* NCTC 6571 and *Escherichia coli* NCTC 10418 were used as controls.

RESULTS

All the 25 clinical samples obtained yielded growth of bacteria. Forty-four bacterial isolates were obtained. Anaerobes were cultured from 24 (96%) specimens and this accounted for 40 (91%) of the number of organisms isolated. Mixed anaerobic/aerobic growth was obtained from 2 (8%) specimens while anaerobes were exclusively cultured from 22 (88%) specimens. One case of submandibular space cellulitis yielded aerobe only (Table 1). Gram-negative anaerobic cocci were the commonest bacteria isolated, predominantly *Prevotella melaninogenicus* (14), *Porphyromonas gingivalis* (8), *Prevotella denticola* (5), and *Peptostreptococcus spp* (6). While *Streptococcus spp* (3) and *Staphylococcus aureus* (1) were the aerobic species isolated.

Table 2 shows the antibiotic profile of the anaerobic and streptococcal isolates. Majority of these organisms were susceptible (75-100%) to the commonly available antibiotics, trimethoprim, chloramphenicol, tetracycline and erythromycin. Clindamycin and ciprofloxacin also displayed excellent *in-vitro* activity against the anaerobic isolates. The least susceptibility to penicillin V was observed in *Peptostreptococcus anaerobius* (33.3%) otherwise, this drug displayed good *in-vitro* activity against the anaerobic bacteria (75-100%).

Streptococcus species were completely susceptible (100%) to trimethoprim, ciprofloxacin, chloramphenicol and erythromycin. The sulphonamides demonstrated the weakest *in-vitro* activity against the aerobic and anaerobic bacterial isolated in this study. Susceptibility testing for *Staphylococcus aureus* was not done.

Table 1: Classification of orofacial infection and the bacterial isolates

Type/source of infection	No of cases	Anaerobic organism	No of isolate	Aerobic organism	No of isolate
Chronic suppurative osteomyelitis (Chronic periodontitis)	5	*P. melaninogenica*	1		
		P. denticola	2		
		P. gingivalis	2		
		P. anaerobius	1		
		P. prevotii	1		
Buccal space abscess (Pulpitis)	4	*P. melaninogenica*	2		
		P. denticola	1		
		P. gingivalis	1		
		P. anaerobius	2		
		P. magnus	1		
		P. productus	1		
		F. nucleatum	1		
		P. intermedia	1		
Lateral pharyngeal space abscess (Pulpitis)	1	*P. melaninogenica*	1	*Strept. spp*	1
		A. viscosus	1		
Acute dentoalveolar abscess (Pulpitis)	5	*P. endodontalis*	1		
		P. melanincgenica	4		
		P. gingivalis	1		
		P. intermedia	1		
		P. Productus	1		
Canine fossa abscess (Pulpitis)	4	*P. gingivalis*	3		
		P. melaninogenica	1		
		P. denticola	1		
		P. productus	1		
Submandibular space abscess (unknown)	3	*P. melaninogenica*		*Strept. spp*	1
		P. denticola			
		P. Gingivalis			
Submandibular space cellulites (unknown)	1			*Staph. aureus*	1
				Strept. spp	1
Chronic suppurative maxillary sinusitis (unknown)	2	*P. gingivalis*	1		
		P. melaninogenica	2		

P = Prevotella, Porphyromonas, Peptostreptococcus, F = Fusobaćteria, A = Actinomyces
Staph = Staphylococcus, Strept = Streptococcus

Table 2: Antibiotic profile of anaerobic/streptococcal isolates

Organism/Antibiotic	Trimeth (%)	Suph (%)	Pen V (%)	Cipro (%)	Tet (%)	Chl (%)	Ery (%)	Clind (%)
P. melaninogenica (13)	10 (76.9)	3 (23.1)	10 (76.9)	13 (100)	12 (92.3)	13 (100)	12 (92.3)	13 (100)
P. gingivalis (8)	6 (75)	0 (0)	6 (75)	7 (87.5)	7 (87.5)	7 (87.5)	7 (87.5)	8 (100)
P. intermedia (2)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
P. denticola (4)	3 (75)	4 (100)	3 (75)	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)
P. endodontalis (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
F. nucleatum (2)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	1 (100)	1 (50)	2 (100)
P. anaerobius (3)	3 (100)	0 (0)	1 (33.3)	3 (100)	3 (100)	2 (100)	3 (100)	3 (100)
P. prevotii (1)	1 (100)	0 (o)	1 (100)	1 (100)	1 (100)	3 (100)	1 (100)	1 (100)
P. productus (2)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	1 (100)	2 (100)	2 (100)
P. magnus (1)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)
A. viscosus (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-
Streptococcus spp. (3)	3 (100)	1 (33.3)	2 (66.7)	3 (100)	2 (66.7)	3 (100)	3 (100)	2 (66.7)

Trimeth = Trimethoprim, Sulp = Sulphonamide, Pen V = Penicillin V, Cipro = Ciprofloxacin, Tet = Tetracycline, Chl = Chloramphenicol, Ery = Erythromycin, Clind = Clindamicin

DISCUSSION

The result of this study demonstrates again the polymicrobial nature of orofacial infections as well as the predominance of anaerobic bacteria in the pathogenesis of these infections. In the present study, the Gram-negative rods and the anaerobic cocci were the commonest anaerobic bacteria isolated. They are *Prevotella, Porphyromonas, Fusobacterium* and *Peptostreptococcus* species. This observation is consistent with result from most studies on orofacial infections (7, 12, 13, 14). *Streptococcus* species are common oral commensals and are frequently isolated in odontogenic infections (7, 14). These organisms were isolated mainly from the odontogenic infections in the present study. *Staphylococcus aureus* found in the nares, perineum and skin. It is not a normal flora of the oral cavity but may be an important pathogenic organism in suppurative non-odontogenic infections of the head and neck region (8). The only *Staphylococcus aureus* isolated in this study was obtained from a case of non-odontogenic submandibular cellulitis.

Resistance to older cheaper antibiotics is becoming increasingly common in Nigeria. In this study, however, it was observed that most of commonly available antibiotics, erythromycin, penicillin V, tetracycline, trimethoprim and chloramphenicol demonstrated very good *in-vitro* activities against most of the anaerobic bacteria. Erythromycin and ciprofloxacin also displayed excellent *in-vitro* activities against the streptococcal isolates. Erythromycin and penicillin V are not only very effective within the environment of the study, but are both cheap and readily available. They should therefore be considered as frontline drugs in the treatment of mild forms of orofacial infections in Nigeria. This will permit the conservation of ciprofloxacin and clindamycin for the management of more complex forms of orofacial infections and other resistant infections. It is important to realize that the successful management of orofacial infections depends on the removal

of sources of infection, establishment of prompt and adequate surgical drainage and the institution of appropriate antibiotic therapy. Antibiotic therapy alone is not a substitute for surgery.

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