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EFFECT OF ANTIMICROBIAL AGENTS ON ORAL MICROORGANISMS

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SOUHRN

Účinek antimikrobiálních prostředků na mikroorganismy dutiny ústní

Zatímco onemocnění zubní dřeně a periodoncia, která představují většinu odontogenních infekcí, jsou vyvolána především endogenní bakteriální mikroflórou dutiny ústní, neodontogenní infekce v téže anatomické krajině mají různé původce v závislosti na povaze a lokalizaci onemocnění. Uvážené podávání antibiotik má význam v prevenci vývoje rezistentních kmenů a dalších vedlejších účinků léků.

Cíl studie: Zjistit (i) prevalenci výskytu bakteriálních druhů v dutině ústní u souboru pacientů s bakteriálními infekcemi léčených na Stomatologické klinice LF UK a FN v Hradci Králové (1996 - 2007), (ii) vztah k věku a pohlaví, (iii) specifické vztahy jednotlivých druhů, (iv) profil rezistence bakterií izolovaných z odontogenních a neodontogenních infekčních lézí.

Materiál a metodika: Retrospektivní hodnocení nálezů laboratorního a klinického vyšetření pacientů, získaných z elektronické databáze Oddělení klinické mikrobiologie LF UK a FN v Hradci Králové v letech 1996 - 2007.

Výsledky: Bakteriální odontogenní či neodontogenní infekce dutiny ústní byla hodnocena u 678 jedinců, z nichž 350 tvořili muži (51,5 %) a 328 ženy (48,2 %). Bakteriální izoláty z dutiny ústní obsahovaly 48 bakteriálních druhů s převahou fakultativních anaerobů 78,5 % (n = 1263) a obligatorních anaerobů 21,5 % (n = 346). Mezi fakultativně anaerobními mikroorganismy dominoval *Haemophilus influenzae* 19,9 % (n = 320). Obligatorně anaerobní mikroorganismy byly vysoce citlivé na většinu antibiotik včetně penicilinů. Pouze některé z nich byly rezistentní na gentamicin a tetracyklin. Více než 95 % orálních streptokoků bylo citlivých na ß-laktamová antibiotika. Mnohem menší citlivost jsme zaznamenali na erythromycin, tetracyklinová antibiotika a kotrimoxazol. Koaguláza-negativní stafylokoky a *Staphylococcus aureus* vykazovaly rovněž nejvyšší citlivost na ß-laktamová antibiotika. Enterobaktérie byly nejcitlivější na piperacillin/tazobactam a cefalosporiny 3. a 4. generace. Velmi rezistentní byly na ampicilin. *Haemophilus influenzae* byl citlivý na řadu ß-laktamových antibiotik a cefalosporiny 2. generace.

Závěr: V hodnoceném souboru bakteriálních izolátů z dutiny ústní dominovaly fakultativně anaerobní mikroorganismy. Mikrobiální nálezy byly u mužů a žen obdobné. Celkový počet bakteriálních druhů se zvyšoval v závislosti na délce studie. Výsledky této studie potvrdily, že ß-laktamová antibiotika, zejména peniciliny a cefalosporiny, jsou stále lékem první volby v léčbě orofaciálních bakteriálních infekcí.

SUMMARY

Effect of antimicrobial agents on oral microorganisms

Disease of the pulp and periodontium which constitute the vast proportion of odontogenic infections are mainly caused by the endogenous bacterial microbiota in the oral cavity while non odontogenic infections in the same area vary depending on the nature and site of infection. The rational use of antibiotics is important to prevent development of resistant strains and other side effects of drugs. **Aim:** To investigate (i) the prevalence of bacterial species in oral samples of patients with bacterial infection reporting at the Department of Dentistry (1996 - 2007), (ii) to assess the age and sex predilection and , (iii) and species specific relationships, (iv) to determine the susceptible-resistant biotype profile of the bacterial isolates from odontogenic and non odontogenic infections. **Materials and methods:** Laboratory and clinical data of patient‘s electronic files at Department of Microbiology, Faculty of Medicine and University Hospital in Hradec Králové for the years 1996-2007 were evaluated retrospectively. **Results:** Bacterial orofacial odontogenic or non odontogenic infection was detected in a total of 678 patients with 350 males (51.6%) and 328 females (48.4%). The bacterial isolates included 48 bacterial species with predominance of facultative anaerobes which accounted for 78.5% (n= 1263) and obligate anaerobes 21.5% (n=346). Among the facultative anaerobes the most common isolate was *Haemophilus influenzae* (n=320, 19.9%). Obligate anaerobes were highly susceptible to most antibiotics including penicillin while resistance to gentamicin and tetracycline was noted among some strains. Greater than 95% susceptibility was demonstrated by oral streptococci to β-lactam antibiotics in comparison to erythromycin and broad spectrum drugs like tetracycline and cotrimoxazole. Coagulase-negative staphylococci and *Staphylococcus aureus* strains also exhibited greater susceptibility to β-lactam antibiotics than broad spectrum drugs. *Enterobacteria* showed the highest susceptibility to piperacillin/tazobactam, 3rd and 4th generation of cephalosporins whereas there was unusually high resistance to ampicillin. Isolates of *Haemophilus influenzae* were susceptible to a wide range of β-lactam antibiotics and 2nd generation of cephalosporins. **Conclusion:** The predominating bacterial pathogen in oral cavity were facultative anaerobes. There was equal gender predilection for the infection. The total species of microbes increased with respect to the study period. The findings in this study suggest that β-lactam antibiotics, mostly penicillins and cephalosporines, are still the mainstay in the antimicrobial management of orofacial infections of bacterial origine.

ANTIBIOTICS ABBREVIATIONS

AMI -Amikacin	ERY- Erythromycin
AMOK- Amoxicillin/	FUR -Furantoin
Clavulanic acid	GEN -Gentamicin
AMP- Ampicillin	IMP -Imipenem
AMPI- Ampicillin/ inhibitor	KYS -Oxolinic acid
AMPS- Ampicillin/ Sulbactam	LIN -Lincomycin
API- Aminopen/ inhibitor	LVF -Levofloxacin
AZL- Azlocillin	MEP -Meropenem
AZR -Aztreonam	MTZ -Metronidazole
AZT- Azithromycin	MUP -Mupirocin
CEF1-Cephalothin	NET -Netilmicin
CETX -Cefotaxime	NOR -Norfloxacin
CFA -Ceftazidime	OFL -Ofloxacin
CFI- Cefpirome	OXA -Oxacillin
CFM- Cefepime	PEN -Penicillin
CFN -Cefazolin	PIP -Piperacillin
CFP -Cefoperazone	PIPT -Piperacillin/ tazobactam
CFPS -Cefoperazone/ sulbactam	ROX- Roxithromycin
CFR -Ceftriaxone	SPI -Spiramycin
CFT- Cefoxitin	TEI -Teicoplanin
CFTX -Ceftizoxime	TET -Tetracycline
CFX -Cefuroxime	TIC -Ticarcillin
CIP -Ciprofloxacin	TICI -Ticarcillin/ inhibitor
CLI -Clindamycin	TMP -Trimethoprim
CMP- Chloramphenicol	TOB -Tobramycin
COL -Colistin	VAN -Vancomycin
COT -Cotrimoxazole	

INTRODUCTION

Orofacial odontogenic infections are common causes of dental consultation worldwide. About 500 distinct bacterial species are found in the oral cavity (7). Orofacial microflora are causative agents for dental caries, pulpitis, abscess, periodontal diseases and halitosis, bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, coronary heart disease and cerebral phalsy (19). Antimicrobial agents are commonly prescribed by dental practitioners for the management of orofacial infections. Their role for treatment is in prevention of spreading infection and in reducing extent of damage (16).

The rational use of antibiotics is important to prevent development of resistant strains and unwanted side effects of drugs. The choice of antibiotic is case-specific and it is important to take into consideration the age and health of the patient, history of allergy, drug absorption and distribution, plasma concentration and laboratory data (26). In addition, the type and site of infection, antibiotic usage prior to an infection, cost effectiveness of the drug, drug metabolism and penetration (26) along with the recent domestic antimicrobial susceptibility patterns are also factors which determine the drug of choice and finally outcome of infection (14). The present study was to report the long-term surveillance of antibiotic susceptibility of the subjects reporting with bacterial infection of odontogenic and non-odontogenic origin at the University Hospital in Hradec Králové from 1996 through 2007.

AIMS OF THE STUDY

(i) To isolate and determine the prevalence of bacterial species in oral samples of patients with bacterial infection reporting at the Department of Dentistry (1996-2007)

(ii) To assess the age, site of infection and sex distribution

(iii) Species-specific relationships

(iv) To determine the most effective antimicrobial therapy for orofacial infections of odontogenic and non-odontogenic origin based on the *in vitro* antibiotic susceptibility test.

MATERIALS AND METHODS

Patient selection and bacterial sampling procedure

The demographic, bacteriologic and antibiotic susceptibility data of patients attending the Department of Dentistry, University Hospital in Hradec Králové with suspected or proven orofacial bacterial infections during the period from 1996 through 2007 were collected retrospectively using the hospital records at the Department of Clinical Microbiology.

Details of dental and medical history were obtained for all cases. Sampling was performed routinely on patients with orofacial odontogenic and non odontogenic infections by swabbing or obtaining a liquid material or pus from oral cavity or neighbouring structures and transported in anaerobic transport devices (sterile test tube for anaerobic transport with stopper or swab containing Amies transport medium (Dispolab) to the laboratories at the Department of Clinical Microbiology.

Culture

After admission all samples were cultivated in accordance with standard methods in microbiology. The culture plates were then examined for bacterial growth each 18-24 hours and quantity or semiquantity was evaluated for each sample. Pure bacterial isolates for identification and antibacterial susceptibility testing were obtained by subculture.

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Identification

Presumptive identification of the pure bacterial colonies of strict/facultative anaerobes/aerobes, gram-positive/negative rods and cocci. Bacterial isolates were identified by standard methods. All gram-negative nonfermentative rods and other unidentified isolates were, if needed, further identified using commercial systems.

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests for the obligate and facultative anaerobes and aerobes were done using disc diffusion test or microdilution broth method. Production of ß-lactamase was identified by nitrocephin test (Lachema), confirmation of MRSA was done by latex aglutination (MRSA-Screen Denka Seiken).

The bacterial strains were manually divided into appropriate susceptibility categories (resistant, intermediate susceptibility, or susceptibility) based on the guidelines for interpretation of diameter of inhibition zone for individual antibiotics (26). Species, drug, zone diameter, susceptibility

category, and quality control results were read manually and the results were recorded into the central laboratory information system.

Exclusion criteria

Cases with negative laboratory results and test results of the same patient but not related to the oral cavity were excluded from the study. In addition, bacteria regarded as normal commensals and duplicate isolates from a given patient with identical species within different samples, and mycological results of the patients were not considered. Samples with mixed isolates without potencial pathogenicity were grouped together as microflora and no attempt was made to find the antibiotic susceptibility profile of their individual species separately in this study.

Statistical Analysis

Data were analysed to evaluate the relationships between specific microbes and gender. Chi-square test and simple linear regression analysis were performed to determine temporal trends in occurence of microbial species. Unpaired *t*-test was done to determine if there was any gender prevalence. Significance was determined at $p<0.05$ level. Relationship between specific microbes and their antibiotic drug-sensitivity profile was also analysed.

RESULTS

A total of 678 cases were included from the 11-year study period (1996 to 2007). 350 (51.6%) were males and 328 (48.4%) females. Overall, 1609 strains were isolated.

Age

The age of the study cohort ranged from 2 to 94 years. The mean age was 41.2 (± 18.03 SD) years for males and 43.7 (+/- 19.5 SD) years for males.

Gender

The proportions of various bacterial species isolated from males and females during the study were comparable (p = 0.082) (Table 1).

Table 1. Gender-specific distribution of microbial isolates among cases

Microbe – group	**Female** %	**Male** %
Moraxella catarrhalis	60.0	40.0
Anaerobes	56.4	43.6
Haemophilus influenzae	56.3	43.8
Oral streptococci	51.8	46.4
Staphylococcus aureus	52.8	47.2
Coagulase-negative staphylococci	51.6	48.4
Streptococcus beta haemolytic	51.3	48.7
Corynebacterium spp.	50.0	50.0
G- non fermentative rods	50.0	50.0
Enterobacteria	47.7	52.3
Indigenous microbiota	41.2	58.8

Site of specimen

Nearly 52 different types of isolates were identified from the specimens. The most frequent sites were throat (18.1%), salivary gland (16.2%) and abscess (14.1%).

Spectrum of microorganisms

A total of 48 species were identified among the 1609 isolates from 678 patients. The spectrum of microorganisms during the study period comprised of predominantly facultative anaerobes 78.5%

(n=1263) and obligate anaerobes 21.5% (n=346). Among the facultative anaerobes the most common species was *H. influenzae* (n=320; 19.9%) followed by enterobacteria (n=235; 14.6%), and beta-haemolytic streptococci (n=193; 12%), *S. aureus* (n=176; 10.9%), coagulase-negative staphylococci (n=122; 7.6%), oral streptococci (n=134; 8.3 %) and Gram negative non-fermentative rods (n=40; 2.5%), *M. catarrhalis* (n=5; 0.3%), and *Corynebacterium* spp. (n=4; 0.3%).

The microflora isolated in this study is profiled in the table 2 and 3

Table 2. Spectrum of bacteria isolated from orofacial infections with their numbers during the study years.

Microbe/s	**Year**												
	1996	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**Total**
Anaerobes	3	16	31	13	3	12	11	51	58	50	72	26	**346**
M. catarrhalis	2	0	1	0	1	1	0	0	0	0	0	0	**5**
Coag-neg staph.	5	7	10	8	5	6	3	10	13	13	20	22	**122**
Corynebacterium	0	1	1	0	0	1	0	0	1	0	0	0	**4**
G- non-fermentive rods	2	1	0	0	0	1	0	18	4	6	2	6	**40**
H. influenzae	19	23	32	37	15	17	15	30	34	31	37	30	**320**
Oral Microbiota	8	3	0	3	0	4	0	0	4	4	4	4	**34**
Staph. Aureus	11	14	26	20	10	9	4	13	20	18	18	13	**176**
Streptococcus beta haemolytic	10	18	12	22	7	12	8	13	22	23	30	16	**193**
Enterobacteria	21	8	13	15	6	20	4	24	21	44	33	26	**235**
Oral streptococci	14	16	11	7	1	4	5	7	14	19	21	15	**134**
Total	**95**	**107**	**137**	**125**	**48**	**87**	**50**	**166**	**191**	**208**	**237**	**158**	**1609**

Note – Details of bacteria names see Table 3

Table 3. Spectrum of bacterial species isolated from orofacial infections

Anaerobes	**Gram negative non fermentative bacilli**
Actinomyces israelii	*Acinetobacter* sp.
Bacteroides fragilis	*Pseudomonas aeruginosa*
Bacteroides melaninogenicus	*Stenotrophomonas maltophilia*
Bacteroides sp.	**Coagulase-negative staphylococci**
Bifidobacterium sp.	*Staphylococcus epidermidis*
Fusobacterium sp.	*Staphylococcus* plasmacoagulase negative
Mobiluncus mulieris	**Oral streptococci**
Peptococcus sp.	Alpha haemolytic *Streptococcus*
Peptostreptococcus micros	*Streptococcus intermedius*
Peptostreptococcus sp.	*Streptococcus milleri*
Porphyromonas endodontalis	*Streptococcus pneumoniae*
Prevotella buccalis - non pigmented	**Beta haemolytic *Streptococcus***
Prevotella melaninogenica - pigmented	Group A beta - haemolytic *Streptococcus*
Propionibacterium propionicum	Group B beta - haemolytic *Streptococcus*
Propionibacterium sp.	Group C beta - haemolytic *Streptococcus*
Veilonella sp.	Group F beta - haemolytic *Streptococcus*
Enterobacteria	Group G beta - haemolytic *Streptococcus*
Citrobacter sp.	Non AB beta - haemolytic *Streptococcus*
Enterobacter sp.	***Corynebacterium*** sp.
Enterococcus sp.	*Corynebacterium pseudodiphteriae*
Escherichia coli	**Others**
Escherichia coli haemolytica	*Staphylococcus aureus*
Klebsiella oxytoca	*Haemophilus influenzae*
Klebsiella pneumoniae	*Moraxella catarrhalis*
Morganella morganii	
Proteus mirabilis	
Proteus vulgaris	
Serratia sp.	

Antimicrobial Susceptibility Results

In general, β-lactam antibiotics like meropenem and ampicillin in combination with β-lactamase inhibitor, macrolide antibiotics like azithromycin and roxithromycin, third generation cephalosporins like ceftizoxime and cefoperazone with β-lactamase inhibitor (sulbactam), fluoroquinolones like ofloxacin and other drugs like nitrofurantoin, mupirocin and teicoplanin demonstrated high levels of antimicrobial activity. Among the different antibiotics used in the study, the maximum resistance was shown by first generation cephalosporins like cefazolin followed by β-lactam antibiotics like ticarcillin, azlocillin, ampicillin and other drugs like metronidazole, cotrimoxazole, tetracycline and erythromycin.

Obligate Anaerobes

Among the 1609 strains of microbes studied, 346 were obligate anaerobes and were highly susceptible to amoxicillin/clavulanic acid combination. 94.1% were susceptible to penicillin. Bacterial isolates (n=4) tested were susceptible also to erythromycin. Available data for 336 isolates of obligate anaerobes demonstrated that they were highly susceptible to imipenem while 2 strains exhibited decreased susceptibility (50%) to tetracycline. All the 9 strains tested of obligate anaerobes were resistant to gentamicin. Less than 1% resistance was observed with chloramphenicol, cefoxitin, and clindamycin. These values are presented in table 4.

Table 4. Antibiotic susceptibility pattern of obligate anaerobes

Obligate anaerobes		
Atb	N	S (%)
GEN	9	0.0
TET	4	50.0
MTZ	322	83.5
PEN	339	94.1
CLI	341	99.4
CFT	344	99.4
CMP	344	99.7
ERY	4	100.0
AMOK	346	100.0
LIN	4	100.0
IMP	336	100.0
CFTX	6	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

Oral streptococci

Oral streptococci remained highly susceptible to chloramphenicol, vancomycin, amoxicillin/ clavulanic acid and teicoplanin but less susceptible to cotrimoxazole (66.3%) and tetracycline (71.6%). Isolates also exhibited good susceptibility to penicillin (95.9%), clindamycin (96.7%) and ampicillin (98.7%) (Table 5).

Table 5. Antibiotic susceptibility pattern of oral streptococci

Oral streptococci		
Atb	N	S (%)
MTZ	2	0.0
COT	102	70.6
TET	122	73.0
ERY	109	93.6
PEN	120	96.7
CLI	40	97.5
AMP	93	98.9
CMP	132	99.2
GEN	1	100.0
AMOK	30	100.0
FUR	2	100.0
CIP	1	100.0
API	6	100.0
CFT	3	100.0
IMP	3	100.0
VAN	95	100.0
OXA	1	100.0
TEI	20	100.0
PIPT	3	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

Staphylococcus aureus*, beta-haemolytic *Streptococcus

The antibiotic susceptibility of *Staph. aureus* strains was as follows: 92% to tetracycline, 93.2% to erythromycin, 97.2% to chloramphenicol, 98.4% to lincomycin, 99.3% to gentamicin, and 99.4% to cotrimoxazole. Isolates of *Staph. aureus* were highly susceptible to all the other tested antibiotics. All the tested antibiotics worked well in the case of beta-haemolytic streptococci (Table 6 and 7).

Table 6. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Atb	**N**	**S** (%)
TET	174	92.0
ERY	176	93.2
CMP	141	97.2
LIN	124	98.4
GEN	137	99.3
COT	176	99.4
AMOK	76	100.0
FUR	1	100.0
CLI	47	100.0
CIP	82	100.0
API	95	100.0
CFT	57	100.0
VAN	138	100.0
OXA	176	100.0
TEI	65	100.0
CEF1	1	100.0
OFL	1	100.0
MUP	1	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

Table 7. Antibiotic susceptibility pattern of beta-haemolytic *Streptococcus*

Atb	N	S (%)
SPI	78	96.2
CLI	108	96.3
ERY	190	98.4
TET	24	100.0
CMP	23	100.0
COT	21	100.0
AMOK	62	100.0
AMP	70	100.0
PEN	193	100.0
VAN	23	100.0
TEI	2	100.0
CEF1	90	100.0
AMPI	1	100.0
AMPS	18	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

Haemophilus influenzae

Azithromycin, cefuroxime, aminopen/ inhibitor, amoxicillin/ clavulanic acid, chloramphenicol and in a small number of cases ampicillin/ inhibitor showed strong antimicrobial activity against

H. influenzae. However, some strains of *H. influenzae* showed greater resistance to cotrimoxazole, tetracycline, and ampicillin. (Table 8)

Table 8. Antibiotic susceptibility pattern of *Haemophilus influenzae*

Haemophilus influenzae		
Atb	N	S (%)
COT	263	79.8
TET	316	98.1
AMP	319	98.1
CMP	80	100.0
AMOK	123	100.0
API	194	100.0
CFX	234	100.0
AZT	293	100.0
AMPI	3	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

Enterobacteria

Enterobacteria were highly susceptible (100%) to drugs like piperacillin/ tazobactam and third and fourth generation cephalosporins like cefoperazone/ sulbactam and cefepime, respectively. Imipenem, piperacillin, vancomycin, and teicoplanin also exhibited high antimicrobial activity against Enterobacteriaceae. More than 75% of isolates were susceptible to several other drugs including amoxicillin/ clavulanic acid. High order of resistance (69.7%) to ampicillin was observed. The bacteria was less susceptible (< 75%) to lincomycin, azlocillin, erythromycin, aminopen/ inhibitor, first generation cephalosporins (cephalothin and cefazolin), tetracycline and ticarcillin. (Table 9)

Table 9. Antibiotic susceptibility pattern of enterobacteria

Atb	N	S (%)
CFT	0	0.0
OXA	2	0.0
AMPS	2	0.0
ROX	1	0.0
AMP	165	30.3
LIN	2	50.0
CEF1	6	50.0
AZL	7	57.1
ERY	19	57.9
API	94	59.6
CFN	134	60.4
TET	162	70.4
TIC	18	72.2
AMOK	33	78.8
CFX	107	86.0
CMP	79	86.1
COL	132	90.2
COT	158	90.5

Atb	n	S (%)
NET	57	94.7
AMI	100	97.0
GEN	142	97.2
CETX	133	97.7
CFA	71	98.6
LVF	77	98.7
CIP	112	99.1
PEN	1	100.0
FUR	1	100.0
IMP	55	100.0
VAN	29	100.0
TEI	12	100.0
PIP	30	100.0
CFPS	58	100.0
OFL	4	100.0
PIPT	59	100.0
MEP	4	100.0
CFP	1	100.0
CFM	56	100.0
TICI	3	100.0

Atb- abbreviation of antibiotic; n- number of tested strains; S- % of susceptible strains

DISCUSSION

Bacteriological profiles

The majority of suppurative odontogenic infections is polymicrobial in nature and consists of both mixed aerobic and anaerobic bacteria (8) with anaerobes two to four times greater in proportion than aerobes (24). Only very few long-term studies have examined the species distribution profiles and gender dominance in oral infections. The aim of this retrospective study was to investigate the prevalence of bacterial species in oral samples of patients with suspected orofacial infection reporting at the Department of Dentistry (1996 - 2007), the species distribution of bacteria, to assess the sex and species specific relation in odontogenic and non-odontogenic infections. A total of 678 culture-positive patients were included in this study with 1609 strains comprising of 48 different species isolated (Table 6).

Age and gender

This study showed an age distribution between 2 and 94 years, with a mean age of 41.2 (± 18.03) years among males and 43.7 (± 19.5) years among females. This is in partial agreement with earlier studies comprising of 25-35, 20-29, and 23-70 years age groups (9). The proportion of males and females in the study were comparable (p=0.082). These findings are in agreement with earlier studies (11). Infections caused by *M. catarrhalis,* anaerobes, *H. influenzae,* oral streptococci, *Staph. aureus,* coagulase-negative staphylococci and beta haemolytic *Streptococcus* were slightly higher among females and enterobacteria in males, however this differences in percentage distribution of isolates among either genders did not show statistical significance. The total number of species of microbes isolated in this study was high. However, a substantial decrease in the number occurred during the years 2000 to 2002 which may be attributed to change in methodology.

Spectrum of microorganisms

Isolates comprised of predominantly facultative anaerobes. Facultative anaerobes and obligate anaerobes accounted for 78.5% (n= 1263) and 21.5% (n=346) respectively. The most frequently isolated facultative anaerobe were identified as *H. influenzae* (n=320, 19.9%) followed by, enterobacteria (n=235, 14.6%), beta-haemolytic *Streptococcus* spp. (n=193, 12%), *Staph. aureus* (n=176, 10.9%), coagulase-negative staphylococci (n=122, 7.6%), oral streptococci (n=110, 6.8%), and Gram-negative non-fermentative rods (n=40, 2.5%). However, *M. catarrhalis* and *Corynebacterium* sp. were the least common. This is in contrast to a study by Heimdahl et al. that

demonstrated predominance of obligate anaerobes like *Bacteroides, Prevotella* and *Fusobacterium* (10). Earlier studies by other investigators have reported *Porphyromonas, Prevotella, Fusobacterium, Peptostreptococcus,* and streptococci, to be the major pathogenic bacteria isolated from dental infections (26).

The results of this study are in agreement with the findings of obligate and facultative anaerobes by Kuriyama et al. (14). In their study involving 664 strains isolated from dentoalveolar infections, periodontitis and pericoronitis, the majority of the isolates belonged to viridans streptococci, *Peptostreptococcus, Gemella,* pigmented and nonpigmented *Prevotella*, *Porphyromonas*, and *Fusobacterium.*

Enteric gram-negative rods, have been isolated from normal oral flora in 27.9% cases with enterobacteria accounting for 57% of isolates in a study by Sedgley et al. (27). These strains have also been found in immunocompromised persons undergoing chemotherapy (5). The proportion of enterobacteria varies depending on the consumption of contaminated food and water and personal hygiene (3). In this study enterobacteria like *Citrobacter, Enterobacter, Enterococcus, Escherichia coli, E. coli haemolytica, Klebsiella oxytoca, Kl. pneumoniae, Morganella morganii, Proteus mirabilis,* and *P. vulgaris* were more commonly isolated. In a study by Gonçalves et al. enteric rods like *E.cloacae* (7 strains), *E. aerogenes* (1 strain), *Pantoea* (*Enterobacter*) *agglomerans* (1 strain), *Serratia marcescens* (5 strains), *Klebsiella pneumoniae* (1 strain) and *Citrobacter freundii* (1 strain) were isolated from periodontal pockets of patients with chronic periodontitis. The isolation of pathogens like *E. coli, Kl. pneumoniae* and *Ps. aeruginosa* from mouth that may cause opportunistic infections in respiratory tract especially in patients who are immunocompromised highlights the importance of early identification of these potentially harmful microorganisms.

Antimicrobial susceptibility profiles

Most odontogenic orofacial infections are caused predominantly by anaerobes but there have been only a few long-term studies that have examined the bacteriologic and antimicrobial susceptibility profiles in oral infection. Administration of antibiotics through oral or other routes affect the microbiota throughout the body and hence it will be useful to compare the susceptibility profiles of oral bacteria.

Obligate anaerobes

In this study, obligate anaerobes did not show resistance to amoxicillin/ clavulanic acid which is similar to the observation reported by Lana et al. (17) wherein all the facultative anaerobic bacterial isolates (34 strains) and majority of obligate anaerobes (52 of 54 strains) showed high susceptibility.

In another study by Fosse et al., amoxicillin with clavulanic acid worked well against Gram-negative bacilli like *Prevotella* except for the presence of one ß-lactamase producing strain (4). *Prevotella* spp. is known to demonstrate resistance to penicillin commonly (1) and this resistance has been found to be similar for both pigmented and non-pigmented species (13).

In this investigation obligate anaerobes showed higher susceptibility to penicillin (94.1%) and the majority of obligate anaerobic strains were susceptible to all the drugs tested, with the exception of tetracycline and gentamicin. Similar observations were made by Kuriyama et al. wherein the susceptibility rates to penicillin G for *Peptostreptococcus, Porphyromonas,* and *Fusobacterium* were 86%, 100%, and 89% respectively while 72% of pigmented and 82% of nonpigmented strains of *Prevotella* showed high susceptibility (14). Certain studies show that in *Prevotella* the resistance mechanisms against tetracycline are genetically-determined like the β-lactam-resistance (4).

In this study it was observed that third generation cephalosporins like ceftizoxime worked very well against all the strains whereas 2 of 342 strains of obligate anaerobes were resistant to second generation cephalosporins like cefoxitin. Greater antimicrobial activity of the newer generation cephalosporins than the older ones may be attributed to the higher stability of the former in the presence of β-lactamases (20).

Thus the high susceptibility to β-lactam antibiotics favours their continued use in the management of infections by obligate anaerobic strains.

Oral streptococci

In this study, oral streptococci exhibited 95.9% susceptibility rate to penicillin. This is in contrast to certain other studies where only 77% of viridans streptococci were susceptible to penicillin G (14). All the tested strains in this study were also susceptible to other drugs except for some resistance to cotrimoxazole and, tetracycline, erythromycin, penicillin, clindamycin and ampicillin.

There have been conflicting reports in the literature regarding the efficacy of penicillins against viridans streptococci and β-lactamase–producing anaerobes (14, 26). In a study by Kuriyama et al. most anaerobes and facultative anaerobes (especially viridans streptococci) were susceptible to penicillin except β-lactamase–positive *Prevotella*. They reported that viridans streptococci and majority of the strains of *Fusobacterium* were resistant to erythromycin while anaerobes were susceptible to clindamycin (14). In patients who have penicillin allergy, alternative drugs like erythromycin and clindamycin are administered (26). Their effectiveness make them suitable for orofacial infections in such patients. In contrast to the bacteriologic data from other studies (14), this study showed that 92% of oral streptococci were susceptible to erythromycin. Previous studies have shown that the serum concentration of the ß-lactam antibiotics and erythromycin is greater than that

achieved in the saliva (21, 28). However oral streptococcal species have been found to be susceptible to low ß-lactam antibiotic concentrations in the saliva (21, 28).

Clindamycin was effective against both oral streptococci and obligate anaerobes which is in agreement with the study by Kuriyama et al. (14). The high bactericidal activity of clindamycin against β-lactamase–producing bacteria coupled with their ability to achieve high alveolar concentrations (11) and clinical efficacy at the recommended dosage (26) make them more suitable for treating infections by β-lactamase–producing obligate anaerobes (26). There is an inhibitory action on the formation of β-lactamase (25) and greater host defence achieved on administration of clindamycin (6) antibiotic-associated colitis, the major side effect, restricts the use of clindamycin to treat severe oral infections or where treatment with penicillin has been ineffective (14).

Previous studies showed that viridans streptococci, *Peptostreptococcus, Porphyromonas,* and *Fusobacterium* were highly susceptible to cefazolin (1st generation cephalosporin) and cefmetazole (2nd generation cephalosporin). However some strains of *Prevotella* showed lower susceptibility only to cefazolin (14). Similar to the above observations, a high susceptibility of obligate anaerobes and a few strains of oral streptococci against cefoxitin (2nd generation cephalosporin) were observed in this study. Cephalosporins show cross-reactivity with β-lactam antibiotics and hence should not be administered to patients with immediate hypersensitivity reactions to penicillin (20). However the broad spectrum and strong bactericidal action against oral pathogens make them drugs of choice in the treatment of dental infections (20).

This study also showed an increased resistance to tetracycline similar to other studies (2), but oral streptococci showed 71.6% susceptibility to tetracycline. However in another study, minocycline worked well against viridans streptococci and strict anaerobic bacteria which is attributed to its powerful bacteriostatic effect than tetracycline (2,14).

The results also demonstrated that alpha haemolytic streptococci were highly susceptible to erythromycin, penicillin, ampicillin, vancomycin but resistance was noted against tetracycline, cotrimoxazole and chloramphenicol. *S. pneumoniae* and other alpha-haemolytic streptococci (23) are known to transfer resistance traits to each other. This inter-species transfer of resistance genes poses great concern in the treatment of resistant strains.

Although some strains among oral streptococci were resistant to penicillin (4.1%) and ampicillin (1.3%), all the strains of alpha-haemolytic streptococci and, beta-haemolytic streptococci tested against penicillin and ampicillin were highly susceptible while cephalosporins were equally effective for oral streptococci and beta-haemolytic streptococci. In another study by Teng et al., among the 207 isolates of alpha-haemolytic streptococci, including *S. mutans*, *S. salivarius*, *S. oralis* and *S. mitis*, only *S. mutans* showed no resistance to penicillin (30). Potgieter et al. reported that a few strains of *S.*

mitis were not susceptible to penicillin, aminoglycosides like gentamicin, kanamycin and tobramycin (22).

Hunt et al. reported susceptibility of streptococci to ampicillin, cephalothin, and penicillin (11). The results in this investigation are in agreement with the above study as ampicillin, cephalosporins and penicillin worked well against oral streptococci, and beta-haemolytic streptococci. However, in contrast to their study, the present study results found a greater antimicrobial activity of erythromycin against all the tested streptococcal species.

Staphylococci

In this study, coagulase-negative staphylococci showed a 98.4% susceptibility to cephalosporin agents like cefoxitin (2^{nd} generation cephalosporin) while all isolates of *Staph. aureus* (n=57) tested with cefoxitin (2^{nd} generation cephalosporin) and 1 of the isolate tested with cephalothin (1^{st} generation cephalosporin) were susceptible. This is in agreement with a previous study by Jacobson et al. (12).

Overall 31.1% of coagulase-negative staphylococci and 6.8% of *Staph. aureus* were resistant to erythromycin. *Staphylococcus aureus* demonstrated greater antimicrobial activity to the tested antibiotics than the coagulase–negative staphylococci. Higher resistance in the range of 50% for streptococcal and staphylococcocal species has also been reported in a study by Hunt and co-workers (11).

In the case of tetracycline, lower susceptibility was demonstrated by all the tested staphylococcal and streptococcal strains except beta-haemolytic streptococci. On the contrary, all the above strains showed higher susceptibility for chloramphenicol. Certain studies have shown a decrease in susceptibility of oral microbiota to minocycline following administration of minimal dose of minocycline. This reveals that antibiotic concentration is closely related with the development of resistant strains within the members of the oral microbiota (29). All the streptococcal and *Staph. aureus* strains were highly susceptible to amoxicillin/ clavulanic acid and vancomycin. Only one vancomycin resistant strain of coagulase negative staphylococci was detected.

This study cohort comprised of a mixed collection of patients and the microorganisms were subjected to a standard set of antibiotics with additional sets of antibiotics used depending on the susceptibility profiles of the data. These have lead to difficulty in direct comparison of susceptibility profiles within each individual species as different sets of antibiotics were used to determine the most appropriate drug of choice for treatment of orofacial infections on a case by case basis. Besides, there can be a difference in the *in vivo* and *in vitro* activity of antibiotics (18). However, the presence of numerous causative organisms for orofacial infections necessitates appropriate antimicrobial for treatment (15).

CONCLUSIONS

The microorganisms most commonly implicated in orofacial infections in this study were facultative anaerobes like *Haemophilus influenzae* and enterobacteria followed by obligate anaerobes. The predominance of facultative anaerobic bacteria and the presence of obligate anaerobes reveal the complex polymicrobial nature of odontogenic and non odontogenic lesions. Both sexes had equal predilection for the disease and there had been no significant change in the male/ female ratio over the 11 year study period. However, there had been an increase in the total number of bacterial species. In future, large-scale oral bacteriological surveillance programmes are required to corroborate the results of the present study.

Obligate anaerobes were highly susceptible to most antibiotics including penicillins while resistance to gentamicin and tetracyline was noted among these species. Greater than 95% susceptibility was demonstrated by oral streptococci to β-lactam antibiotics in comparison to erythromycin and broad-spectrum drugs like tetracycline and cotrimoxazole. However, most isolates of alpha and beta-haemolytic streptococci showed greater susceptibility to antimicrobials than the oral streptococcal species. The susceptibility rate of coagulase-negative staphylococci was significantly lower than that of the *Staphylococcus aureus* strains although both groups exhibited greater susceptibility to β-lactam antibiotics than broad spectrum drugs. Enterobacteria showed the highest susceptibility to piperacillin/tazobactam and third and fourth generation cephalosporins, whereas there was unusually high resistance to ampicillin. Gram-negative non-fermentative bacilli were more susceptible to 3^{rd} generation cephalosporins and polypeptide antibiotics. Isolates of *H. influenzae* were susceptible to a wide range of β-lactams, broad-spectrum antibiotics like chloramphenicol and 2^{nd} generation cephalosporins. *Branhamella catarrhalis* and *Corynebacterium* species were also found to be susceptible to β-lactam antibiotics.

The findings in this study suggest that β-lactam antibiotics are still the mainstay in the antimicrobial management of orofacial infections as they are effective in eradicating strict and facultative anaerobes but appropriate and adequate antibiotic regimen on a case-specific basis is essential to prevent the emergence of resistance to antimicrobials in the future. Towards this goal, large scale surveillance programs will help in improving patient outcome and formulating public health policies.

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