ORIGINAL RESEARCH

Incidence of Anaerobic Bacteria in 118 Patients with Deep-space Head and Neck Infections from the People's University Hospital of Maxillofacial Surgery, Bhopal, India

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ABSTRACT

Aim: The aim of this study was to assess the incidence and susceptibility to antibacterial agents of anaerobic strains in 118 patients with head and neck abscesses (31) and cellulitis (87).

Materials and methods: In total, 118 pus specimens from 118 consecutive patients with abscesses (31 cases) and cellulitis (87) of the head and neck were evaluated from 2006 to the end of 2011. The patients were admitted to the University Hospital of Maxillofacial Surgery, Bhopal, India, and comprised 76 men and 42 women: Four children, 103 adults and 11 elderly people.

Results: Anaerobic bacteria (174 strains within 18 genera) were found in 88 (74.6%) of the 118 specimens. Anaerobes only were present in 23 (19.5%) specimens, aerobic/facultative bacteria only in 20 (16.9%) and mixed aerobic/anaerobic flora in 65 (55.1%). No growth was detected in 10 (8.5%) specimens. Two or more anaerobes per specimen were found in 56 (63.6%) of the specimens yielding anaerobes. The incidence of isolation of anaerobes from patients with identified odontogenic sources of infection was 82.2% (60 of 73 cases) and that in patients with other sources of infection was 71.4% (15 of 21, $p > 0.20$).

Conclusion: The start of empirical treatment could influence the frequency or rate of isolation of *Fusobacterium* species. The involvement of the *Bacteroides fragilis* group in some head and neck infections should be considered.

Keywords: Anaerobic, Odontogenic, Space infection.

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INTRODUCTION

Anaerobic bacteria are important pathogens in head and neck infections.[1] According to several authors resistance rates to some antibacterial agents (such as ampicillin/sulbactam and clindamycin) have shown a tendency to increase.[2,3] The treatment of infections in maxillofacial surgery involves surgical procedures and application of antibacterial agents.[4]

Most head and neck infections are endogenous and mixed in nature.[5] Thus, the antibacterial treatment of mixed infections should cover both aerobes and anaerobes. Resistance rates for anaerobes vary within species as well as within sources of the isolates. Anaerobic bacterias are a common cause of infections, some of which can be serious and life-threatening. Because anaerobes are the predominant components of the skin's and mucous membranes normal flora, they are a common cause infections of endogenous origin. Because of their fastidious nature, anaerobes are hard to isolate and are often not recovered from infected sites. The administration of delayed or inappropriate therapy against these organisms may lead to failures in eradication of these infections. The isolation of anaerobic bacteria requires adequate methods for collection, transportation and cultivation of clinical specimens. The management of anaerobic infection is often difficult because of the slow growth of anaerobic organisms, which can delay their identification by the frequent polymicrobial nature of these infections and by the increasing antimicrobial resistance of anaerobic bacteria to antimicrobials.

The aim of this study was to know the incidence and susceptibility patterns to antibacterial agents of anaerobes in patients with abscesses and cellulitis of the head and neck over a period of 5 years and to assess the influence of the start of empirical treatment on the isolation rates of the anaerobes.

MATERIALS AND METHODS

Patients

In total, 118 pus specimens were randomly selected from 118 patients with abscesses (31 cases) and cellulitis (87) of the head and neck which were evaluated from 2006 to the end of 2011. The patients were admitted to the People's University Hospital of Maxillofacial Surgery, Bhopal, India and comprised 76 men and 42 women: Four children, 103 adults and 11 elderly people.

The sites of infection were submandibular or parapharyngeal (80 cases, 50 of them affecting the floor of the mouth), neck (6) and facial (32). The site of origin of the infections was identified in 94 patients (79.7% of all patients). The mean age of the patient was 42 years. Pregnant women and patient with blood dyscrasias were excluded from the study. Informed consent was taken. The most common source was odontogenic infection, occurring in 73 cases (77.7%). Other sources involved salivary gland infections (7 cases), trauma (6), upper airway (5) and other infections (3). Three patients suffered from diabetes and three patients had malignant diseases. Most (73.7%, 87 of 118) patients were evaluated after the start of empirical treatment in the hospital with

β-lactams (24 cases), metronidazole (5), both agents (52) and other antibacterial drugs (6) for 1 to 3 days.

Strain isolation and culture: After skin disinfection with 70% ethanol and iodophor, pus aspirates were taken by needle aspiration or during incision. The specimens were placed in Stuart transport medium (Merck) and processed within 2 hours of collection. The specimens were inoculated onto Brucella agar (Becton Dickinson) enriched with hemin, vitamin K (Becton Dickinson) and 5% sheep blood. Part of each specimen was placed in Komkova anaerobic broth [National Centre of Infectious and Parasitic Diseases (NCIPD)], which was boiled for 5 to 10 minutes used and cooled prior to use. Komkova broth is a cooked-meat medium, containing glucose, gelatin and 0.3% agar.[6] After inoculation, the Komkova anaerobic broth was overlaid with 1 to 2 ml sterile liquid paraffin and incubated at 37°C. The broth was subcultured after 48 to 72 hours on enriched Brucella blood agar. A direct smear was made and examined after Gram staining with 0.1% basic fuchsin as a counterstain. The specimens were plated on blood agar plates as an aerobic control. Anaerobic media were incubated using GasPak anaerobic system envelopes (Becton Dickinson) or Anaerobe Pack (NCIPD) at 37°C for up to 7 or 14 days, when actinomycosis was clinically suspected. Anaerobic strains were identified by Gram stain, colonial morphology, aerobic control, susceptibility to special potency disks, catalase, spot indole and API system Rapid ID 32 A.[2]

Antibacterial Susceptibility Testing

The special potency disks (Rosco and Becton Dickinson) contained oxgall, kanamycin (1,000 µg), colistin (10 µg) and metronidazole (5 µg).

The antibacterial susceptibility of 151 anaerobic strains was evaluated by using an agar dilution method with two to three consecutive concentrations.[7] Enriched Brucella blood agar plates containing the following agents were used (µg ml^{-1}): Amoxicillin (0.5, 1 and 2), clindamycin (2 and 4), ampicillin/sulbactam (8/4 and 16/8) and metronidazole (8, 16 and 32).

The bacteria inoculum corresponded to 0.5 McFarland standard and the final inoculum was about 10^5 CFU per spot.[7] When no growth was observed on the plate after 48 hours of anaerobic incubation, the isolate was considered to be susceptible to the agent. Breakpoints for intermediate susceptibility and resistance to amoxicillin (for Gram-negative anaerobes), clindamycin, ampicillin/sulbactam and metronidazole were 1 and 2, 4 and 8, 16/8 and 32/16 and 16 and 32 µg ml^{-1}, respectively.[7]

According to *in vitro* data, the minimum inhibitory concentrations (MICs) for ampicillin and amoxicillin against anaerobes have been reported to be identical.[7] Amoxicillin breakpoints have been considered to be equivalent to ampicillin breakpoints.

Enriched *Brucella* blood agar plates without antibacterial agents were used for growth and purity controls for the strains (by anaerobic incubation) and aerobic/facultative contaminant control (by aerobic incubation). The control strains used were two laboratory anaerobic isolates (*Prevotella intermedia* and *Clostridium perfringens*) with known antibiotic MICs.

STATISTICAL ANALYSIS

Differences between groups were compared by using Chi-square test with or without Yates' correction factor. Yates' correction factor for continuity was included in the calculation of Chi-square values for 2 × 2 tables when the expected frequency was <10 in one or more cells.

RESULTS

Anaerobic bacteria (174 strains within 18 genera) were found in 88 (74.6%) of the 118 specimens. Anaerobes only were present in 23 (19.5%) specimens, aerobic/facultative bacteria only in 20 (16.9%) and mixed aerobic/anaerobic flora in 65 (55.1%). No growth was detected in 10 (8.5%) specimens. Two or more anaerobes per specimen were found in 56 (63.6%) of the specimens yielding anaerobes. The incidence of isolation of anaerobes from patients with identified odontogenic sources of infection was 82.2% (60 of 73 cases) and that in patients with other sources of infection was 71.4% (15 of 21, $p \geq 0.20$).

The predominant anaerobic bacteria were *Prevotella* (49 strains), *Fusobacterium* species (22), *Actinomyces* species (21), anaerobic cocci (20) and *Eubacterium* species (18) (Table1). Microaerophilic streptococci were found in 28 (23.7%) of the specimens and, in most cases (89.3%), were associated with anaerobes.

Amoxicillin resistance in Gram-negative anaerobes from patients treated with β-lactams was slightly more common (34%, 17 of 50) than in those from other patients (14.3%, 4 of 28; $p \geq 0.10$) (Table 2). Low rates of nonsusceptibility to both amoxicillin and metronidazole were detected in Gram-negative anaerobes (1.3%, 1 of 78 strains). However, it is important to stress that β-lactamase testing of anaerobic organisms is useful and recommended,[7] because all β-lactamase-positive Gram-negative anaerobes should be considered as resistant, independently of their ampicillin MIC values. In addition, for Gram-positive anaerobes, there is no ampicillin breakpoint. The susceptibility breakpoint for Gram-positive anaerobes should be higher than that for Gram-negative anaerobes.[8] Therefore, although in the present study three Gram-positive anaerobic strains exhibited amoxicillin MICs of >1µg ml^{-1}, they should not be considered as amoxicillin-resistant strains.

Table 1: Anaerobic bacteria isolated from head and neck infections treated or not treated before sampling

Organism/group	*Total no. (%) of strains*	*No. (%) of strains from nontreated patients*	*No. (%) of strains from treated patients*
Gram-negative	**90 (51.7)**	**25 (54.3)**	**65 (50.8)**
Bacteroides fragilis group	7 (4.0)	2 (4.3)	5 (3.9)
Fusobacterium species	22 (12.6)	10 (21.7)	12 (9.4)
F. nucleatum	16 (9.2)	6 (13.0)	10 (7.8)
F. necrophorum	3 (1.7)	2 (4.3)	1 (0.8)
F. mortiferum	3 (1.7)	2 (4.3)	1 (0.8)
Prevotella spp.	49 (28.2)	10 (21.7)	39 (30.5)
P. melaninogenica	13 (7.5)	2 (4.3)	11 (8.6)
P. intermedia	16 (9.2)	5 (10.9)	11 (8.6)
P. oris	4 (2.3)	0 (0)	4 (3.1)
P. loescheii	2 (1.1)	1 (2.2)	1 (0.8)
P. disiens	2 (1.1)	0 (0)	2 (1.6)
P. denticola	2 (1.1)	0 (0)	2 (1.6)
P. corporis	1 (0.6)	0 (0)	1 (0.8)
Nonpigmented *Prevotella* spp.	9 (5.2)	2 (4.3)	7 (5.5)
Porphyromonas spp.	3 (1.7)	1 (2.2)	2 (1.6)
*Sutterella wadsworthensis**	2 (1.1)	0 (0)	2 (1.6)
Capnocytophaga spp.*	3 (1.7)	1 (2.2)	2 (1.6)
Veillonella spp.	4 (2.3)	1 (2.2)	3 (2.3)
Gram-positive	**84 (48.3)**	**21 (45.6)**	**63 (49.2)**
Actinomyces spp.*	21 (12.1)	6 (13.0)	15 (11.7)
A. odontolyticus	11 (6.3)	3 (6.5)	8 (6.2)
A. israelii	2 (1.1)	1 (2.2)	1 (0.8)
Other *Actinomyces* spp.	8 (4.6)	2 (4.3)	6 (4.7)
Bifidobacterium spp.	3 (1.7)	1 (2.2)	2 (1.6)
Clostridium tertium	1 (0.6)	0 (0)	1 (0.8)
Eubacterium spp.	18 (10.3)	3 (6.5)	15 (11.7)
Eggerthella lenta	5 (2.9)	3 (6.5)	2 (1.6)
GPAC†	16 (9.2)	2 (4.3)	14 (10.9)
Finegoldia magna	6 (3.4)	2 (4.3)	4 (3.1)
Micromonas micros	2 (1.1)	0 (0)	2 (1.6)
Peptostreptococcus anaerobius	3 (1.7)	0 (0)	3 (2.3)
Other and nonidentified GPAC	5 (2.9)	0 (0)	5 (3.9)
Lactobacillus spp.*	13 (7.5)	5 (10.9)	8 (6.2)
Propionibacterium spp.*	7 (4.0)	1 (2.2)	6 (4.7)
All anaerobic strains	**174**	**46**	**128**

*Microaerophilic species, †GPAC: Gram-positive anaerobic cocci.

Prevotella intermedia, *Fusobacterium nucleatum*, *Prevotella melaninogenica* and the *Bacteroides fragilis* group were the most common Gram-negative anaerobic species, accounting for 9.2, 9.2, 7.5 and 4% respectively, of all anaerobic strains. *Bacteroides fragilis* group strains included *Bacteroides fragilis* (two strains), *Bacteroides vulgatus* (one), *Bacteroides distasonis* (one) and three other strains. Gram-positive anaerobic cocci (GPAC) were detected in 16 (13.6%) specimens and *Finegoldia magna* accounted for 37.5% of all GPAC strains. About half of the 21 *Actinomyces* strains belonged to *Actinomyces odontolyticus*. Among the aerobic/facultative isolates from the patients of the Peoples University Hospital of Maxillofacial Surgery in 2006 to 2011, 68% were Gram-positive cocci, 30.5% were Gram-negative bacteria and 1.5% were *Candida* species.

DISCUSSION

Abscesses and cellulitis of the head and neck are severe diseases. A nontreated 79-year-old man with cellulitis of the floor of the mouth died 2 hours after admission to the hospital. His specimen yielded *Fusobacterium necrophorum*, *Finegoldia magna*, microaerophilic streptococci and *Bifidobacterium* and *Lactobacillus* species. The resistance rate to amoxicillin of Gram-negative anaerobes was 26.9% (21 of 78 strains). Resistance rates to clindamycin and metronidazole of Gram-negative anaerobes were 5.4% (4 of 74) and 2.5% (2 of 79) respectively, and those of Gram-positive species were 4.5% (3 of 66) and 58.3% (42 of 72), respectively. Only one strain was not susceptible to ampicillin/sulbactam.

In the present study, the predominant anaerobic species were similar to those reported by Brook (2004);[9] however, isolates of *Porphyromonas* species were relatively rare. The involvement of microaerophilic streptococci was considered as, recently, members of the '*Streptococcus milleri*' group have been recognized as important pathogens in head and neck abscesses.[10]

The detection rate of anaerobes from patients with deep-space head and neck infections was relatively lower than that

Table 2: Resistance patterns of anaerobic isolates from two patients' groups (not treated and empirically treated) with abscess or cellulitis of the head and neck

Organism (no. of strains)	*Agent*			*Not treated*			*Empirically treated*		
				No. of strains	*% R*	*% I*	*No. of strains*	*% R*	*% I*
Bacteroides fragilis group(7)	Amoxicillin	2	2	0	5	5	0	7	7
	Metronidazole	2	0	0	5	0	0	7	0
	Clindamycin	2	0	2	4	1	1	6	1
	Ampicillin/sulbactam	2	0	0	4	0	0	6	0
Prevotella and *Porphyromonas* spp.(49)	Amoxicillin	10	1	0	39	10	1	49	11
	Metronidazole	10	0	0	39	0	0	49	0
	Clindamycin	10	0	0	37	2	3	47	2
	Ampicillin/sulbactam	10	0	1	33	0	0	43	0
Fusobacterium species(15)	Amoxicillin	8	0	1	7	1	2	15	1
	Metronidazole	8	0	0	7	0	0	15	0
	Clindamycin	8	1	0	6	0	0	14	1
	Ampicillin/sulbactam	8	0	0	7	0	0	15	0
Other Gram-negative anaerobes(8)	Amoxicillin	2	1	0	5	1	0	7	2
	Metronidazole	2	0	1	6	2	0	8	2
	Clindamycin	2	0	0	5	0	0	7	0
	Ampicillin/sulbactam	1	0	0	4	0	0	5	0
Gram-positive anaerobic cocci(14)	Metronidazole	2	0	0	12	1	0	14	1
	Clindamycin	2	0	1	10	0	0	12	0
	Ampicillin/sulbactam	2	0	0	12	0	0	14	0
Clostridium tertium(1)	Metronidazole	0	0	0	1	1	0	1	1
	Clindamycin	0	0	0	1	0	0	1	0
	Ampicillin/sulbactam	0	0	0	1	0	0	1	0
Gram-positive nonspore-forming rods(57)	Metronidazole	15	11	0	42	29	0	57	40
	Clindamycin	15	1	0	38	2	2	53	3
	Ampicillin/sulbactam	15	0	0	41	0	0	56	0

% I: Percentage intermediately susceptible; % R: Percentage resistant

(82-100%) observed by Jousimies-Somer et al (2002),[2] but was higher than that (21-59.3%, according to the sources of infection) reported by Huang et al (2006).[11] Detection rates of anaerobes were similar in children (75%, 3 of 4 cases), adults (74.8%, 77 of 103) and the elderly (72.7%, 8 of 11; $p \geq 0.20$). The rate of isolation of anaerobes from empirically treated patients was slightly lower (72.4%, 63 of 87) than that from nontreated patients (80.6%, 25 of 31; $p \geq 0.20$).

The rate of isolation of *Fusobacterium* species from non-treated patients (32.2%, 10 of 31) was higher than that from treated patients (13.8%, 12 of 87, $p \leq 0.05$), whereas no significant difference ($p \geq 0.10$) was observed between groups for *Prevotella* species. The start of empirical treatment appears to influence the frequency or rate of isolation of *Fusobacterium* species.

Species of the *Bacteroides fragilis* group have been detected in single cases of head and neck (0.9%) and pleuropulmonary (0.3%) infections (Jousimies-Somer et al 2002).[2] In the present study, *Bacteroides fragilis* group species were isolated more often (in 5.9%, 7 of 118 specimens) and accounted for 4% of all anaerobic strains. Similarly, these organisms accounted for 5.7% of anaerobic isolates from the respiratory tract, according to Piérard et al (2003).[12]

Clostridia are unusual isolates in head and neck infections (Jousimies-Somer et al 2002).[2] In the present study, a metronidazole-resistant *Clostridium tertium* strain was found in association with *Prevotella corporis* and *Propionibacterium acnes* in a treated patient with cellulitis of the floor of the mouth. Metronidazole resistance has been reported in *Clostridium tertium* and some other clostridial species.[13-15]

For the Gram-negative anaerobes, the rates of non-susceptibility to amoxicillin (32%, 25 of 78 strains) and clindamycin (13.5%, 10 of 74) were lower than those to penicillin (81.8%) and clindamycin (31.1%) in Greece (Papaparaskevas et al 2005).[3] Penicillin resistance has been found in 83% of Prevotella isolates (Aldridge et al 2001),[1] as well as in 32 to 35% of those in odontogenic infections (Kuriyama et al 2001).[5]

In the present study, amoxicillin resistance was present in 10 (21.7%) of 46 *Prevotella* strains. One (6.7%) of 15 *Fusobacterium* strains was amoxicillin resistant and three (20%) strains were intermediately susceptible to the agent. β-lactam-resistant *Porphyromonas* species have been reported by Aldridge et al (2001),[1] but have not been detected in other studies (Kuriyama et al 2001, Bahar et al 2005).[5,16] In the

present work, one of three *Porphyromonas* strains was amoxicillin resistant.

The susceptibility rate of *Prevotella* species to clindamycin (88.6%, 39 of 44 strains) was similar to that (90%) reported in odontogenic abscesses by Sobottka et al (2002).[4] However, clindamycin resistance in *Prevotella* strains (4.5%, 2 of 44 strains) was lower than that (22.2%) observed by Wexler et al (2002).[17] Clindamycin resistance rates were relatively low in both Gram-negative (5.4%) and Gram-positive (4.5%) anaerobes. Ampicillin/sulbactam was the most active agent evaluated. Orofacial anaerobes are usually susceptible to ampicillin/sulbactam and amoxicillin/clavulanate (Kuriyama et al, 2000),[5] although recent studies have reported a decreased activity of these agents against 5 to 8% of *Bacteroides fragilis* group strains and some *Peptostreptococcus anaerobius* isolates (Aldridge et al 2001; Kato et al, 2000; Koeth et al, 2004).[1,18,19]

Intermediate susceptibility to amoxicillin/clavulanate has been detected in single *Prevotella* strains by Wexler et al (2002).[17] In the present study, one *Prevotella oralis* strain was both amoxicillin resistant and intermediately susceptible to ampicillin/sulbactam. No resistance to ampicillin/sulbactam was observed in *Bacteroides fragilis* group strains, although one ampicillin/sulbactam-resistant *Bacteroides fragilis* group isolate was detected in a patient (not involved in the study) with malignancy and maxillofacial wound infection in 2009.

CONCLUSION

The wide diversity and susceptibility patterns of anaerobic species motivate the use, wherever possible, of anaerobic microbiology in maxillofacial surgery departments.

The limitation of the study was that when prior antibiotic coverage was given to the patient, isolation of microbial species was negative. The start of empirical treatment could influence the frequency or rate of isolation of *Fusobacterium* species. Involvement of the *Bacteroides fragilis* group in some severe head and neck infections should be considered.

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