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Anaerobic bacteria in 118 patients with deep-space head and neck infections from the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria

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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). Odontogenic infection was the most common identified source, occurring in 73 (77·7 %) of 94 patients. The incidence of anaerobes in abscesses and cellulitis was 71 and 75·9 %, respectively, and that in patients before (31 patients) and after (87) the start of empirical treatment was 80·6 and 72·4 %, respectively. The detection rates of anaerobes in patients with odontogenic and other sources of infection were 82·2 and 71·4 %, respectively. In total, 174 anaerobic strains were found. The predominant bacteria were *Prevotella* (49 strains), *Fusobacterium* species (22), *Actinomyces* spp. (21), anaerobic cocci (20) and *Eubacterium* spp. (18). *Bacteroides fragilis* strains were isolated from 7 (5·9 %) specimens. The detection rate of *Fusobacterium* strains from non-treated patients (32·2 %) was higher than that from treated patients (13·8 %). Resistance rates to clindamycin and metronidazole of Gram-negative anaerobes were 5·4 and 2·5 %, respectively, and those of Gram-positive species were 4·5 and 58·3 %, respectively. One *Prevotella* strain was intermediately susceptible to ampicillin/sulbactam. In 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.

INTRODUCTION

Anaerobic bacteria are important pathogens in head and neck infections (Jousimies-Somer *et al.*, 2002). The treatment of infections in maxillofacial surgery involves surgical procedures and application of antibacterial agents (Sobottka *et al.*, 2002). Most head and neck infections are endogenous and mixed (Kuriyama *et al.*, 2001). 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. According to several authors (Aldridge *et al.*, 2001; Jousimies-Somer *et al.*, 2002; Papaparaskevas *et al.*, 2005), resistance rates to some antibacterial agents (such as ampicillin/sulbactam and clindamycin) have shown a tendency to increase.

The aim of this study was to evaluate 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 4 years and to assess the influence of the start of empirical treatment on the isolation rates of the anaerobes.

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METHODS

Patients. In total, 118 pus specimens from 118 consecutive patients with abscesses (31 cases) and cellulitis (87) of the head and neck were evaluated from 2002 to the end of 2005. The patients were admitted to the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria, and comprised 76 men and 42 women: 4 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 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 1–3 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 h of collection. The specimens were inoculated onto Brucella agar (Becton Dickinson) enriched with haemin, vitamin K (Becton Dickinson) and 5 % sheep blood (Jousimies-Somer *et al.*, 2002). Part of each specimen was placed in Komkova anaerobic broth [National Centre of Infectious and Parasitic Diseases (NCIPD)], which was boiled for 5–10 min and cooled prior to use. Komkova broth is a cooked-meat medium, containing glucose, gelatin and 0·3 % agar (Tiagunenko & Marina, 1990). After inoculation, the Komkova anaerobic broth was overlaid with 1–2 ml sterile liquid paraffin and incubated at 37 °C. The broth was subcultured after 48–72 h 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 discs, catalase, spot indole and API system Rapid ID 32 A (bioMérieux) (Jousimies-Somer *et al.*, 2002). The special potency discs (Rosco and Becton Dickinson) contained oxgall, kanamycin (1000 μg), vancomycin (5 μg), colistin (10 μg) and metronidazole (5 μg).

Antibacterial susceptibility testing. The antibacterial susceptibility of 151 anaerobic strains was evaluated by using an agar dilution method with two to three consecutive concentrations (National Committee for Clinical Laboratory Standards, 2004). 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). Antimicrobial agents were obtained from Sigma (amoxicillin, metronidazole and clindamycin) and Pfizer (ampicillin/sulbactam). The bacterial inoculum corresponded to 0·5 McFarland standard and the final inoculum was about 10^5 c.f.u. per spot (National Committee for Clinical Laboratory Standards, 2004). When no growth was observed on the plate after 48 h 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 (National Committee for Clinical Laboratory Standards, 2004). Amoxicillin breakpoints have been considered to be equivalent to ampicillin breakpoints because, according to *in vitro* data, the MICs for ampicillin and amoxicillin against anaerobes have been reported to be identical (National Committee for Clinical Laboratory Standards, 2004).

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 AND DISCUSSION

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, $P>0·20$).

The predominant anaerobic bacteria were *Prevotella* (49 strains), *Fusobacterium* species (22), *Actinomyces* spp. (21), anaerobic cocci (20) and *Eubacterium* spp. (18) (Table 1). Microaerophilic streptococci were found in 28 (23·7 %) of the specimens and, in most cases (89·3 %), were associated with anaerobes. *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 University Hospital of Maxillofacial Surgery in 2002–2005, 68 % were Gram-positive cocci, 30·5 % were Gram-negative bacteria and 1·5 % were *Candida* species.

Abscesses and cellulitis of the head and neck are severe diseases. A non-treated 79-year-old man with cellulitis of the floor of the mouth died 2 h after admission to the hospital. His specimen yielded *Fusobacterium necrophorum*, *Finegoldia magna*, microaerophilic streptococci and *Bifidobacterium* and *Lactobacillus* spp.

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); however, isolates of *Porphyromonas* spp. 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 (Han & Kerschner, 2001).

The detection rate of anaerobes from patients with deep-space head and neck infections was relatively lower than that (82–100 %) observed by Jousimies-Somer *et al.* (2002), but was higher than that (21–59·3 %, according to the sources of infection) reported by Huang *et al.* (2006). Detection rates of anaerobes were similar in children (75 %, 3 of 4 cases),

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 non-treated 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)
Non-pigmented *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 non-identified 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.

adults (74·8 %, 77 of 103) and the elderly (72·7 %, 8 of 11; $P>0·20$). The rate of isolation of anaerobes from empirically treated patients was slightly lower (72·4 %, 63 of 87) than that from non-treated patients (80·6 %, 25 of 31; $P>0·20$).

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, $P<0·05$), whereas no significant difference ($P>0·10$) was observed between groups for *Prevotella* spp. 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). 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).

Clostridia are unusual isolates in head and neck infections (Jousimies-Somer *et al.*, 2002). 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 (Miller *et al.*, 2001; Peláez *et al.*, 2002; Speirs *et al.*, 1988).

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). Penicillin resistance has been found in 83 % of *Prevotella* isolates (Aldridge *et al.*, 2001), as well as in 32–35 % of those in odontogenic infections (Kuriyama *et al.*, 2001). 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), but have not been detected in other studies (Bahar *et al.*, 2005; Kuriyama *et al.*, 2001). In the present work, one of three *Porphyromonas* strains was amoxicillin resistant.

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; $P > 0{\cdot}10$) (Table 2). Low rates of non-susceptibility 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 (National Committee for Clinical Laboratory Standards, 2004), 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 (Dubreuil *et al.*, 1999). Therefore, although in the present study three Gram-positive anaerobic strains exhibited amoxicillin MICs of >1 μg ml^{-1}, they should not be considered as amoxicillin-resistant strains.

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). However,

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

% I, Percentage intermediately susceptible; % R, percentage resistant.

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

clindamycin resistance in *Prevotella* strains (4·5 %, 2 of 44 strains) was lower than that (22·2 %) observed by Wexler *et al.* (2002). 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), although recent studies have reported a decreased activity of these agents against 5–8 % of *Bacteroides fragilis* group strains and some *Peptostreptococcus anaerobius* isolates (Aldridge *et al.*, 2001; Kato *et al.*, 2000; Koeth *et al.*, 2004). Intermediate susceptibility to amoxicillin/clavulanate has been detected in single *Prevotella* strains by Wexler *et al.* (2002). 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 2002.

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