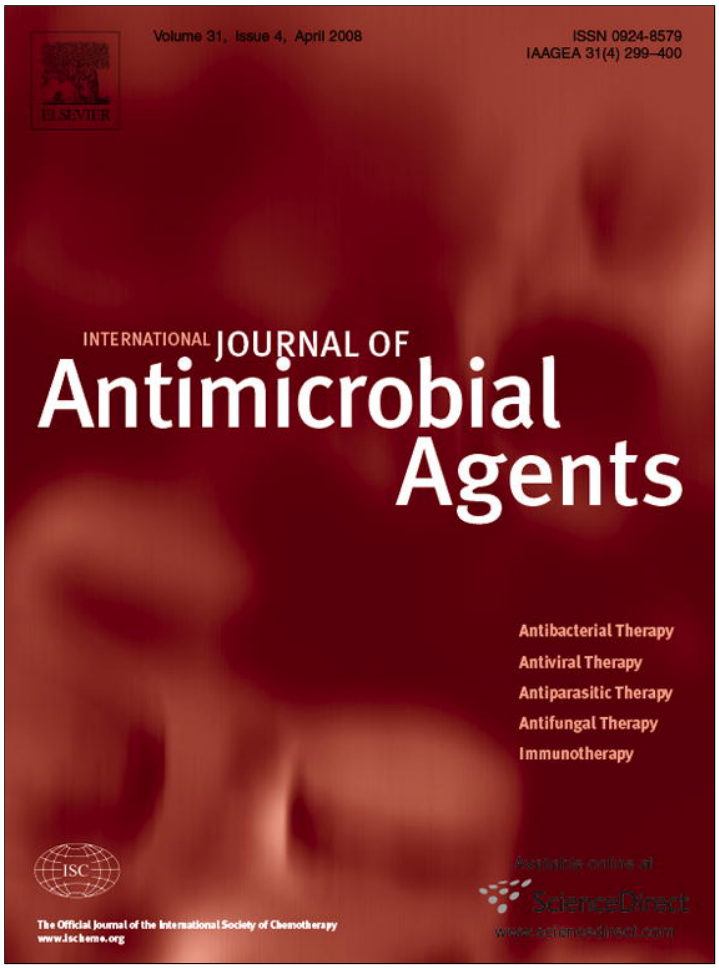

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European surveillance study on antimicrobial susceptibility of Gram-positive anaerobic cocci

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Abstract

Gram-positive anaerobic cocci (GPAC) are a heterogeneous group of microorganisms frequently isolated from local and systemic infections. In this study, the antimicrobial susceptibilities of clinical strains isolated in 10 European countries were investigated. After identification of 299 GPAC to species level, the minimum inhibitory concentrations of penicillin, imipenem, clindamycin, metronidazole, vancomycin and linezolid were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute. The majority of isolates were identified as *Finegoldia magna* and *Parvimonas micra* (formerly *Peptostreptococcus micros*), isolated from skin and soft tissue infections. All isolates were susceptible to imipenem, metronidazole, vancomycin and linezolid. Twenty-one isolates (7%) were resistant to penicillin ($n = 13$) and/or to clindamycin ($n = 12$). Four isolates were resistant to both agents. The majority of resistant isolates were identified as *F. magna* and originated from blood, abscesses and soft tissue infections.

Keywords: Antimicrobial resistance; Clinical isolates; Anaerobic cocci

1. Introduction

Gram-positive anaerobic cocci (GPAC) are a heterogeneous group of microorganisms widely distributed as members of the normal human microflora. They are isolated from the oropharynx, respiratory tract, skin, gut and urogenital tract [1]. GPAC are also opportunistic pathogens and are frequently isolated both from local and systemic infections [2,3]. The strict anaerobic Gram-positive cocci were previously included in the genera *Peptococcus* and *Peptostreptococcus. Peptococcus* strains are rarely isolated from human clinical specimens [1]. Taxonomical revisions are ongoing and proposals have been made that the genus *Peptostreptococcus* should consist of the species *Peptostreptococcus anaerobius* and *Peptostreptococcus stomatis* [4,5]. *Peptostreptococcus magnus* and *Peptostreptococcus micros* have been proposed to be reclassified as *Finegoldia magna* and *Micromonas micros* [6]. However, the *Micromonas* are microalgae and *P. micros* was again considered as the valid name [1]. Recently, *Parvimonas micra* was proposed as an alternative [7]. The remaining species have been suggested to be included in the genera *Anaerococcus, Peptoniphilus* and *Gallicola* [8].

GPAC are involved in approximately one-quarter of all anaerobic isolates from human clinical infections [2]. In most cases infections are polymicrobial, although *F. magna* strains are often isolated in pure cultures [2]. The prevalence of GPAC as pathogens is increasing and information regarding

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their antimicrobial susceptibilities is relatively limited compared with that of other anaerobic species. The aim of this study was therefore to examine the current status of antimicrobial resistance in clinical isolates of GPAC in Europe.

2. Materials and methods

2.1. Bacterial isolates

Collaborators in each participating country (Austria, Croatia, Czech Republic, Denmark, Finland, France, Great Britain, Greece, Hungary and Sweden) collected consecutive clinical isolates of GPAC and sent them to the Division of Clinical Microbiology, Karolinska Institutet (Karolinska University Hospital Huddinge, Stockholm, Sweden). Non-duplicate isolates of each species were collected from each patient and the day of collection and sampling site were recorded. The isolates were identified by Gram staining, biochemical tests (Rapid ID 32A anaerobe identification kit; bioMérieux SA, Marcy l'Etoile, France) and gas–liquid chromatography of volatile metabolites from peptone–yeast–glucose broth. The code from the Rapid ID test and the terminal volatile fatty acid profile of each isolate were used for identification according to the Anaerobe Reference Laboratory, National Public Health Service Wales, Microbiology Cardiff, University Hospital of Wales, Cardiff, UK.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of benzyl penicillin (AstraZeneca, Södertälje, Sweden), imipenem (Merck, Sharp & Dohme, Sollentuna, Sweden), clindamycin (Sigma–Aldrich, Stockholm, Sweden), metronidazole (Rhône-Poulenc Rorer, Alfortville, France), vancomycin (Abbott Scandinavia AB, Solna, Sweden) and linezolid (Pharmacia & Upjohn, Milan, Italy) were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) [9]. Testing was performed using Brucella agar plates supplemented with 5 μg of hemin and 1 μg of vitamin K per millilitre plus 5% laked sheep blood. The plates were incubated for 48 h at 37 °C in anaerobic jars (GasPak® Anaerobic System; BBL, Cockeysville, MD). The reference strains were *Bacteroides thetaiotaomicron* ATCC 29741 and *Eubacterium lentum* ATCC 43055. The susceptibility breakpoints used were those recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.srga.org/eucastwt/mictab/index.html) when available (imipenem MIC > 2 mg/L; vancomycin MIC > 4 mg/L; and linezolid MIC > 2 mg/L); otherwise those from the CLSI were used (benzyl penicillin MIC > 0.5 mg/L; clindamycin MIC > 2 mg/L; and metronidazole MIC > 8 mg/L).

2.3. Determination of β-lactamase production

Production of β-lactamase was determined by inoculating 1 μL of bacteria with 100 μL of 1.5 mM nitrocefin at room temperature. The mixture was checked for changes in colour after 15 min, 30 min and 24 h.

3. Results

3.1. Bacterial isolates

Among the isolates sent to Karolinska Institutet, 299 were identified as GPAC and 23 isolates as other species (mainly *Streptococcus* and *Veillonella*). Forty-nine isolates did not grow on blood agar or in chopped-meat broth. Most isolates were collected in Sweden (22%), Finland (19%) and Great Britain (18%) and the majority of isolates were from skin and soft tissue infections (Table 1). The majority of isolates were identified as *F. magna* (111; 37.1%), *P. micra* (53; 17.7%), *Peptoniphilus harei* (44; 14.7%), *Anaerococcus vaginalis* (21; 7.0%) and *P. anaerobius* (20; 6.7%). Nineteen isolates (6.4%) could not be identified to species level and were described as *Peptostreptococcus* sp. The remaining isolates were identified as *Peptoniphilus asaccharolyticus* ($n = 8$), *Peptoniphilus ivorii* ($n = 5$), *Peptoniphilus indolicus* ($n = 8$), *Peptoniphilus lacrimalis* ($n = 5$), *Anaerococcus octavius* ($n = 1$), *Anaerococcus prevotii* ($n = 2$), *Anaerococcus tetradius* ($n = 1$) and *Anaerococcus lactolyticus* ($n = 1$).

3.2. Antibiotic susceptibility testing

The in vitro susceptibilities to the antimicrobial agents of the GPAC clinical isolates are shown in Table 2. All isolates were susceptible to imipenem, metronidazole, vancomycin and linezolid. A total of 21 isolates (7.0%) were resistant to penicillin ($n = 13$) and/or clindamycin ($n = 12$). Four isolates were resistant to both agents. The majority of resistant strains were collected in Great Britain ($n = 11$), whilst only single resistant isolates originated from Austria ($n = 2$), Croatia ($n = 1$), Czech Republic ($n = 2$), Finland ($n = 3$), France ($n = 1$) and Sweden ($n = 1$). Eight of the isolates were identified as *F. magna*, of which four were resistant to clindamycin, three to both penicillin and clindamycin and one isolate to penicillin only. The remaining resistant isolates were identified as *P. anaerobius* ($n = 3$; all resistant to penicillin), *P. indolicus* ($n = 3$; one isolate resistant to penicillin, one to clindamycin and one to both agents), *P. micros* ($n = 2$; resistant to either penicillin or clindamycin) and five resistant isolates belonged to five different species with variable resistance. The principal origins of resistant isolates were blood (patients with septicaemia), abscesses and soft tissue infections (Table 3).

Table 1
Distribution and origin of isolates of anaerobic Gram-positive cocci

Origin of isolates (*n*)	Country (no. of isolates)									
	AUT	CRO	CZE	DNK	FIN	FRA	GBR	GRC	HUN	SWE
Septicaemia (31)			1	4	1		23		2	
Bone (12)	1		1		1		1		4	4
Arthritis (3)									1	2
Head and neck (15)	3				3	7	1			1
Meningitis (2)							2			
Brain abscess (1)			1							
Dental infection (16)			1			11			4	
Eye (1)	1									
Ear (6)	1				2					3
Lung (1)							1			
Pleura (2)	1						1			
Abdominal abscess (18)	2		3		1			1	7	4
Peritoneal fluid (6)			3		1				2	
Soft tissue (49)	11		5		16			6		11
Skin (43)	3		1		11		2			26
Diabetic ulcer (10)					4			3		3
Other abscess (33)	1		1		9		14	2	1	5
Other (42)	9	9	2		8		9		1	4
Not specified (8)						6				2
Total (299)	33	9	19	4	57	24	54	12	22	65

AUT, Austria; CRO, Croatia; CZE, Czech Republic; DNK, Denmark; FIN, Finland; FRA, France; GBR, Great Britain; GRC, Greece; HUN, Hungary; SWE, Sweden.

Table 2
In vitro activity of antimicrobial agents against isolates of Gram-positive anaerobic cocci ($n = 299$)

Antimicrobial agent	MIC (mg/L)			*S* (%)	*R* (%)
	MIC_{50}	MIC_{90}	Range		
Penicillin[a]	0.016	0.125	<0.008–4.0	96	4
Imipenem[b]	0.032	0.064	<0.016–1.0	100	–
Clindamycin[a]	0.064	0.5	<0.008 to ≥256	96	4
Metronidazole[a]	0.5	4.0	<0.064–8.0	100	–
Vancomycin[b]	0.125	0.5	0.016–1.0	100	–
Linezolid[b]	0.5	2.0	<0.25–2.0	100	–

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC for 50% and 90% of the organisms, respectively; *S*, susceptible, *R*, resistant and intermediate-susceptible.

[a] Breakpoints recommended by the Clinical and Laboratory Standards Institute.

[b] Breakpoints recommended by the European Committee on Antibiotic Susceptibility Testing.

3.3. β-Lactamase production

No isolates produced β-lactamases.

4. Discussion

In this study, clinical isolates of GPAC resistant to penicillin and/or clindamycin were found. The overall resistance rate was 7%. There were variations in the proportion of resistant isolates between the European countries included in the study, ranging from 20% in isolates from Great Britain to 0% in Denmark, Greece and Hungary. There was large variation in the numbers of isolates collected in the participating countries and therefore no analyses were performed on differences of resistance rates in separate regions.

Table 3
Resistant isolates of Gram-positive anaerobic cocci from 10 European countries and their clinical origin

Country (no. of isolates)	No. (%) of resistant isolates	Source of isolation (no. of resistant isolates)
AUT (33)	2 (6%)	Bone (1), soft tissue (1)
CRO (9)	1 (11%)	Unknown (1)
CZE (19)	2 (11%)	Blood (1), soft tissue (1)
DNK (4)	–	–
FIN (57)	3 (5%)	Abscess (3)
FRA (24)	1 (4%)	Dental infection (1)
GBR (54)	11 (20%)	Blood (5), lung (1), skin (1), abscess (1), unknown (3)
GRC (12)	–	–
HUN (22)	–	–
SWE (65)	1 (2%)	Abdominal abscess (1)

AUT, Austria; CRO, Croatia; CZE, Czech Republic; DNK, Denmark; FIN, Finland; FRA, France; GBR, Great Britain; GRC, Greece; HUN, Hungary; SWE, Sweden.

Similar resistance rates of anaerobic cocci have been reported previously [10,11], although a higher prevalence of resistance in particular to clindamycin has been observed in some studies [12–14]. However, in contrast to the results of some earlier investigations, no isolates resistant to metronidazole were found [10,15,16]. In more recent studies, no resistance to metronidazole has been detected in GPAC [11,17]. It is also worth noting that differences in resistance rates between different species of GPAC, such as *P. anaerobius* and *P. stomatis,* have been reported [18].

In the present study, none of the isolates produced β-lactamase; penicillin resistance in Gram-positive cocci appears to be due to modifications in the penicillin-binding proteins [19]. Resistance to clindamycin has been showed to be caused by an RNA methylase that modifies the site of action of the drug [20].

In a recent study by Wildeboer-Veloo et al. [21] using 16S rRNA-based probes for identification, it was reported that the most common GPAC from human infections are *F. magna, P. micra* and *P. harei*, which was also demonstrated in the present study. However, the investigators failed to detect *P. asaccharolyticus*, which has been regarded as a common species in clinical specimens harbouring GPAC. Previous studies using phenotypic methods may have misidentified *P. harei* as *P. asaccharolyticus*. In the present study, eight isolates were identified as *P. asaccharolyticus*.

Although antibiotic resistance within anaerobic bacterial populations is rising, the clinical implications are not obvious since most infections involving anaerobes are polymicrobial and also contain aerobic and facultative anaerobic microorganisms [22]. The importance of antibiotic resistance in anaerobic microorganisms has further been confounded by the empirical use of broad-spectrum antimicrobial agents and as mixed infections often respond to debridement and drainage [23]. Reduced susceptibilities to penicillin and in particular to clindamycin as well as frequent reports of resistance to metronidazole render the treatment options for infections involving GPAC with empirical antimicrobial therapy hazardous. In conclusion, susceptibility testing of anaerobic isolates in patients with severe infections as well as continuous surveillance of antimicrobial susceptibility in GPAC seem highly justified.

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