Antibiotic susceptibilities of Gram-positive anaerobic cocci: results of a sentinel study in England and Wales

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Objective: A sentinel study was carried out to determine the antimicrobial susceptibilities of Gram-positive anaerobic cocci (GPAC) freshly isolated from clinical material in diagnostic laboratories in England and Wales.

Methods: A total of 113 GPAC isolates consisting predominantly of current or former members of the genus Peptostreptococcus was obtained from 17 sentinel laboratories in England and one in Wales. Minimum inhibitory concentrations (MICs) of 10 antimicrobial agents were determined by the Etest method. The agents tested were: penicillin, tetracycline, erythromycin, cefoxitin, clindamycin, chloramphenicol, imipenem, co-amoxiclav, piperacillin/tazobactam and metronidazole. MIC50 and MIC90 values for each drug-species combination were calculated whenever suitable numbers of each species were obtained.

Results: Excellent spectra of activity (0% resistance) against GPAC were seen for metronidazole, piperacillin/tazobactam, cefoxitin, imipenem and chloramphenicol. Low degrees of resistance to co-amoxiclav (3.5%), clindamycin (7.1%), penicillin (7.1%) and significant degrees of resistance to tetracycline (41.6%) and erythromycin (27.4%) were detected. Some examples of putative macrolide-lincosamide linked resistance were noted in seven (6.2%) isolates of GPAC.

Conclusion: This study is one of the largest susceptibility studies specifically on GPAC carried out to date and the resulting data may be of value to those involved in the empirical treatment of infections involving Gram-positive anaerobic cocci.

Keywords: Peptostreptococcus spp., Etests, anaerobes, MICs

Introduction

Gram-positive anaerobic cocci (GPAC) are a heterogeneous group of organisms that form part of the normal endogenous flora of man. They are often present in deep-seated anaerobic infections of soft tissues, decubitus ulcers, infections of bones and joints, and in cases of obstetric and gynaecological sepsis.1 As a group, GPAC have been largely understudied, and are often reported in clinical specimens as anaerobic streptococci (sic). Until recently, most GPAC from human clinical material were included in the genus Peptostreptococcus but this genus has been reclassified into three new genera, namely: Micro- monas (Micromonas micros formerly Peptostreptococcus micros), Anaerococcus (Anaerococcus prevotii formerly P. prevotii) and Peptoniphilus (Peptoniphilus asaccharolyticus formerly P. asaccharolyticus).2 The only remaining member of the genus Peptostreptococcus is the type strain P. anaerobius. Similarly, P. magnus has been reclassified as Finegoldia magna and this GPAC has emerged as one of the most commonly isolated anaerobic pathogens, particularly in orthopaedic and joint infections, cases of septic arthritis and prosthetic implant infections.1 As appreciation of the significance of GPAC such as F. magna grows, it becomes more important to have knowledge about their susceptibilities to antimicrobial agents that may be employed in treatment of GPAC infections. Data on the antimicrobial susceptibilities of GPAC in the literature is often included in general anaerobic susceptibility studies and have come predominantly from the USA.3-6 This study sought to gain susceptibility data on GPAC isolated from fresh clinical material from the UK.

Materials and methods

Source laboratories

Eighteen sentinel clinical diagnostic microbiology laboratories were recruited for the study. They consisted of 17 former Public Health Labo-
Gram-positive anaerobic cocci susceptibilities

ratories (PHL) or PHL collaborating laboratories in England and one in Wales. The participating laboratories were in: Cambridge, Carlisle, Coventry, Gloucester, Hereford, Ipswich, Leicesters, Lincoln, Manchester, Nottingham, Peterborough, Plymouth, Preston, RhyL, Salisbury and Southampton. The former PHL collaborating laboratories were St George’s Hospital and University College Hospital, London.

Selection of GPAC isolates

Participants were asked to collect up to 10 Gram-positive anaerobic cocci (GPAC) isolated from clinical material over a 1 month period during February 2002, irrespective of their potential clinical significance. Selection criteria for GPAC was based on Gram stain reaction, cellular morphology and the inability to grow in air or 5% carbon dioxide in air at 37°C. Participants were requested not to base selection on the presence of a zone of susceptibility to a metronidazole 5 μg disc (which is commonly placed directly on primary isolation plates as an indicator of the presence of anaerobes) as this would prejudice against bona fide metronidazole-resistant GPAC. Isolates of putative GPAC were sent to the Anaerobe Reference Laboratory (ARL) in Cardiff for identification and determination of minimum inhibitory concentrations (MICs) of 10 antimicrobial agents.

Identification of GPAC

Isolates were identified according to the criteria of Holdeman et al.1, including analysis of volatile metabolic end-products by gas–liquid chromatography supplemented with the criteria of Murdoch1 using the API ATB 32A Rapid ID anaerobe identification kit (bioMérieux Laboratories, Marcy l’Étoile, France). In total, 113 isolates were confirmed as GPAC and included in the study.

Isolates were identified as: F. magna, n = 43; P. anaerobius, n = 25; A. vaginalis, n = 11; M. micros, n = 5; P. harei, n = 4; P. asaccharolyticus, n = 3; P. ivorii, n = 3; A. prevotii, n = 2; P. lacrimalis, n = 1; Slackia heliotrinireducens (formerly P. heliotrinireducens), n = 1; and 15 unidentified butyrate-producing species of GPAC. MIC determination of penicillin, tetracycline, erythromycin, cefoxitin, clindamycin, chloramphenicol, imipenem, co-amoxiclav, piperacillin/tazobactam and metronidazole was by the Etest method (AB Biodisk, Solna, Sweden) which is the preferred method used in the ARL. Organisms for testing, usually in A. vaginalis species, were determined as an MIC above the breakpoint for each drug as listed in Table 1 below. The overall activity of erythromycin in GPAC was quite poor with 37.2% resistance, activity against P. anaerobius individually was better with only 1/25 (4%) resistant. Examples of macrolide-lincosamide linked resistance in the study of Reig et al.14 and Sanchez et al.3 who noted >10% resistance of P. magna to clindamycin, were higher than the 7.1% overall GPAC resistance and 7% resistance of F. magna found in this study in the UK. However, Wren12 recorded 9% of P. magna (F. magna) resistant to clindamycin in London, a level similar to our results suggesting that clindamycin resistance is lower in the UK than in France or the USA. However, it should be considered that methodological differences may have influenced these results.

Amongst the individual species or groups of GPAC, there were significant levels of resistance to certain antibiotics. For example, 15/25 (60%) of P. anaerobius were resistant to tetracycline, as were 16/43 (37.2%) of F. magna and 3/5 (60%) of M. micros. Thirteen of 43 (30.2%) F. magna were also resistant to erythromycin. Although the overall activity of erythromycin in GPAC was quite poor with 27.4% resistance, activity against P. anaerobius individually was better than all other species or groups of putative macrolide-lincosamide linked resistance of >256 mg/L to both erythromycin and clindamycin. These were A. prevotii (2), F. magna (2), butyrate-producing GPAC (2) and one strain of P. harei. There were 12 examples (10.6%) representing dissociated resistance (clindamycin MIC < 1.0 mg/L; erythromycin MIC > 8 mg/L) compared with 5.1% examples of dissociated resistance in the study of Reig et al.14 Reig et al.15

Discussion

The highest percentage of overall resistance detected amongst GPAC was 41.6% resistance to tetracycline, followed by 27.4% resistance to erythromycin. The resistance of Putative GPAC resistance to erythromycin, and 3.5% GPAC resistance to co-amoxiclav. There was no resistance amongst GPAC to piperacillin/tazobactam, chloramphenicol, cefoxitin, imipenem or metronidazole.

This sentinel antimicrobial susceptibility study on 113 fresh clinical isolates is one of the largest carried out specifically on GPAC. Previous susceptibility data on GPAC were recorded mainly from susceptibility studies on a range of anaerobes and most of these were carried out outside the UK as summarized in the review article by Murdoch.1 This study is also one of the largest in terms of the number of antimicrobial agents tested against GPAC, equalling Bowker et al.’s8 study of nine agents plus trovafloxacin against 75 GPAC isolates. Other studies include that of Panchini et al.,10 who tested seven agents against 32 GPAC isolates and the report of Greenwood & Palfreyman11 on the susceptibility of 50 GPAC isolates to penicillin and vancomycin in 1987.
Table 1. MIC values (mg/L) for GPAC species or groups against 10 antimicrobial agents

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<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
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reported that an incidence of 80% macrolide resistance in 21 Peptostreptococcus strains was due to the erm(TR) gene and suggested these anaerobic members of the normal oropharyngeal flora may be an important reservoir of this gene for transfer to macrolide resistant Streptococcus pyogenes. Chopra & Roberts 16 also highlighted the potential mobility of the genetic determinants associated with resistance to tetracycline and this study suggests that GPAC may act as reservoirs of tetracycline resistance for transfer to other endogenous bacteria. Although the numbers are small, there is a suggestion that some GPAC species are commonly resistant to certain agents as both A. prevotii isolates were resistant to erythromycin, clindamycin and tetracycline; similarly 75% (three of four) of P. harei isolates were resistant to tetracycline. This compares with no resistance to any agents in P. lacrimalis, S. heliotrinireducens and P. ivorii. Further investigations are warranted on greater numbers of these species.

Acknowledgements

We gratefully acknowledge the contribution of fresh clinical isolates of GPAC from the sentinel laboratories without whom this study could not have been undertaken, and the technical assistance of Carol Davis.

References