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Antimicrobial susceptibility of anaerobic bacteria

Evaluation of the routine antimicrobial susceptibility testing results of clinically significant anaerobic bacteria in a Slovenian tertiary-care hospital in 2015

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ABSTRACT

The aim of our study was to determined antimicrobial susceptibility profiles of 2673 clinically significant anaerobic bacteria belonging to the major genera, isolated in 2015 in a large tertiary-care hospital in Slovenia. The species identification was performed by MALDI-TOF mass spectrometry. Antimicrobial susceptibility was determined immediately at the isolation of the strains against: penicillin, co-amoxiclav, imipenem, clindamycin and metronidazole, using gradient diffusion methodology and EUCAST breakpoints. The most frequent anaerobes were *Bacteroides fragilis* group with 31% (n = 817), Gram positive anaerobic cocci (GPACs) with 22% (n = 589), *Prevotella* with 14% (n = 313) and *Propionibacterium* with 8% (n = 225). Metronidazole has retained full activity (100%) against all groups of anaerobic bacteria intrinsically susceptible to it. Co-amoxiclav and imipenem were active against most tested anaerobes with zero or low resistance rates. However, observed resistance to co-amoxiclav (8%) and imipenem (1%) is worrying especially among *B. fragilis* group isolates. High overall resistance (23%) to clindamycin was detected in our study and was highest among the genera *Prevotella, Bacteroides, Parabacteroides*, GPACs and *Clostridium*. Routine testing of antimicrobial susceptibility of clinically relevant anaerobic bacteria is feasible and provides good surveillance data.

1. Introduction

Anaerobic bacteria are common human pathogens and can cause serious and life threatening infections. However, infections caused by anaerobic bacteria may easily be overlooked, mainly because of the special requirements needed for their isolation, with the emphasis on appropriate collection and transportation of specimens and anaerobic techniques employed by the laboratory. The use of specialized culture media, anaerobic atmosphere generation and prolonged time of cultivation are the most critical elements for successful isolation of anaerobes in the laboratory [1]. Furthermore, treatment of anaerobic infections may be complicated because many antimicrobial agents have poor activity against anaerobic bacteria, but also because of the underlying condition of localized or generalized tissue anaerobiosis, prerequisite for the infection, that needs to be reversed for successful therapy [2,3]. Additionally, majority of clinically significant anaerobic isolates are involved in mixed bacterial infections together with aerobic bacteria being an important synergistic element in the pathogenesis [4]. Finally, there is growing resistance of anaerobic bacteria to antimicrobial agents with intrinsic activity against anaerobes [3].

The antimicrobial susceptibility testing of anaerobic bacteria is rarely performed in most clinical microbiology laboratories and there are several reasons for that [3]. Besides laboratory considerations which include special incubation conditions, isolation and identification procedures, there is in fact relatively few data that supports clinical correlation between the *in vitro* susceptibility results and patient outcome [5,6]. However, a pivotal study from Nguyen et al. showed that such correlation exists for patients with *Bacteroides* bacteremia and that patients receiving active antimicrobial therapy have higher microbiological and clinical cure and lower mortality than patient receiving inactive therapy [7]. Therefore, majority of recommendations today advocate for limited antimicrobial susceptibility testing of anaerobic bacteria, only in special clinical circumstances and for epidemiological and

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surveillance reasons [3,5]. However, the antimicrobial resistance of some anaerobic bacteria has increased in the past decades and resistance phenotypes have become increasingly less predictable [8]. Additionally, new developments in the field of anaerobic bacteriology, especially the introduction of a very reliable and rapid identification techniques, the MALDI-TOF mass spectrometry (MS) [9], efforts to standardize readily available and inexpensive disc diffusion method for antimicrobial susceptibility testing of some anaerobic species, especially the rapidly growing *B. fragilis* group [10] and, surprisingly, efforts to overcome the need for incubation in anaerobic atmosphere for isolation of anaerobic bacteria with the development of quasi-universal culture media [11,12] may narrow the gap between the two bacteriological worlds and increase the possibilities for routine antimicrobial susceptibility testing of anaerobes in the future.

Limited data about the antimicrobial susceptibility of anaerobic bacteria is available worldwide. The objective of our study was to collect antimicrobial susceptibility data during the routine work and use these to determine current status of antimicrobial susceptibility of clinically significant anaerobic bacteria in Slovenia in 2015 and to compare them with the available data from other sources.

2. Materials and methods

2.1. Bacterial isolates

The study was performed at the Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, which is the largest microbiology laboratory in Slovenia and receives samples from two major tertiary-care hospitals, the University Medical Centre Ljubljana and the Institute of Oncology Ljubljana with approximately 2400 hospital beds in total. Anaerobic bacterial strains isolated from all types of human clinical specimens in 2015 were included. Repeated isolates from the same patients were excluded, irrespective of body site and susceptibility profile [13].

2.2. Isolation and identification of anaerobic bacteria

All clinical samples with exception of blood were inoculated onto Schaedler agar (SCS) and Schaedler agar supplemented with neomycin and vancomycin (SNVS) (bioMèrieux, Mercy I'Etoile, France) using the four quadrant streaking technique and thioglycollate broth (Becton Dickinson, Sparks, USA). The inoculated plates were incubated in an anaerobic atmosphere (80% N_2, 10% CO_2, 10% N_2) generated with the Anoxomat system (Mart Microbiology BV, Lichtenvoorde, Netherlands) at 37 °C for a minimum of 48 h. Incubation time was extended to 14 days in case of specimens from implant associated infections. Blood samples were inoculated to BacT/Alert FN Plus or BACTEC-Lytic blood culture bottles and incubated for a maximum of 5 days in the BacT/Alert 3D (bioMèrieux, Mercy I'Etoile, France) and BACTEC 9000 (Becton Dickinson, Sparks, USA) blood culture systems, respectively.

Identification was performed using MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) following recommendations from the manufacturer. Briefly, pure cultures were spotted on a stainless-steel target using wooden tooth pick in duplicates. One spot per strain was covered with 1 μL of 70% formic acid and upon drying, both spots were covered with 1 μL of HCCA matrix (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid solution), left to dry and analyzed with the Biotyper software version 3.1. Log (score)s ≥ 2.0 and ≥ 1.7 were interpreted as high confidence and low confidence correct identification [14].

2.3. Antimicrobial susceptibility testing

The susceptibility against five antimicrobial agents was determined with gradient diffusion method using Etests (bioMerieux, Marcy l'Etoile, France) for penicillin, imipenem, and metronidazole and MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) for co-amoxiclav and clindamycin. The method was based on CLSI standard procedure [15]. Briefly, all tested anaerobic strains were initially sub-cultured from the primary agar plates to Brucella agar supplemented with 5% laked sheep blood, hemin and 10 μg/mL vitamin K_1 (Becton Dickinson, Sparks, USA). Subsequently, bacterial suspension of 1 McFarland standard was prepared in saline solution and spread uniformly to agar plate. Antimicrobial test strips were placed on the surface and the plates were incubated in an anaerobic atmosphere at 37 °C for 24–48 h. *B. fragilis* ATCC 25286 was used for quality control on weekly basis. Antimicrobial susceptibility results were interpreted as susceptible, intermediate or resistant according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints [16]. The susceptibility results were initially imported to laboratory information system on the day the test was evaluated and exported from there for the purpose of this study. The MIC range, MIC_{50} and MIC_{90}, percentage of resistant isolates and the number of isolates were calculated.

3. Results

A total of 2673 non-repetitive clinically relevant anaerobic strains were included in this study isolated in 2015 from 1350 patients, 58% (n = 782) males, average age was 60 years (0–101 years). Majority of them were from skin and soft tissue infections, 57% (n = 1568), abdominal infections, 21% (n = 529) and head and neck infections, 5% (n = 131). Isolates from positive blood cultures represented 4% (n = 113) (Table 1).

Anaerobic Gram negative bacilli represented 54% (n = 1448) of all isolates, with genera *Bacteroides*, *Prevotella* and *Fusobacterium* being most common among them with 31% (n = 817), 14% (n = 313) and 7% (n = 178), respectively. Gram positive anaerobic cocci (GPACs) were the second most common isolates as a group with 22% (n = 589) and *Finegoldia*, *Peptoniphilus* and *Anaerococcus* being most frequently isolated genera among them with 8% (n = 204), 6% (n = 165) and 5% (n = 124), respectively. Finally, Gram positive anaerobic bacilli represented 21% (n = 553) of isolates with *Propionibacterium* 8% (n = 225), *Clostridium* 7% (n = 176) and *Actinomyces* 4% (n = 103). The distribution of isolated anaerobic genera is shown in Table 2.

The cumulative susceptibility results were calculated only for anaerobic species with 30 or more non-repetitive isolates and are shown in Table 3. Briefly, within *B. fragilis* group the most frequently isolated species were *B. fragilis*, *B. thetaiotaomicron*,

Table 1
Origin of anaerobic isolates involved in the antibiotic susceptibility evaluation.

Type of infection	n	%
Skin and soft tissues	1568	57
Abdominal	529	21
Head and neck	131	5
Blood culture	113	4
Urogenital	103	4
Bone and joint	84	3
Implant associated	48	2
Ocular	25	1
Thoracic	17	1
Other	55	2
Total	**2673**	**100**