ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-NEGATIVE ANAEROBIC BACTERIA FROM 2 HOSPITALS IN SMOLENSK, RUSSIA

Dmitry GALKIN, Olga KRETCHIKOVA, Marina SUKHORUKOVA, Roman KOZLOV
Institute of Antimicrobial Chemotherapy (IAC) of the Smolensk State Medical Academy, Smolensk, Russia

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REVISED ABSTRACT

Objective: To analyze resistance patterns of gram-negative anaerobic bacteria isolated during the first half of the year 2005 in several hospitals in a Russian city.

Methods: Clinical anaerobic isolates from 2 hospitals in Smolensk (Central Russia) were tested using agar dilution method in accordance with NCCLS/CLSI M11-A6 guidelines.

Results: Overall 69 gram-negative anaerobic bacteria from 41 patients were studied. Isolation sites were represented by intra-abdominal - 25 (60.9%), soft tissue - 7 (17.1%), prostate fluid - 5 (12.2%), bone - 3 (7.4%), and dental - 1 (2.4%) infections. Susceptibility of 31 (44.9%) *Prevotella* spp., 23 (33.3%) *Bacteroides* spp. (predominantly *Bacteroides fragilis* group - 18 strains), 7 (10.2%) *Fusobacterium* spp., 4 (5.8%) *Porphyromonas* spp. and 4 (5.8%) *Veilonella* spp. to ampicillin, clindamycin, metronidazole, imipenem, ertapenem, amoxicillin/clavulanic acid and cefoperazone/sulbactam was determined. All species were susceptible to carbapenems. In *Prevotella* spp. there were 64% and 3% strains resistant to ampicillin and clindamycin and 4% strains with intermediate resistance to metronidazole. Among *Bacteroides* spp. 92% of strains were resistant to ampicillin and 22% to clindamycin. No resistance to metronidazole was detected in *Bacteroides* spp. MIC50/90 of 0.5/2.0 and 4.0/8.0 was achieved for amoxicillin/clavulanic acid and cefoperazone/sulbactam respectively, in this group. All *Fusobacterium* spp. were susceptible to clindamycin, 2/7 strains were resistant to ampicillin and 1/7 strain demonstrated intermediate susceptibility to metronidazole. Amoxicillin/clavulanic acid and cefoperazone/sulbactam showed $MIC_{50/90}$ of 0.125/2.0 and 1.0/4.0, respectively. Among *Porphyromonas* spp. strains, 1/4 strain was resistant to ampicillin, clindamycin and metronidazole. One *Veillonella* spp. strain was resistant to ampicillin, with no resistance to metronidazole and clindamycin. $MIC_{50/90}$ of amoxicillin/clavulanic acid and cefoperazone/sulbactam were, respectively 0.125/16.0 and 1.0/32.0 for *Porphyromonas* spp., 0.125/0.5 and 1.0/1.0 to *Prevotella* spp., 0.06/0.5 and 1.0/4.0 to *Veillonella* spp.

Conclusion: Metronidazole, carbapenems, and inhibitor-protected beta-lactams are preferred for the therapy of anaerobic infections.

INTRODUCTION

Routine antimicrobial susceptibility testing (AST) of anaerobic bacteria is not justified for all clinical microbiological laboratories. Mainly this is explained by a high cost of microbiological work up of anaerobes. On the other hand AST of anaerobes is not always crucial since adequate antimicrobial therapy in many clinical situations maybe administered empirically.

However recent studies reveal a tendency towards growing resistance of anaerobic bacteria which is linked to clinical failures of empirical antimicrobial therapy without adequate anaerobic coverage [1 - 3]. Thus there is an obvious need for conduction of periodic local and multicenter surveillance studies of resistance patterns of anaerobic bacteria to commonly used antimicrobials. The main objectives of these surveillance studies are:

1) to obtain information necessary for choosing an antibiotic regimen for empirical therapy

2) to evaluate and resistance profile of clinically significant anaerobes to antibiotics in order to examine possibilities of their use in anaerobic infections.

For this purpose we conducted surveillance AST of anaerobic bacteria isolated in hospitals in one city in the Central part of Russia.

STUDY OBJECTIVE

To analyze resistance patterns of gram-negative anaerobic bacteria isolated from patients with clinical infections during the first half of the year 2005 in several hospitals in Smolensk, Central Russia.

METHODS

All strains were isolated in two largest clinics Red Cross Hospital and the Smolensk Regional Hospital. Intra-abdominal fluid, biopsy material from skin and soft tissue, bone and joint infections as well as punctuate in chronic prostatitis and aspirate in dental infections served as a clinical material. All clinical specimens were transferred to and processed in the reference laboratory of the IAC, Smolensk, Russia.

- Agar supplemented with 5% sheep blood prepared on the basis of Brucella and Columbia agars (Becton Dickinson, USA) was used for isolation of anaerobes.
- Cultures were incubated in the atmosphere with NO_2 - 80%, CO_2 - 10%, H_2 - 10%; in the anaerobic chamber (Bugbox, Jouan, France) under +37^{0}C during 7 days.
- Preliminary identification was performed on the basis of morphology and Gram stain as well as with application of commercially available disks with erythromycin (60 mcg), penicillin (2 IU), rifampin (15 mcg), vancomycin (5 mcg), canamycin (1000 mcg), colisitin (10 mcg), polyanetolsulfonate (Oxoid, England). For the final identification we used commercial systems Rapid ID 32 A (bioMerieux, France).
- Isolated strains were kept on tryptic-soy broth (bioMerieux, France) with addition of 10% sterile glycerin under -70^{0}C.

AST was performed according to the recent NCCLS/CLSI M11-A6 recommendations with agar dilution method being used to determine MICs [4]. Brucella agar, Becton Dickinson, USA with addition of hemin (5 mcg/ml), vitamin K1 (1 mg/ml) (Becton Dickinson, USA) and laked sheep blood was used. Double serial dilutions of corresponding antibiotic substances were applied.

For preparation of bacterial suspension we used a pure 48-hrs culture of microorganisms in a sterile broth (Brucella broth, Becton Dickinson, USA) diluted to a 0.5 McFarland turbidity standard (Remel Diagnostics, USA).

Inoculum was incorporated onto plates with antibiotics with automatic multipoint inoculator (Multipoint Inoculator, Mast Diagnostics, Germany). Incubation under +37^{0}C during 42-48 hrs was performed in the anaerobic chamber. Quality control strains *Bacteroides fragilis* ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741, and *Eubacterium lentum* ATCC 43055 were used at each set of susceptibility testing.

RESULTS

During the study period 69 gram-negative anaerobic bacteria from 41 patients were isolated. Isolation sites are presented in the Fig. 1.

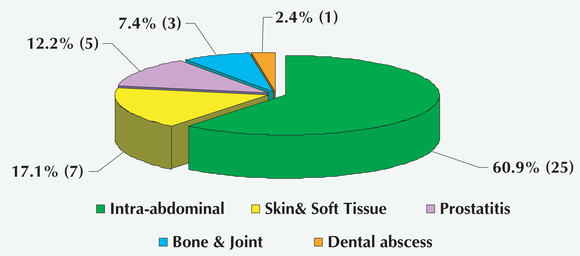

Fig. 1 Structure of the clinical material in respective infections (n=69)

From one to four anaerobic strains were isolated from one clinical specimen. Overall we tested 31 (44.9%) *Prevotella* spp., 23 (33.3%) *Bacteroides* spp. (predominantly *Bacteroides fragilis* group - 18 strains), 7 (10.2%) *Fusobacterium* spp., 4 (5.8%) *Porphyromonas* spp. and 4 (5.8%) *Veilonella* spp.

Among others recommended, we have also chosen to test susceptibility of anaerobes to amoxicillin/sulbactam which was recently registered in Russia and cefoperazone/sulbactam which is widely used as a monotherapy in mixed aerobic-anaerobic infections of various sites.

The results of *in vitro* activity of various antibiotics against isolated gram-negative anaerobes are presented in the tables 1 and 2.

Organism name	Antimicrobial agent	MIC (mcg/mL) MIC_{50}	MIC_{90}	% S	I	R
Bacteroides spp. (n=23)	Ampicillin	16	32	4.3	4.3	91.3
	Amoxicillin/ clavulanic acid	0,5	2	95.7	0	4.3
	Amoxicillin/ sulbactam	1	2	-	-	-
	Cefoperazone	32	128	39.1	26.1	34.8
	Cefoperazone/ sulbactam	4	8	-	-	-
	Imipenem	0.125	1	100	0	0
	Meropenem	0.125	0.25	100	0	0
	Ertapenem	0.25	1	100	0	0
	Clindamycin	1	128	73.9	4.3	21.7
	Lincomycin	16	128	-	-	-
	Ciprofloxacin	4	16	-	-	-
	Moxifloxacin	0,5	2	100	0	0
	Gatifloxacin	0,5	1	100	0	0
	Metronidazole	1	2	100	0	0
Prevotella spp. (n=31)	Ornidazole	1	1	-	-	-
	Chloramphenicol	4	8	100	0	0
	Ampicillin	2	16	35.5	0	64.5
	Amoxicillin/ clavulanic acid	0.125	0.5	100	0	0
	Amoxicillin/ sulbactam	0.5	1	-	-	-
	Cefoperazone	2	16	93.5	3.2	3.2
	Cefoperazone/ sulbactam	1	1	-	-	-
	Imipenem	0.03	0.125	100	0	0
	Meropenem	0.03	0.125	100	0	0
	Ertapenem	0.06	0.25	100	0	0
	Clindamycin	0.03	0.06	96.8	0	3.2
	Lincomycin	0.03	0.06	-	-	-
	Ciprofloxacin	1	8	-	-	-
	Moxifloxacin	1	2	100	0	0
	Gatifloxacin	0.25	1	100	0	0
	Metronidazole	0.5	1	96.8	3.2	0
	Ornidazole	1	1	-	-	-
	Chloramphenicol	2	4	100	0	0

Table 1. In vitro activity of various antimicrobial agents against most commonly isolated gram-negative anaerobic species (*Bacteroides* spp., *Prevotella* spp).

Table 2. In vitro activity of various antimicrobial agents against less commonly isolated gram-negative anaerobic species (*Fusobacterium* spp., *Porphyromonas* spp., *Veilonella* spp.)

Organism name	Antimicrobial agent	MIC (mcg/mL) MIC_{50}	MIC_{90}	% S	I	R
Fusobacterium spp. (n=7)	Ampicillin	1	4	42.9	28.6	28.6
	Amoxicillin/ clavulanic acid	0.125	2	100	0	0
	Amoxicillin/ sulbactam	0.06	1	-	-	-
	Cefoperazone	4	8	100	0	0
	Cefoperazone/ sulbactam	1	4	-	-	-
	Imipenem	0.125	1	100	0	0
	Meropenem	0.03	2	100	0	0
	Ertapenem	0.03	0.5	100	0	0
	Clindamycin	0.06	1	100	0	0
	Lincomycin	0,5	32	-	-	-
	Ciprofloxacin	2	4	-	-	-
	Moxifloxacin	0.125	4	100	0	0
	Gatifloxacin	0.125	1	100	0	0
	Metronidazole	0.25	16	85.7	14.3	0
	Ornidazole	0.5	16	-	-	-
	Chloramphenicol	1	1	100	0	0
Porphyromonas spp. (n=4)	Ampicillin	0.25	16	75.0	0	25.0
	Amoxicillin/ clavulanic acid	0.125	16	75.0	0	25.0
	Amoxicillin/ sulbactam	0,06	16	-	-	-
	Cefoperazone	1	256	75.0	0	25.0
	Cefoperazone/ sulbactam	1	32	-	-	-
	Imipenem	0.03	1	100	0	0
	Meropenem	0.03	0,5	100	0	0
	Ertapenem	0.03	0,5	100	0	0
	Clindamycin	0.06	128	75.0	0	25.0
	Lincomycin	0.125	128	-	-	-
	Ciprofloxacin	0.5	4	-	-	-
	Moxifloxacin	0.25	1	100	0	0
	Gatifloxacin	0.125	1	100	0	0
	Metronidazole	0.125	16	75.0	25.0	0.0
	Ornidazole	0.25	16	-	-	-
	Chloramphenicol	2	4	100	0	0
Veillonella spp. (n=4)	Ampicillin	0.25	1	75.0	25.0	0
	Amoxicillin/ clavulanic acid	0.06	0.5	100	0	0
	Amoxicillin/ sulbactam	0.06	0.5	-	-	-
	Cefoperazone	1	16	100	0	0
	Cefoperazone/ sulbactam	1	4	-	-	-
	Imipenem	0.03	0.25	100	0	0
	Meropenem	0.03	0.03	100	0	0
	Ertapenem	0.03	0.06	100	0	0
	Clindamycin	0.06	0.125	100	0	0
	Lincomycin	0.125	0.25	-	-	-
	Ciprofloxacin	0.125	2	-	-	-
	Moxifloxacin	0.25	1	100	0	0
	Gatifloxacin	0.125	0.5	100	0	0
	Metronidazole	2	4	100	0	0
	Ornidazole	2	4	-	-	-
	Chloramphenicol	1	2	100	0	0

CONCLUSIONS

1. The highest anti-anaerobic activity against gram-negative strains was observed in:
   - nitroimidazoles (metronidazole, ornidazole);
   - inhibitor protected β-lactams (amoxicillin/clavulanic acid, amoxicillin/sulbactam, and cefoperazone/sulbactam);
   - carbapenems;
   - IV generation fluoroquinolones (gatifloxacin, moxifloxacin).

   These antimicrobials can be administered empirically for the therapy of anaerobic infections.
2. Microbiological activity of amoxicillin/sulbactam was comparable to that of amoxicillin/clavulanic acid.
3. Gatifloxacin was at 1 - 2 dilutions more active than moxifloxacin and was much more active against tested gram-negative strains in comparison to ciprofloxacin.
4. Despite a good activity of lincosamydes against *Prevotella* spp. and *Fusobacterium* spp., the resistance rate to clindamycin in most clinically significant anaerobes - *Bacteroides* spp., is growing and the percentage of non susceptible strains (I+R) was 26%.

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