## SESSION XIII—ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

Antimicrobial Susceptibility of Anaerobic Bacteria in the United States 2
*Hecht, D.W.**

Antimicrobial Susceptibility Patterns of Anaerobic Bacteria in Europe 3
*Nord, C.E.**

Molecular Basis of Promiscuous DNA Transfer by *Bacteroides* sp. 4
*Vedantam, G.**

Clinical and Environmental Anaerobes are Potential Reservoirs for Acquired Mobile Macrolide and Tetracyline Resistant Genes 5
*Roberts, M.C.;* Soge, O.O.; Meschke, J.S.*

The *Bacteroides fragilis* RecA Protein is Involved in Repair of DNA Damage Caused by Metronidazole and Ultraviolet Light 6
*Steffens, L.S.; Paul, L.;* Abratt, V.R.*

Anaerobic Bacteria in Pyogenic Infections: Incidence and Susceptibility to Anti-Anaerobic Agents 7
*Amer, F.A.;* Mohtady, H.A.; El-Behedy, E.M.; El-Ahmady, M.*

ß-Lactamase Production Among Anaerobic Bacteria Isolated from Intra-Abdominal Infections 8
*Mamal Torun, M.; Canberk, M.B.; Ozdemir, S.; Altinkum, S.; Cilingir, H.; Bahar, H.**

Susceptibility Profiles and Detection of Resistance Genes in *Bacteroides* and *Parabacteroides* Genera 9
*Boente, R.F.;* Ferreira, E.O.; Ferrerira, L.Q.; Falcão, L.S.; Miranda, K.R.; Guimarães, P.L.S.; Santos Filho, J.; Barroso, D.E.; Emond, J.P.; Paula, G.R.; Vieira, J.M.B.D.; Domingues, R.M.C.P.*

Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) Profile and Antimicrobial Susceptibility Pattern in *Fusobacterium nucleatum* Associated with Chronic Periodontitis in Nigerian Patients 10
*Nwaokorie, F.O.;* Coker, A.O.; Ogunsola, F.T.; Avila-Campos, M.J.; Gaetti-Jardim, Jr., E.; Salvage, K.O.*

Surveillance of Antimicrobial Resistance Among Clinical Isolates of Anaerobes in Kuwait 11
*Jamal, W.Y.; Shahin, M.; Rotimi, V.O.**

Description of *Bacteroides* Strains Exhibiting Heteroresistance to Cefoxitin and Carbapenems 12
*Sóki, J.;* Hedberg, M.; Urbán, E.; Terhes, G.; Nagy, E.*

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# ANTIMICROBIAL SUSCEPTIBILITY OF ANAEROBIC BACTERIA IN THE UNITED STATES

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Susceptibility testing of anaerobic bacteria has been standardized for over 20 years, yet testing remains infrequently performed in clinical laboratories. As a result, clinicians must frequently rely upon published susceptibility results to infer activity of various antimicrobials against anaerobic pathogens they are treating at their medical centers. Among reference laboratories in the United States using the same reference agar dilution method, it is clear that antibiotic resistance among *Bacteroides* species in the United States remains high for clindamycin, cefoxitin, piperacillin, and moxifloxacin, with moderate resistance to tigecycline, and little to no resistance among the β-lactam/ β-lactamase combinations, carbapenems, and metronidazole. Among non-Bacteroides anaerobes including *Prevotella, Peptostreptococci*, and *Clostridium* species including *C. difficile*, antibiotic resistance is rising as well, albeit to a lesser extent.

Compiling susceptibility testing data from several laboratories may be helpful in identifying changing patterns of antibiotic resistance among anaerobic bacteria. The anaerobe working group of the Clinical and Laboratory Standards Institute (CLSI) is currently planning to propose publication of compiled data from various laboratories in the United States in both the M11 and M100 documents at the June 2008 CLSI meeting. This surrogate antibiogram could then be updated as needed in future years and should be helpful to clinical microbiology laboratories and clinicians in the selection of antimicrobial agents. Results of the compiled data and the recommendations of the working group will be presented.

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# ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF ANAEROBIC BACTERIA IN EUROPE

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Clinical usage of antimicrobial agents has been accompanied by the isolation of antimicrobial-resistant bacteria. During the last years, there have been reports in Europe showing increasing numbers of anaerobic bacteria resistant to different antimicrobial agents. Resistance in anaerobic bacteria has a significant impact on the selection of antimicrobial agents for empirical therapy. The development of antibiotic resistance in anaerobic bacteria has been documented for beta-lactam drugs, clindamycin, macrolides, tetracyclines, fluoroquinolones and nitroimidazoles. The *Bacteroides fragilis* group is more resistant to antimicrobial agents than most other anaerobic bacteria. The *Bacteroides* genus and the genera *Prevotella* and *Porphyromonas* have become increasingly resistant to many anti-anaerobic agents. *Fusobacterium* strains resistant to beta-lactam drugs are relatively frequent. Resistant anaerobic cocci and *Propionibacterium acnes* have also been reported. Recently, fluoroquinolone-resistant *Clostridium difficile* strains producing three toxins have been isolated from patients with severe *C. difficile* diseases. The ESCMID Study Group for Antimicrobial Resistance in Anaerobic Bacteria (ESGARAB) has investigated the antimicrobial resistance patterns in anaerobic bacteria in Europe, is investigating the antimicrobial resistance mechanisms in anaerobic bacteria, and is developing standardisation methods for testing antimicrobial agents against anaerobic bacteria. The ESGARAB has recently investigated the antimicrobial susceptibility of Gram-positive anaerobic cocci in Europe. The antimicrobial susceptibilities of clinical strains isolated in 10 European countries were investigated. After identification of 299 anaerobic cocci 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. Antimicrobial susceptibility testing of anaerobic cocci in patients with severe infections as well as continuous surveillance of antimicrobial susceptibility in Gram-positive anaerobic cocci seem highly justified.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# MOLECULAR BASIS OF PROMISCUOUS DNA TRANSFER BY *BACTEROIDES* SP.

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*Bacteroides* sp organisms transfer DNA efficiently by conjugation, and are reservoirs of antibiotic resistance genes. Tn*5520* is a *B. fragilis* mobilizable transposon encoding only two proteins, an integrase and a mobilization (relaxase) protein BmpH. Tn*5520* can insert into non-mobile plasmids that carry antibiotic resistance genes, and make them mobile. A 71bp origin of DNA transfer (*oriT*) is located upstream of *bmpH*. BmpH alone is sufficient for transfer of this *oriT*. BmpH is thus a multifunctional protein involved in *oriT* nicking and covalent attachment (the first step in DNA transfer), and that also interacts with mating channel proteins during *oriT*-DNA translocation.

In recent studies, we have identified the BmpH active-site, and shown that it contains the conserved residues H132, Y184 and Y191 that are required for both DNA nicking activity and covalent interaction with nicked substrates. Active-site mutants of BmpH proteins do not localize to the bacterial membrane, confirming that the covalent interaction of *oriT* and relaxase (relaxosome) is required for productive DNA translocation. Further, we have shown that purified wild-type BmpH and BctA (a critical *B. fragilis* mating channel protein) interact *in vitro* (far-western analyses) and *in vivo* (bacterial two-hybrid analyses). We have also confirmed that BmpH strongly interacts *in vitro* with an *E. coli* conjugal plasmid protein TraG that is known to couple relaxosomes to the mating channel.

This is the first demonstration of a multifunctional relaxase efficiently interacting with mating channel proteins of different bacteria, and our results provide molecular evidence for a mechanism of promiscuous DNA transfer within and from *Bacteroides* sp.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# CLINICAL AND ENVIRONMENTAL ANAEROBES ARE POTENTIAL RESERVOIRS FOR ACQUIRED MOBILE MACROLIDE AND TETRACYLINE RESISTANT GENES

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In the past few years, the number of environmental anaerobic genera bacteria carrying acquired tetracycline (*tet*) and/or macrolide-lincosamide-streptogramin (MLS) resistance genes has increased from 15 to 22 genera carrying *tet* genes and from 10 to 17 genera carrying MLS genes. Most resistance genes are associated with mobile elements, plasmids, and transposons, which are transferable. However, the distribution and the diversity of the *tet* and MLS resistance genes in anaerobic bacteria is more limited and differs from the distribution and diversity found in aerobic/facultative species. Of the 38 known *tet* genes, 13 are found in 22 anaerobic genera [14 Gram-negative and 8 Gram-positive]. This includes the *tetA*(P) and *tetB*(P) genes which are unique to the genus *Clostridium*, while the remaining 36 *tet* genes are found in anaerobic, aerobic, and facultative Gram-positive and Gram-negative genera. Anaerobic bacteria carry 8 of 11 ribosomal protection *tet* genes and 5 of 23 *tet* efflux genes with only three of the genes exclusively found in Gram-negative bacteria. Currently there are 66 MLS genes with 11 found in 17 anaerobic genera [8 Gram-negative and 9 Gram-positive]. These include 9 rRNA methylase [*erm*] genes which confer macrolide, lincosamide, and streptogramin B resistance, two transport genes [*mef*(A), *msr*(D)] which confer macrolide resistance, and 2 lincosamide transferases [*lnu*(A), *lnu*(B)] which confers lincosamide resistance. Previous studies have demonstrated antibiotic resistance gene transfer between clinical donor anaerobic bacteria and related or unrelated recipients for many of the 13 *tet* and 11 MLS genes. However, previous studies have shown transfer from *C. perfringens* donors and to *C. perfringens* recipients and *Clostridium* spp. recipients. In our recent work, using DNA-DNA hybridization, PCR assays and sequencing we have demonstrated that US environmental *C. perfringens* carry multiple *tet* and MLS genes, and selected isolates were able to transfer *tet*(M), coding for tetracycline resistance, and *mef*(A) and *msr*(D) genes, both coding for macrolide resistance, to a recipient *Enterococcus faecalis* under laboratory conditions. *C. perfringens* are ubiquitous in the environment, and this work suggests that environmental *C. perfringens* are could act as reservoirs for these antibiotic resistance genes in the environment just as other anaerobes most likely do in human and animals.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# THE *BACTEROIDES FRAGILIS* RecA PROTEIN IS INVOLVED IN REPAIR OF DNA DAMAGE CAUSED BY METRONIDAZOLE AND ULTRAVIOLET LIGHT

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*Bacteroides fragilis,* an anaerobic commensal of the human gut, is also an opportunistic pathogen causing abscess formation and anaerobic septicaemia outside of this environment. Metronidazole, a DNA damaging agent, is used to treat *B. fragilis* infections. This antibiotic causes single and double stranded DNA breaks, which are thought to be repaired through recombinational repair. The RecA protein is required to facilitate recombinational repair and, therefore, might be important for cell survival following metronidazole damage. The role of the *B. fragilis* RecA protein in repairing metronidazole-damaged DNA was investigated genetically and functionally by mutagenesis of the *B. fragilis* 638R *recA* gene. Bioinformatic analysis revealed that the two genes upstream of *recA* encode a putative bacterioferritin comigratory protein (BCP), possibly involved in the survival of oxidative stress, as well as a hypothetical protein. RT-PCR of this gene cluster proved that the *recA* gene is expressed together with them as part of an operon. A *B. fragilis recA* mutant was generated using gene targeted insertional inactivation, and the insertion was confirmed by PCR and Southern hybridization. On a microscopic level, the mutant formed elongated cells after exposure to metronidazole, indicating a typical DNA repair stress response phenotype. The mutant exhibited increased sensitivity to metronidazole and ultraviolet radiation, and this was reversed by complementation with a copy of the wild type *recA* gene, indicating that the gene is involved in the repair of both these types of DNA damage. In addition, over-expression of the functional gene in the wild-type *B. fragilis* caused increased survival in the presence of metronidazole and also increased the growth rate of the bacterium. *B. fragilis* RecA is, therefore, important for recombinational repair of the DNA damage caused by metronidazole, and over-expression of the protein may lead to metronidazole resistance in *B. fragilis.*

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# ANAEROBIC BACTERIA IN PYOGENIC INFECTIONS: INCIDENCE AND SUSCEPTIBILITY TO ANTI-ANAEROBIC AGENTS

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**Purpose:** To identify the incidence of anaerobic bacteria in pyogenic infections and to determine their susceptibility to the most commonly-used anti-anaerobic agents.

**Methods:** This study was carried out over a period of 18 months in the Hospital and the Microbiology and Immunology Department, Zagazig Faculty of Medicine, Egypt. Samples of pus and wound swabs were collected from 157 patients suffering from infections suspected of having anaerobic aetiology. Samples were processed according to standard microbiological procedures and anaerobes were identified by API20A for anaerobes. Susceptibility of anaerobes to anti-anaerobic agents was carried out by the agar dilution method according to CLSI guidelines. The following antimicrobials were used: linezolid, quinupristin/dalfopristin, clindamycin, metronidazole, vancomycin, teichoplanin, penicillin, chloramphenicol, piperacillin, cefoxitin, imipenem, and tetracycline.

**Results:** Anaerobic bacteria were isolated from 72 samples out of the 157 samples studied (46%). Out of the 72 samples positive for anaerobes, 79% showed mixed aerobic and anaerobic bacteria, while only 21% showed anaerobic bacteria. Anaerobic bacteria were isolated from patients suffering from infected tumours, abscesses, surgical site infection, infected tracheostomy wound, intra-abdominal infections, diabetic foot, endometritis, and pelvic inflammatory disease. Isolated anaerobes in the order of frequency were: *Bacteroides fragilis*, Gram-positive cocci, *Bacteroides melaninogenicus, Fusobacterium* spp., *Bacteroides ovatus, Bacteroides ruminicola,* and *Bacteroides urelyticus*. Linezolid, vancomycin, teichoplanin and quinupristin/dalfopristin were mainly active against Gram-positive, with no useful activity against Gram-negative anaerobes. Metronidazole, imipenem and chloramphenicol had the best activity against *Bacteroides* spp. cefoxitin and imipenem against G-positive cocci, while metronidazole, piperacillin, cefoxitin, imipenem and tetracycline demonstrated the highest acyvity against *Fusobacterium* spp.

**Conclusions:** Anaerobes are contributors to types of infections studied. Samples collected from such cases should be considered for anaerobic cultivation as well. Susceptibility tests for anaerobes needs to be considered on periodic basis to monitor changes of susceptibility profiles of anaerobes.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# β-LACTAMASE PRODUCTION AMONG ANAEROBIC BACTERIA ISOLATED FROM INTRA-ABDOMINAL INFECTIONS

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**Background:** Beta-lactam antibiotics are critical agents in the treatment of anaerobic infections. The aim of this study was to determine the occurrence of β-lactamase production in the predominant anaerobic bacteria isolated from patients with secondary peritonitis and abdominal abscesses.

**Materials and Methods:** Pus of 34 intra-abdominal abscesses and peritoneal fluids of 68 patients with secondary peritonitis were studied for the presence of anaerobic bacteria. Specimens were inoculated on the basal medium prepared with brucella agar supplemented with hemin, vitamin K1, and 5% sheep blood, and additionnaly inoculated on phenylethyl alcohol anaerobic agar, on kanamycin vancomycin anaerobic agar prepared with this basal medium. Media were incubated 72 hours on anaerobic conditions obtained with Anaero-Gen (Oxoid & Mitsubishi Gas Company) in anaerobic jars (oxoid). Anaerobic bacteria were identified by API 32 ID (BioMerieux) and nitrocefin disks were used to determine the β-lactamase production of each strain.

**Results:** *Bacteroides fragilis* was found as the predominant bacteria with a total of 73 (71.5%) strains. 38 (37.2%) strains of *Petostreptococcus* spp which 26 of them were *Peptostreptococcus anaerobius* and 12 of them *Fingoldia manga* were isolated as a secondary predominant group. *Clostridium* spp with a total of 22 (21.5%) strains including 7 strains of *C. perfringens*, 2 strains of *C. ramosum,* 5 strains of *C. butyricum* and 8 other *Clostridium* sp were in the third range. β-lactamase production was found in 61 (83%) strains of *B. fragilis*, and in 1 (4.5%) strain of *C. butyricum*. β-lactamase production was not found in 38 *Peptostreptococcus* strains.

**Conclusion:** The emergence of aerobic and anaerobic β-lactamase producing bacteria play an important role in the transmission of β-lactamase gene between bacteria. β-lactamase in anaerobic Gram positive bacteria is not frequent and need more studies to understand the mechanisms and the geographic emergence.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# SUSCEPTIBILITY PROFILES AND DETECTION OF RESISTANCE GENES IN *BACTEROIDES* AND *PARABACTEROIDES* GENERA

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Susceptibility to 5 antimicrobials were determined by agar dilution method for *Bacteroides* spp. and *Parabacteroides distasonis* strains isolated from human intestinal microflora and clinical settings in Brazil (n= 42) and France (n= 18). All *Bacteroides* spp and *P. distasonis* isolates were uniformly susceptible to metronidazole. The resistance rates to ampicillin, cefoxitin, tetracycline, and clindamycin were 98.3%, 11.7%, 68.3% and 23.3%, respectively. All *P. distasonis* strains were resistant to ampicilin and cefoxitin, tetracycline, and clindamycin; the rates were 37.5%, 87.5% and 50%, respectively. The *erm*F and *nim* genes were not found in the genus *Parabacteroides,* and 37.5%, 12.5%, 12.5%, and 87.5% of the *Parabacteroides* strains possessed *cep*A, *cfi*A, *cfx*A and *tet*Q resistance genes, respectively. The genes *cep*A, *cfi*A, *cfx*A, *tet*Q, *erm*F and *nim* were found in 69.2%, 17.3%, 11.5%, 50%, 7.7%, and 3.8% of the *Bacteroides* strains, respectively. Ten strains of *Bacteroides* and *Parabacteroides*, positive for the presence of *cfi*A gene were submitted to susceptibility testing by E-test with imipenem and amoxicilin-clavulanate (amox-clav). The resistance rate to imipenem was 12.5% and 8.3% to amox-clav. Strains resistant to imipenem presented an active metallo-β- lactamase by E-test MBL assay. Tests will be performed in order to detect and categorize the insertion sequence responsible for the imipenem resistance. These results reinforce the importance of surveying susceptibility patterns and detection of resistance determinants.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# ARBITRARILY PRIMED POLYMERASE CHAIN REACTION (AP-PCR) PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN *FUSOBACTERIUM NUCLEATUM* ASSOCIATED WITH CHRONIC PERIODONTITIS IN NIGERIAN PATIENTS

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*Fusobacterium nucleatum* is the most common anaerobe isolated from the oral cavity, and also the most common oral species frequently isolated from infections involving other body sites. Very little work has been done on oral fusobacteria in Nigeria. The aim of this study was to evaluate the genetic diversity and antimicrobial susceptibility of 48 strains of *Fusobacterium nucleatum* recovered from 50 Nigerian patients with chronic periodontitis. Genetic diversity was assayed using AP-PCR method with the arbitrary primer OPA-05. Strains were placed into five groups (A, B, C, D and E) based on their AP-PCR profile and all the strains showed 80% of similarity. The Minimum inhibitory concentration was determined by agar dilution method using a Brucella blood agar. Beta-lactamase production was evaluated using the nitrocefin method. Plasmid extraction was carried out using Purelink-Quick Plasmid Miniprep Kit (Invitrogen, Sao Paulo, Brasil). All isolates were susceptible to metronidazole, clindamycin, cefoxitin, tetracycline, and amoxycillin/clavulanate. Only one strain was resistant to amoxicillin (MIC ≥ 32 μg/ml) and was β–lactamase-positive. Since none of the strains harbored plasmids, this could suggest a chromosomal resistance. AP-PCR analysis showed heterogeneity among strains.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# SURVEILLANCE OF ANTIMICROBIAL RESISTANCE AMONG CLINICAL ISOLATES OF ANAEROBES IN KUWAIT

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**Introduction:** Anaerobes are exotic and important human pathogens especially in those with impaired body defense systems. In this surveillance study, we investigated the trend in the antibiotic resistance pattern of anaerobes submitted to the Anaerobe Reference Laboratory over a period of 6 years, 2002-2007.

**Methods:** Clinically significant isolates are sent from all the government hospitals in Kuwait for identification and susceptibility testing. The identification were carried out using conventional, API 20A and GLC methods. Susceptibility to amoxicillin-clavulanic acid, clindamycin, imipenem, meropenem, metronidazole, piperacillin, piperacillin-tazobactam, and penicillin (as appropriate) was determined by estimating the minimum inhibitory concentrations (MIC) by E test method. Metronidazole-resistant strains were investigated for carriage of *nim* genes by PCR technique and the PCR product subjected to PCR-RFLP analysis.

**Results:** During this period, 2619 isolates were received, out of which 803 (30.7%) were *Bacteroides fragilis*, 667 (25.5%) *Bacteroides* spp., 543 (20.7%) *Prevotella* spp., 24 (0.9%) *Porphyromonas* spp., 496 (18.9%) *Peptostreptococcus* spp. and 22 (0.8%) *Clostridium* spp. *B. fragilis* and the *Bacteroides* spp. were the most resistant isolates. Antibiotics with the poorest activities were clindamycin with resistance rates of 39.3%, 49.4%, 28%, and 19.6% against *B. fragilis, Bacteroides* spp., *Prevotella* and *Clostridium* spp., respectively, followed by piperacillin with resistance rates of 31.6%, 48.1%, 20.1%, and 1.5%, respectively. The overall resistant rate of *B. fragilis* to metronidazole was 1.4%. *B. ovatus* resistance rates to this drug were 1.4, 5.3, 6.7, 3.2, 0, and 0% in 2002-2007, respectively. Resistance to the carbapenems was seen mostly in *B. fragilis* with rates as high as 3.9%, and 6.1% for imipenem and meropenem, respectively. The highest level of resistance was noted in year 2005 and lowest in 2007 with all isolates. All the metronidazole-resistant strains of *Bacteroides* spp. tested carried *nim*A and *nim*E genes.

**Conclusion:** Metronidazole resistance among the clinical isolates remains low in spite of the sporadic isolation of *nim* gene-carrying *Bacteroides* spp. encountered in 2005. Resistance to clindamycin, piperacillin, and, to a lesser extent, amoxicillin-clavulanic acid remains unacceptably high.

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## ANTIMICROBIAL SUSCEPTIBILITY & RESISTANCE

# DESCRIPTION OF BACTEROIDES STRAINS EXHIBITING HETERORESISTANCE TO CEFOXITIN AND CARBAPENEMS

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**Objective:** Heteroresistant phenotypes are already known for methicillin and vancomycin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*, our present aim was to document and characterize the heteroresistant phenotype of *Bacteroides* strains to cefoxitin or carbapenems noticed using Etests for antimicrobial susceptibility testing.

**Methods:** 8 heteroresistant *B. fragilis* strains collected during the previous 10 years and 100 clinical *B. fragilis* group strains recently isolated in Hungary were studied. For *in vitro* susceptibility measurements with cefoxitin and carbapenems, agar dilution, and the Etest were used. Heteroresistant colonies growing in the ellipse between the Etest strip and main population were further analyzed. The occurrence of *cfxA* and *cfiA* resistance genes and their regulatory regions were investigated by PCR and nucleotide sequencing. Population analysis profiles were also examined.

**Results:** The already discovered 8 *B. fragilis* strains proved heteroresistant to carbapenems on the use of Etest susceptibility measurements (from 0.25-4 μg/ml of continuous growth up to 16-32 μg/ml). All of them were *cfiA*-positive but, they did not harbor insertion sequence elements in the upstream region of the resistance genes. Among the 100 recent *Bacteroides* isolates, 21 strains heteroresistant to cefoxitin were observed. Their Etest patterns generally displayed a continuous growth of the less susceptible subpopulation from 8-128 μg/ml up to 256 μg/ml. Of these 21 strains, 11 harbored *cfxA* genes and their upstream regions were usually altered to the common 1.2 kb fragment, as seen in our previous studies. The population analysis profile analysis demonstrated the presence of more resistant subpopulations in the cultures of strains corresponding to the more resistant colonies in the Etest ellipse zones. Repeated experiments with subcultures from single colonies taken from the heteroresistant zones resulted in the original heteroresistant appearance when the Etest method was applied.

**Conclusion:** Heteroresistance to important β-lactam antibiotics appears among *Bacteroides* strains, but the phenotype can not yet be linked to any particular genetic constitution.
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