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REVIEW

Extended role for insertion sequence elements in the antibiotic resistance of *Bacteroides*

József Sóki

József Sóki, Institute of Clinical Microbiology, Albert Szent-Györgyi Clinical Center, Faculty of Medicine, University of Szeged, H-6725 Szeged, Hungary
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Correspondence to: József Sóki, PhD, Institute of Clinical Microbiology, Albert Szent-Györgyi Clinical Center, Faculty of Medicine, University of Szeged, Semmelweis u. 6., H-6725 Szeged, Hungary. soki.jozsef@med.u-szeged.hu
Telephone: +36-62-545399 Fax: +36-62-545712
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Abstract

The *Bacteroides* species are important micro-organisms, both in the normal physiology of the intestines and as frequent opportunistic anaerobic pathogens, with a deeply-rooted phylogenetic origin endowing them with some interesting biological features. Their prevalence in anaerobic clinical specimens is around 60%-80%, and they display the most numerous and highest rates of antibiotic resistance among all pathogenic anaerobes. In these antibiotic resistance mechanisms there is a noteworthy role for the insertion sequence (IS) elements, which are usually regarded as representatives of 'selfish' genes; the IS elements of *Bacteroides* are usually capable of up-regulating the antibiotic resistance genes. These include the *cepA* (penicillin and cephalosporin), *cfxA* (cephamycin), *cfiA* (carbapenem), *nim* (metronidazole) and *ermF* (clindamycin) resistance genes. This is achieved by outward-oriented promoter sequences on the ISs. Although some representatives are well characterized, *e.g.*, the resistance gene-IS element pairs in certain resistant strains, open questions remain in this field concerning a better understanding of the molecular biology of the antibiotic resistance mechanisms of *Bacteroides*, which will have clinical implications.

Key words: Antibiotic resistance; Antibiotic resistance genes; *Bacteroides fragilis*; Insertion sequence elements

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SIGNIFICANCE OF *BACTEROIDES*

The species of the genus *Bacteroides* are the most prominent human pathogenic anaerobic bacteria. Additionally, they have other important specialities: they are one of the most important members of the mammalian normal intestinal microbiota and they are the best-studied organisms of a separate and early diverged phylum, Bacteroidetes, of Bacteria. As regards their pathogenic nature, they account for 60%-70% of the total anaerobic pathogens cultivated from clinical samples, and despite the relatively low number of such materials, they often cause high mortality in various infectious processes, such as abscesses and other soft tissue infections, and often cause anaerobic sepsis[1].

Their phylum is a phylogenetic relative of the group of green-sulfur photosynthetic bacteria, the Chlorobiales, and the best-known and most frequently isolated species, *Bacteroides fragilis (B. fragilis)*, as a type species for anaerobic bacteria, is often referred to as the anaerobic *Escherichia coli*[2]. *B. fragilis* was first isolated as '*B. fragilis*' and later renamed as *B. fragilis*. Until the late 1970s, almost all Gram-negative anaerobic bacilli were classified

Table 1 A list of the related species comprising the '*Bacteroides fragilis* group' at present

Bacteroides			*Parabacteroides*
B. acidifaciens	*B. fluxus*	*B. propionifaciens*	*P. distasonis*[1,2]
B. barnesiae	*B. fragilis*[a,b]	*B. pyogenes*	*P. goldsteinii*[1]
B. caccae[1,2]	*B. galacturonicus*	*B. rodentium*	*P. gordonii*
B. cellulosilyticus	*B. gallinarium*	*B. salanitronis*	*P. johnsonii*
B. chinchillae	*B. graminisolvens*	*B. salyersiae*[1]	*P. merdae*[1,2]
B. clarus	*B. helcogenes*	*B. sartorii*	
B. coagulans	*B. heparinolyticus*	*B. stercoris*[1,2]	
B. coprocola	*B. intestinalis*[1]	*B. thetaiotaomicron*[1,2]	
B. coprophilus	*B. massiliensis*[1]	*B. uniformis*[1,2]	
B. coprosuis	*B. nordiia*	*B. vulgatus*[1,2]	
B. dorei	*B. oleiciplenus*	*B. xylanisolvens*	
B. eggerthii[1,2]	*B. ovatus*[1,2]	*B. xylanolyticus*	
B. faecis	*B. paurosaccharolyticus*	*B. zoogleoformans*	
B. finegoldii	*B. plebeius*		

[1]The main pathogenic species of *Bacteroides* that were included in an antibiotic susceptibility study and are most frequently isolated from clinical specimens; [2]The 10 *Bacteroides* species earlier comprising the *B. fragilis* group are marked with a superscript b.

in this genus, and only the more recent molecular techniques, such as DNA-DNA homology measurements and 16S rRNA sequence comparisons, allowed a more accurate classification. Thus, the genera of *Bacteroides*, *Prevotella* and *Porphyromonas* were formed from the earlier *Bacteroides* genus during the late 1980s. 16S rRNA phylogeny and other molecular classification methods were then applied, making the picture more diverse[3]. The parent genus *Bacteroides* now contains 41 described and well-characterized species. Some other former *Bacteroides* species were reclassified into the newly formed *Parabacteroides* genus[3], which belongs in the *Porphyromonadaceae* family (*P. distasonis* and *P. merdae*) (Table 1). *Bacteroidaceae*, *Marinilabiaceae*, *Porphyromonadaceae*, *Prevotellaceae* and *Rikenellacaea* are the families of the Bacteroidales order. Together with other important but aerobic taxa (the Cytophagales, Flavobacteriales and Sphingobacteriales orders), they form the Bacteroidetes phylum. The current situation regarding the species of the *Bacteroides* and *Parabacteroides* genera, the subjects of the current review, is summarized in Table 1 with some implications in respect of their pathogenic potential. In a recent study, the phylogenetic relations between *Bacteroides* species were analysed by multilocus sequence analysis, and thus these species could be ranked into 10 subgroups also showing some common characteristics regarding their pathogenic nature and sites of isolation[4].

Genomic studies have revealed the important genetic characteristics of this group of anaerobic bacteria and contributed to extensive metagenomic analyses of their habitat, the intestines and the participating microbiota[5]. These studies have reconfirmed that *Bacteroides* species are important symbionts there and opened up new ways for the investigation of this firmly interacting ecosystem. Besides the earlier cultivation and microscopic methods, metagenomic analyses have also proved that the two most abundant taxa there are Bacteroidales and Firmicutes (low G + C Gram-positives)[5,6]. The composition of the mammalian intestinal microbiota depends on the type of food intake (herbivorous, carnivorous or omnivorous)[7], but in the case of human beings three enterotypes can be distinguished as regards the prevalence of the main abundant constituents (*Bacteroides*, Prevotella and *Ruminococcus*); it is suspected that this is determined by the host and does not depend on the geographic origin[8]. The *Bacteroides* as one of the groups of predominant constituents of the human intestines exert beneficial effects for the host. However, experiments involving the application of metagenomics suggest that the intestinal microbiota, including *Bacteroides*, can affect not only the food intake, but also the development and physiology of the intestines and the immune system, and such distant organs as the liver, muscles, circulation and central nervous system. Thus, their roles regarding participation in illness states such as obesity and inflammatory bowel diseases have been the subjects of previous and ongoing investigations[9-11].

Virulence mechanisms of Bacteroides spp.

Though *Bacteroides* can be regarded as only opportunistic pathogens since they reside in the intestines in high cell numbers and cause diseases with underlying predisposition circumstances such as trauma, circulation defects and immunosuppression, they usually possess a pathogenic repertoire with which they participate in infections. *B. fragilis*, the earliest identified and thus the type species, is isolated most frequently from anaerobic infections with a prevalence of 60%-70%. As it is estimated to have a prevalence in the intestines of only 0.5%-5.0% and to be localized to the epithelium rather than to the lumen, it can be regarded as the most pathogenic species among the *Bacteroides*, and this is supported by the experimental data[1]. The most frequent infections that it causes are intra-abdominal and intra-pelvic, lung and brain abscesses, appendicitis, diarrhea, inflammatory bowel disease, lower respiratory and soft-tissue infections, and sepsis. The main predisposing factors are usually surgery, mixed aerobe-anaerobe infections, immunosuppression, diabetes and circulatory defects. However, besides the prominent pathogenic role of *B. fragilis,* most *Bacteroides* are capable of adhering, evading and destroying the tissues with their direct and indirect virulence mechanisms, which are production of capsules, fimbriae and adhesins, tissue destruction enzymes (fibrinogenases, haemolysins, neuraminidase and enterotoxin) and properties of aerotolerance, evasion of the host immune system, and antibiotic resistance mechanisms[1].

The most potent virulence mechanism of *B. fragilis* has been demonstrated to involve certain capsular polysaccharide (CPS) species[12]. In the mid-1980s, the use of CPS material of *B. fragilis* was shown to evoke abdominal abscesses experimentally in a rat model, and the nature of the immuno-modulation involved the induction of a humoral response[13]. The chemical structures

of two CPS species, CPS-A and B, of *B. fragilis* NCTC 9343 were later determined and their abscess-inducing properties were proved to be due to a zwitterionic structure[14,15]. The capsules participate in immuno-modulation by other usual modes of interactions, the inhibition of phagocytosis and complement action. Electron microscopically, *B. fragilis* may be seen to have small or large capsules or only an electron-dense layer which is implicated in complement resistance[16-18]. Studies have led to the cloning of these CPS operons[19,20], and subsequently altogether 8 operons with similar structures have been found in the genomic sequence of *B. fragilis* NCTC9343 that have a common regulatory property, the possession of invertible promoters[21]. This special regulatory feature may result in numerous variable surface compositions through activation of on-off switches (about 2^8) in the case of a single strain. The examination of *B. fragilis* YCH46 and 638R genomes demonstrated that at least 10 CPS operons can be located on these genomes, which may have different alleles (n = 28), allowing a much higher number of variations in possible surface compositions[22]. The large and small capsule phenotypes are suspected of being regulated by the expression of the gene *BF2782* (or *BF2790* in *B. fragilis* 638R), which is a putative sugar transferase participating in the synthesis of the CPS species and is also the subject of invertible promoter structure[22]. Similar CPS operons are suspected of functioning under the regulation of invertible promoters in other *Bacteroides* species (*B. caccae*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, *B. vulgatus*, *P. merdae* and *P. distasonis*), but not in the oral *Bacteroides* relatives (*Prevotella* and *Porphyromonas*)[23]. The CPS-A of *B. fragilis* is capable of regulating the maturation of the immune system which, in turn, is an important contribution to the overall symbiotic interactions between *Bacteroides* and the host[24,25].

Another important virulence factor is the enterotoxin of *B. fragilis*, which may cause diarrhea especially in young mammalians. This enterotoxin is a metallo-protease capable of the specific cleavage of the E-cadherin protein in the zonula adhaerens portion of the intestinal epithelium. This causes specific processes leading to the symptoms of diarrhea; disorganization of the actin cytoskeleton, epithelial fluid loss, inflammation, and possible penetration of the enterotoxinogenic *B. fragilis* cells into the nearby and distant tissues[26]. The inflammatory action of the *B. fragilis* enterotoxin may be so pronounced that the malignant transformation it causes can be detected both clinically and experimentally[27,28]. The genes of the enterotoxin, consisting of three main types (*bft1-3*), lie on a specific portion of a 'pathogenicity island', which is a conjugative transposon resembling other *B. fragilis* genome-borne conjugative transposons[29-31]. The similar CTn*86* and CTn*9343* elements have molecular variants resulting from (1) insertion of a ca. 6 kb region containing the *bft* genes into CTn*86* (accordingly, *bft*-positive CTn*86*s are enterotoxinogenic, whereas *bft*-negative CTn*86*s are not); (2) replacement of the 3' regions of both CTns; and (3) insertion of a novel ca. 7 kb region into some CTn*9343*s[32,33]. A more detailed summary of the pathogenicity and virulence factors of *Bacteroides* is to be found in an excellent recent review[1].

Antimicrobial resistance of Bacteroides spp. and its genetic background

As the *Bacteroides* are the most significant human anaerobic pathogens, detection of their antimicrobial susceptibilities has a significant history, and trends have been observed in the most frequent resistance rates and the most numerous resistance mechanisms among their clinical isolates. As time has passed, these latter resistance trends have become more pronounced. In the 1960s and 1970s, the strains were much less resistant to all groups of antibiotics than more recently[34]. In the meantime, the recommended susceptibility measurement methods have changed. Since the 1980s, the recommended method for the detection of their antibiotic susceptibilities has been agar dilution[35]. Regular studies have been carried out, especially in the United States and in Europe[36,37], and the breakpoint recommendations of the NCCLS (National Committee for Clinical Laboratory Standards, currently the Clinical Laboratory Standards Institute-CLSI, www.clsi.org) in the United States have been widely used for resistance categorization; additionally, we now have the recommendations of another influential body, EUCAST (www.eucast.org). Since the *Bacteroides* in the intestines are readily exposed to antibiotics administered orally and excreted into the bile, a continuous increase in resistance rates has been observed for all major antibiotics. The resistance to tetracycline has changed most profoundly, which may be explained by the intensive use of tetracycline and the fact that the spread of tetracycline resistance elements is highly enhanced by tetracycline (see the explanation below)[34].

The *Bacteroides* have displayed a significant rate of resistance to 'normal β-lactams' (penicillins and 1st and 2nd generation cephalosporins) throughout the studied periods, but some increases have also been observed. One important issue relating to the 'normal β-lactam' resistance is the breakpoint categorization, since the MIC values for all such drugs are scattered widely, ranging from the low 0.25 μg/mL to the very high 256 μg/mL. Thus in a 1990 European study, only 12% of *B. fragilis* strains were found to be resistant to ampicillin at a breakpoint of 32 μg/mL[38], whereas in a study in 2000 with 2/64 μg/mL as breakpoints, 99.3/27% were categorized as resistant[39]. It was additionally observed that the distribution of 'normal β-lactam', especially ampicillin, resistance distribution is bimodal, with modes at about 32 and ⩾ 256 μg/mL[38]. Since the majority of *Bacteroides* isolates exhibit β-lactamase activities, this was proposed to be the main resistance mechanism[34]. The gene *cepA* of an Ambler Class A β-lactamase is very prevalent[40] (about 70%, according to our own unpublished observations) among *B. fragilis* and other *Bacteroides* strains. Little is known concerning the mechanisms of

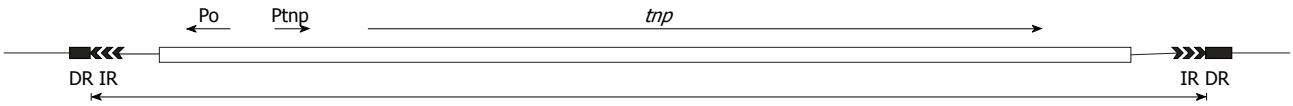

Figure 1 Schematic structure of an insertion sequence element. DR: Direct repeats; IR: Inverted repeats; Po: Outward-oriented promoter; Ptnp: Promoter of the transposase. The transposase gene is denoted as tnp. The borders of the insertion sequence (IS) are indicated by the closed arrowheads below.

resistance to β-lactam/β-lactamase inhibitor combinations; however, the rates in the United States (< 1%) and in Europe (10.3%) have been increasing continuously in recent years, probably because of the extensive use of such drugs[36,37].

Cefoxitin was earlier a very effective antibiotic for the treatment of *Bacteroides* infections, but the levels of resistance to this drug rose by 6% and 10.3% in Europe and the United States, respectively[39,41], during the 1990s, though this has been followed by a decrease (12% *vs* 9%) in the past decade in the United States[36]. The main resistance mechanisms involve the decreased affinity of the penicillin-binding proteins to cefoxitin and the production of another Ambler Class A β-lactamase capable of hydrolyzing cephamycins[42]. The gene for this latter β-lactamase, *cfxA*, has been located on a mobilizable transposon MTn*4555*[43], which has been proved to have several variants at its 3' end[44,45].

Some *B. fragilis* isolates are also resistant to the wide-spectrum carbapenems, due to a metallo-β-lactamase coded by the *cfiA* (*ccrA*) gene[46,47]. Despite the low prevalence of carbapenem-resistant *B. fragilis* isolates (about 1%), this has displayed a continuous rise since the introduction of these drugs[37,41]. It is very interesting that the *cepA* and *cfiA* genes are found mutually exclusively among *B. fragilis* isolates and define two genetic groups (Division I -cepA-positive and Division II -*cfiA*-positive)[48,49] that can also be differentiated by the levels of DNA-DNA homologies[50], PCR typing methods[48], ribotyping[51], multi-locus enzyme electrophoresis[49] and MALDI-TOF mass spectrometry[52,53].

The rate of resistance to the macrolide-lincosamide-streptogramin antibiotics of 32.4% is not an exception; the rates of resistance presumed to be caused by *ermF* genes has a steep rise[37]. Our recent investigations based on susceptibility measurements and resistance gene detection (unpublished) of clinical *Bacteroides* isolates revealed that other resistance genes (*ermB*, *ermG*, *mefA* and *msrSA*)[54,55] may participate significantly in the development of clindamycin resistance.

Resistance to 5-nitroimidazoles is caused either by alterations in the cellular redox system that can diminish the lethal action of these drugs or by 5-nitroimidazole reductases that reduce the nitro group of 5-nitroimidazoles to an amino group without the formation of toxic intermediates[56-61]. The 5-nitrimidazole reductases are coded by *nim* genes that bear about 60%-70% mutual homologies and have 9 representatives (*nimA-I*)[60,62-64]. The form *nimI* has been found only among *Prevotella baroniae* isolates, while some *Bacteroides*-specific *nim* genes are present in other source organisms too[65-69]. The rates of resistance to metronidazole are fortunately very low among *Bacteroides* strains in most places (< 1%).

Tetracycline resistance has been estimated to be approaching 100%; the resistance gene is *tetQ*, coding a ribosomal protection protein. The *tetQ* genes are found on conjugative transposons[70]. Interestingly, *Bacteroides* carry another tetracycline resistance gene *tetX* (or its amino-terminally truncated, 60% homologous variant *tetX1*)[71], which is capable of oxidizing the tetracycline molecule[72], but since it requires oxygen for this process, its role in the tetracycline resistance of *Bacteroides* is very limited. *Bacteroides* is resistant at a low level (1.7%) to tigecycline, a synthetic minocycline, glycylcycline derivative. In such tigecycline-resistant cases, a direct role of the *tetX* and *tetX1* genes has not been confirmed for the *Bacteroides*[73].

The *Bacteroides* are now becoming resistant to the once fully effective fluoroquinolones such as trovafloxacin and moxifloxacin, reaching resistance rates of > 40% and 13.6% in the United States and Europe, respectively. Additional data on the antibiotic resistance rates of *Bacteroides* are to be found in a recent exhaustive review[74].

INSERTION SEQUENCE ELEMENTS

There are a huge variety of transposable and conjugally mobile genetic elements, in particular among prokaryotes. IS elements are short (from 600 to 2000 bp long), double-stranded integrative DNA sequences that code for only a transposase gene, bordered by inverted repeat sequences; during their integration, they usually cause target site duplications of a small number of nucleotides. A general scheme relating to their organization is presented in Figure 1. They are to be found in all three domains of life (Archaea, Bacteria and Eukarya). Their classification is based on the ends of their inverted repeats and the conserved amino acid residues of the transposase genes[75]. In this way, about 25 families are distinguished among prokaryotes and are usually named after their earliest and best-examined members. Some families fulfil the above-mentioned description criteria, but molecularly represent a more divergent type of elements, *e.g.*, the application of different transposition mechanisms to the main groups of IS elements which harbor transposases with an indispensable aspartate-aspartate-glutamate (DDE) motif forming the active catalytic center. Similar motifs can be found in the integrase proteins of retroviruses and, among others, in

Table 2 The 5-nitroimidazole resistance *nim* genes of interest for *Bacteroides*

Nim gene type	Carrying genetic element	Activating IS	No. of isolates[1]
nimA			
	pIP417 (7.7 kb)	IS*1168*	10[102,114,115]
	10 kb uncharacterized plasmid	IS*1168*	2[102]
	8.2 kb uncharacterized plasmid	IS*614*	1[102]
	Chromosomal	IS*1168* or Unknown	3[102]
	Unknown	IS*1168*	12[115]
	Unknown	IS*1169*	1[116]
nimB			
	Chromosomal	IS*1168* or IS*612* or IS*614*	8[102,114]
	Unknown	IS*1168*	3[116]
nimC			
	pIP419 (10 kb)	IS*1170*	4[115]
	Chromosomal	IS*1170*	2[102]
	Unknown	IS*1170*	2[63,116]
nimD			
	pIP421 (7.3 kb)	IS*1169*	1[102,117]
	Chromosomal	Unknown	1[102]
	Unknown	IS*1169*	6[116]
nimE			
	pBF388c (pWAL610, 8.3 kb)	IS*Bf6*	5[102,118]
nimF			
	Chromosomal	Unknown	1[116]
nimG			
	Unknown	Unknown	1[63]
nimH			
	Unknown	Unknown	-[2]

[1]The number if isolates with the given genotypes are indicated with references; [2]Taken from GenBank (www.ncbi.nl.nih.gov, acc. no. FJ969397).

RNase H ribonuclease, in the DNA polymerase I 3'-5' proofreading activity domain and in the RuvC recombinase proteins of bacteria forming the RNase H enzyme superfamily. The reader can find further data on the classification and transposition mechanisms in some older and more recent reviews[75,76] and in the IS Finder database (www-is.biotoul.fr)[77].

The simple genetic organization in *sensu stricto* is sufficient for IS element dispersal, and they can therefore be regarded as appropriate examples of selfish genetic elements. However, thorough examinations of their prevalence, genetic structure and transposition suggest that they not only parasitize their hosts, but sometimes participate in firm interaction with them. Such interaction with the host may be accomplished *via* (1) a promoter supply for the host genes; (2) increased evolution rates; and (3) a metabolic load. The activation of the expression of nearby genes by promoter supply is mediated by outward-oriented promoters and is specific for a small subset of ISs. This way, various bacterial genes can be activated, resulting in antibiotic resistance most notably, and the ISs act among others on various antibiotic resistance genes, *e.g.*, *bla*$_{TEM(pBR322)}$ of *Escherichia coli*[78], *bla*$_{OXA-51}$ *Acinetobacter baumannii*[79] and *oprD* of *Pseudomonas aeruginosa*[80]. They can influence the evolution potential of their host by their mutagenization of the host genomes by hopping activity[81]. It is also known that the introduction of a copy of an additional accessory genetic element, *e.g.*, a plasmid, and the amplification of their physiological copy number means a fitness decrease first and then an adaptation[82], which is also valid for IS elements. IS elements participate in activation of *Bacteroides* antibiotic resistance genes.

The discovery that erythromycin and clindamycin resistance is due to an MLS$_B$ resistance mechanism (capable of causing cross-resistance to the chemically different macrolid, lincosamide and streptogramin B antibiotics), mediated by *ermF* genes, and the subsequent linking of these genes to similar compound transposons, was the first indication of IS element involvement in antimicrobial resistance among *Bacteroides*. Clindamycin resistance plasmids were first detected in clindamycin-resistant isolates[83]. Such plasmids as pBF4 (pIP410), pBFTM10 (pCP1) and pBI136 were characterized very well molecularly in the 1980s[84]. pBF4 (41 kb) harbored Tn*4351* bordered by inverted copies of IS*4351* and in between *ermF* and an aerobic-type tetracycline resistance gene, *tetX*[85]. Tn*4551* accounts for a large portion (about 30%) of pBFTM10 (15 kb) and contains *ermF* in direct repeats of IS*4351*[84]. pIB136 (80 kb) also contains Tn*4551*, but with a high preponderance to lose this structure[86].

The 1990s revealed other important links between IS elements and the antibiotic resistance of *Bacteroides*. After cloning of the determinant for the carbapenem resistance, *cfiA*[46,47], PCR detection and parallel molecular methods have demonstrated that carbapenem-resistant mutants can arise in single-step mutations from *cfiA*-positive but carbapenem-susceptible *B. fragilis* isolates[87], which proved to be insertions of IS*1186*[88] and IS*942*[47]. Later studies confirmed these findings and the roles of a series of other IS elements were identified in carbapenem-resistant strains from such different geographic regions and countries as Europe (France, the United Kingdom, Hungary, Sweden, Switzerland, Norway and Italy), the United States, Japan, Korea and Kuwait[48,89-95]. The 5-nitroimidazole resistance genes, *nim*s, also carry various IS elements in their upstream regions. In these cases, the *nim* gene types, the carrying genetic elements and the activating ISs were linked (Table 2). The presence of IS elements has been demonstrated in the upstream regions of other β-lactamase genes, *cepA* and *cfxA*. In a high cephalosporinase-producer strain, *B. fragilis* CS30, this feature was caused by a specific DNA sequence that contained an IS21-like region (IS*Bf1*) at its 3' end adjacent to the *cepA* gene[96]. In the case of a representative strain (*B. vulgatus* CLA341) for *cfxA*-mediated cephamycin resistance, the upstream region of the *cfxA* gene also contained an IS element (IS*Bf8*) that was identified later by bioinformatics analysis[97,98]. The majority of the *cfxA* genes of *Bacteroides* do not normally contain this (IS*Bf8*) and another mobile element (MITE*Bf2*) in their upstream region[45]. Interestingly, again in high β-lactamase-

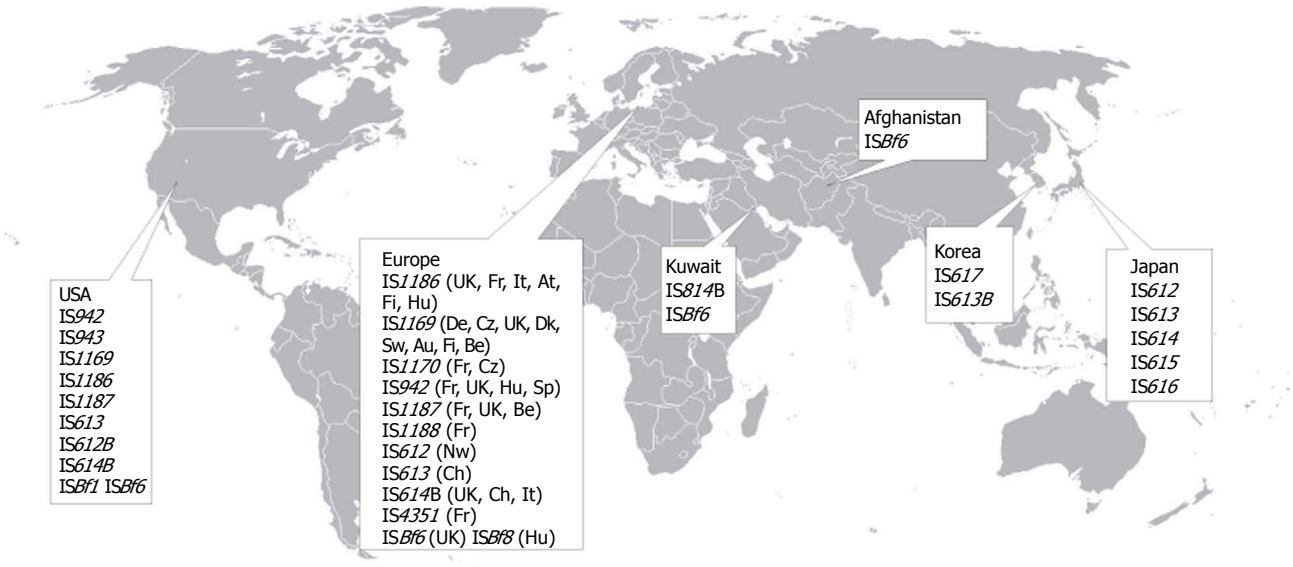

Figure 2 Insertion sequence elements found worldwide in antibiotic-resistant *Bacteroides* isolates. For Europe, the following abbreviations are used to identify the countries in which the insertion sequences were isolated: At: Austria; Be: Belgium; Ch: Switzerland; Cz: Czech Republic; De: Germany; Dk: Denmark; Fi: Finland; Fr: France; Hu: Hungary; It: Italy; Nw: Norway; Sp: Spain; Sw: Sweden; UK: United Kingdom.

producer *cfxA*-positive strains, the presence of IS*614*-like elements has been revealed in the upstream region of the resistance gene by inverse PCR[44].

For *cfiA* and *cfxA,* a heterogeneous resistance phenotype has been detected by diffusion methods (especially the Etest) in strains that have elevated agar dilution MICs and do not have IS elements in the upstream region of the resistance genes[45,99].

Though specific for some representative strains and resistance genes, the *Bacteroides* IS elements have been shown to be capable of activating all IS-requiring resistance genes. In this way, IS*4351* can activate *cfiA*[48], the IS elements of the *nim* and *cfiA* genes are interchangeable, and an IS element discovered for *cfiA*, IS*614* (or its variant), has been found to activate the *cfxA* gene also[44]. However, little is known about the prevalence and epidemiology of the resistance gene-activating ISs apart from their being found in resistant isolates. The best-studied examples are the *nim* and *cfiA*-carrying strains, but these differ considerably with respect to the prevalence of 'silent' and activated genes. Thus, all well-characterized *nim* genes are associated with an IS element (Table 2), but the majority of the *cfiA* genes are 'silent', and not associated with ISs[52,87,89,100,101]. Examinations of the insertion sites of ISs among *nim* and *cfiA* genes revealed that for *nim*s the insertion sites are well defined and conservative for a particular *nim* gene type[102], whereas for the *cfiA* genes they vary[91,92]. This means a well-known mechanism for the emergence of nitroimidazole resistance by the *nim* and IS combination, which was investigated and discussed recently, especially for the β-lactam resistance mechanisms of *Enterobacteriaceae*[103,104], involves the consecutive steps of emergence, adaptation and spreading. For the *Bacteroides* the *nim* gene IS combinations first emerged, which were then inserted into specific replicons (plasmids and chromosomes) and subsequently were spread in the *Bacteroides* population.

Another epidemiological concern besides the interchangeability of the IS elements is their geographical distribution (Figure 2). A number of studies of IS elements in resistant strains indicated that there is little geographical restriction to their spreading worldwide, *e.g.*, IS*614* or IS*614*-like elements were found ubiquitously, though some local tendencies can also be observed (Figure 2, cf. Japan and Korea). It could also be that these IS elements vary in the nucleotide sequence, giving rise to isoforms (not mentioned in full detail here) and could be mosaics/combinations of other elements. This can be explained by the homologous nature of these elements and the fact that they can be harbored coincidentally in an unknown proportion of the strains.

While the role of IS elements in emerging antibiotic-resistant *Bacteroides* strains is well documented, the process of the movement/skipping of the IS elements from their proper positions has been investigated only poorly. Podglajen *et al*[87] studied this process *in vitro* and reported a rough estimation of the development of imipenem-resistant strains, with 10^{-8} to 10^{-7}/cell frequencies in a given culture. Edwards *et al*[89] detected this process *in vivo* when the initially susceptible strain in a patient with a *B. fragilis* infection treated with imipenem became resistant[105].

Overall, the IS elements found among *Bacteroides* species belong in 9 IS families, members of 5 families being capable of activating antibiotic resistance genes. An overview of these elements is provided in Table 3.

THE PROMOTERS CARRIED BY THE BACTEROIDES IS ELEMENTS

Although IS element insertion correlates well with an-

A Consensus sequences

Regions	-33	-7
	TtTG	tnnTAnnTTTGY

B

Gene	IS element			
cepA	IS*Bf1*	TTTG	16 nt	TcaTAccTTTGTtgga˅
cepA	-	aTTGaaTT	16 nt	TcaTAccTTTGTtgga
cfxA	IS*Bf8*	TTTG	17 nt	atgTAccTTTGTcggc˅
cfxA	-	TTTc	10 nt	atgTAccTTTGTcggc
cfiA	IS*942*	agTG	17 nt	TtgTActTTTGCca˅
	IS*1186*	TTTG	16 nt	gctTAacTTTaCgcaa˅
	IS*1187*	TTG	17 nt	gacgAatTTTGCa˅
	IS*1188*	TTG	17 nt	TtgTAtcTTTGCaca˅
cfiA	-	TaTa	8 nt	atgTtagTTTGaatac˅
ermF	IS*4351*	aTTG	18 nt	TtaTAtgTTTGCtca˅
nimA	IS*1168*	TTTG	18 nt	gctTAacTTTaCgca˅
tetQ		TTTG	16 nt	gtgTAatTTTGTaatc˅

Figure 3 The nucleotide sequences of the promoters of some important Bacteroides antibiotic resistance genes. The consensus sequence with the conserved regions (A), and the actual promoter sequences (B). The match with the consensus is shown in bold capital letters, proven transcriptional initiation sites are marked in bold with an arrowhead next to them; n denotes any nucleotide, and small letters in the consensus indicate less conserved bases. The own promoters of *cepA* and *cfxA* were searched for bioinformatically and are not IS elements next to them in the list. The own promoter sequence of *cfiA* is from our unpublished preliminary experiments made by 'rapid amplification of cDNA ends' capable of amplifying in PCR the 5' end of the mRNA. Underlined -33 regions are parts of compound promoters and these parts originate only from insertion sequence elements in the cases of these promoters.

tibiotic resistance gene expression, the main reason for their up-regulation is that the IS elements carry outward-oriented promoters capable of driving the expression of the genes. The initial hypothesis for this up-regulation was the lending of IS activation mechanisms from other antibiotic resistance genes for aerobic species, but only *E. coli* promoter sequences could be investigated for these *Bacteroides* IS elements at that time. There was a straightforward result concerning the requirements for transcription in *Bacteroides* when Bayley *et al*[106] recognized the nucleotide composition of the promoter sequences for several antibiotic resistance and other *Bacteroides* genes. The promoter consensus sequence for *Bacteroides* is depicted in Figure 3A. This highly different requirement in promoter sequence was later explained by the results of Vingadassalom *et al*[107], who proved that the primary σ subunit of the *Bacteroides fragilis* RNA polymerase is unusual and different from that of other bacteria; whereas it is able to start transcription from original *Bacteroides* promoters in reconstitution experiments, it clusters firmly together with the suspected primary σ-factors of other Bacteroidetes species, but only distantly to the primary and stationary σ-factors of other bacteria.

Several antibiotic resistance gene promoter sequences were recognized during these investigations. The first was for the *cepA* gene of *B. fragilis* CS30 in the original work of Bayley *et al*[106] Similarly, those authors determined the promoter for the *cfxA* gene on MTn*4555* of *B. vulgatus* CLA341[96]. Interestingly, this promoter is a compound one: the -7 region originates from a prototype MTn*4555* backbone and the -33 region from an IS element (IS*Bf8*). MTn*4555* insertion of the IS*614* elements is associated with increased resistance to cefoxitin, though the exact transcription initiation site for this IS element and the promoter remain to be elucidated[44]. Among these rare data relating to the promoter structures of *Bacteroides*, those carried by IS*612*, IS*613*, IS*614*, IS*615* and IS*616* elements activating the *cfiA* genes have been recognized, thereby furnishing us with important confirmatory data[90,93]. Podglajen *et al*[108] also determined the outward-oriented promoters of some important IS elements (IS*1186*, IS*942*, IS*1187* and IS*1188*) participating in activation of the *cfiA* genes of some carbapenem-resistant *B. fragilis* isolates. Although the recognition of the requirements for the *Bacteroides* promoter nucleotide sequence facilitated an understanding of their antibiotic resistance mechanisms, there was also research into other aspects of their properties, *e.g.*, the CPS on-off regulation[21]. Figure 3B lists the known and some proposed sequences of promoters of antibiotic resistance genes of *Bacteroides*.

Table 3 The insertion sequence elements involved in the up-regulation of antibiotic resistance genes in *Bacteroides*

IS family[1]	Group[1]	IS[2]	Activated genes
IS4	ISPepr1		
		IS*943*	*cfiA*
		IS*Bf8*	*cfxA*
IS5	IS5		
		IS*1186* (IS*1168*)	*cfxA, cfiA, nimA, nimB*
		IS*1169*	*cfiA, nimA, nimD*
	IS1031		
		IS*Bf6*	*nimE*
IS21	-		
		IS*Bf1*	*cepA*
IS982	-		
		IS*1187*	*cfiA*
IS31380	IS942		
		IS*942*	*cfiA*
		IS*1170*	*nimC*
		IS*612*	*cfiA, nimB*
		IS*613*	*cfiA*
		IS*614*	*cfxA, cfiA, nimB*
		IS*615*	*cfiA*
	-		
		IS*1188*	*cfiA*
		IS*4351*	*ermF, cfiA*
		IS*616*	*cfiA*

[1]The IS families and the subgroups within them (taken from IS Finder)[77]; - indicates no further classification; [2]The species of IS elements activating the resistance genes of *Bacteroides* spp.; the mosaics and isoforms are not indicated. IS: Insertion sequence.

Other resistance mechanisms

Despite the dominance of IS element-borne activation of the antibiotic resistance genes of *Bacteroides*, natural resistance (to aminoglycosides, 1st and 2nd-generation fluoroquinolones and aztreonam), resistance emerging by point mutations, and the enforcement of internal regulatory mechanisms of the genes should be mentioned.

Point mutations in the *gyrA* gene (coding for a subunit of topoisomerase Ⅱ) can cause ciprofloxacin, moxifloxacin and trovafloxacin resistance[109]. A special, well-characterized resistance mechanism of *Bacteroides* is coded by tetracycline resistance conjugative transposons harboring the *tetQ* genes. The *tetQ* genes have their own promoters that can be up-regulated by tetracycline, as observed in the 1970s and exhaustively analyzed since the 1990s[70]. This is mediated by an attenuation mechanism where the transcription stalls at a leader upstream of *tetQ* in the absence of tetracycline, but in the presence of tetracycline the transcription proceeds. The *tetQ* gene is in an operon with the regulatory proteins of *rteABC*, which upon tetracycline exposure up-regulate the excision, mobilization and conjugation genes[110]. For this regulation to be effective, other regulatory processes are also involved, whose absence makes the conjugative transposons constitutive with respect to tetracycline[111]. Some other important resistance genes code efflux pumps, *e.g.*, *bexA* (fluoroquinolones)[112], *mefA* and *msrSA* (clindamycin)[55], and an endogeneous efflux mechanism, mediated by the *bmeABC* genes, can be up-regulated by mutations in the amino acid sequence of the coded effector proteins[113].

CONCLUDING REMARKS

Bacteroides species are noteworthy participants and contributors to human health and disease. They comprise a group of bacteria with additional molecular biological specific features as regards their promoter and RNA polymerase structures and a huge number of surface variations due to the invertible promoters at their CPS operons. The regulation of their antibiotic resistance genes is in most cases also specific; they need up-regulatory IS elements for antibiotic resistances to develop. However, there is a paucity of data about the observed associations in resistant strains: the promoters of less characterized IS elements are still to be determined, the roles of up-regulatory IS elements in other resistance genes could be investigated, and the frequencies with which the IS elements move to the upstream positions of the resistance genes could be examined in greater detail. These latter approaches would promote a better understanding of the whole picture of the rather prevalent antibiotic resistances of the *Bacteroides* species, which in turn would facilitate the design of better antimicrobial therapies against this important group of bacteria in the future.

REFERENCES

1 **Wexler HM**. Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 2007; **20**: 593-621 [PMID: 17934076 DOI: 10.1128/CMR.00008-07]

2 **Smith CJ**, Rocha ER, Paster BJ. The medically important Bacteroides spp. In health and disease. In: Dworkin M FS, Rosenberg E, Schleifer K-H, Stackebrandt E, editors. New York: Springer, 2006: 381-427

3 **Shah HN**, Jacinto RC, Ahmod N. The genus Bacteroides. In: Encyclopedia of Life Sciences. Chichester: John Wiley & Sons, Ltd, 2008

4 **Sakamoto M**, Ohkuma M. Identification and classification of the genus Bacteroides by multilocus sequence analysis. *Microbiology* 2011; **157**: 3388-3397 [PMID: 21948050 DOI: 10.1099/mic.0.052332-0]

5 **Turnbaugh PJ**, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* 2007; **449**: 804-810 [PMID: 17943116 DOI: 10.1038/nature06244]

6 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

7 **Ley RE**, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* 2008; **6**: 776-788 [PMID: 18794915 DOI: 10.1038/nrmicro1978]

8 **Arumugam M**, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174-180 [PMID: 21508958 DOI: 10.1038/nature09944]

9 **Mazmanian SK**, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; **453**: 620-625 [PMID: 18509436 DOI: 10.1038/nature07008]

10 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540 19043404]

11 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]

12 **Shapiro ME**, Onderdonk AB, Kasper DL, Finberg RW. Cellular immunity to Bacteroides fragilis capsular polysaccharide. *J Exp Med* 1982; **155**: 1188-1197 [PMID: 6174672]

13 **Gibson FC**, Tzianabos AO, Onderdonk AB. The capsular polysaccharide complex of Bacteroides fragilis induces cytokine production from human and murine phagocytic cells. *Infect Immun* 1996; **64**: 1065-1069 [PMID: 8641762]

14 **Baumann H**, Tzianabos AO, Brisson JR, Kasper DL, Jennings HJ. Structural elucidation of two capsular polysaccharides from one strain of Bacteroides fragilis using high-resolution NMR spectroscopy. *Biochemistry* 1992; **31**: 4081-4089 [PMID: 1567854]

15 **Tzianabos AO**, Onderdonk AB, Rosner B, Cisneros RL, Kas-

per DL. Structural features of polysaccharides that induce intra-abdominal abscesses. *Science* 1993; **262**: 416-419 [PMID: 8211161]
16 **Patrick S**, Reid JH, Coffey A. Capsulation of in vitro and in vivo grown Bacteroides species. *J Gen Microbiol* 1986; **132**: 1099-1109 [PMID: 3760820]
17 **Lutton DA,** Patrick S, Crockard AD, Stewart LD, Larkin MJ, Dermott E, McNeill TA.. Flow cytometric analysis of within-strain variation in polysaccharide expression by Bacteroides fragilis by use of murine monoclonal antibodies. *Journal of medical microbiology* 1991; **35**: 229-237 [PMID: 1719202]
18 **Kasper DL**. Chemical and biological characterization of the lipopolysaccharide of Bacteroides fragilis subspecies fragilis. *J Infect Dis* 1976; **134**: 59-66 [PMID: 939922 DOI: 10.1093/infdis/134.1.59]
19 **Comstock LE**, Coyne MJ, Tzianabos AO, Pantosti A, Onderdonk AB, Kasper DL. Analysis of a capsular polysaccharide biosynthesis locus of Bacteroides fragilis. *Infect Immun* 1999; **67**: 3525-3532 [PMID: 10377135]
20 **Coyne MJ**, Kalka-Moll W, Tzianabos AO, Kasper DL, Comstock LE. Bacteroides fragilis NCTC9343 produces at least three distinct capsular polysaccharides: cloning, characterization, and reassignment of polysaccharide B and C biosynthesis loci. *Infect Immun* 2000; **68**: 6176-6181 [PMID: 11035722 DOI: 10.1128/IAI.68.11.6176-6181.2000]
21 **Krinos CM**, Coyne MJ, Weinacht KG, Tzianabos AO, Kasper DL, Comstock LE. Extensive surface diversity of a commensal microorganism by multiple DNA inversions. *Nature* 2001; **414**: 555-558 [PMID: 11734857 DOI: 10.1038/35107092]
22 **Patrick S**, Blakely GW, Houston S, Moore J, Abratt VR, Bertalan M, Cerdeño-Tárraga AM, Quail MA, Corton N, Corton C, Bignell A, Barron A, Clark L, Bentley SD, Parkhill J. Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of Bacteroides fragilis. *Microbiology* 2010; **156**: 3255-3269 [PMID: 20829291 DOI: 10.1099/mic.0.042978-0]
23 **Coyne MJ**, Comstock LE. Niche-specific features of the intestinal bacteroidales. *J Bacteriol* 2008; **190**: 736-742 [PMID: 17993536 DOI: 10.1128/JB.01559-07]
24 **Mazmanian SK**, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; **122**: 107-118 [PMID: 16009137]
25 **Lee YK**, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science* 2010; **330**: 1768-1773 [PMID: 21205662 DOI: 10.1126/science.1195568]
26 **Sears CL**. The toxins of Bacteroides fragilis. *Toxicon* 2001; **39**: 1737-1746 [PMID: 11595636]
27 **Toprak NU**, Yagci A, Gulluoglu BM, Akin ML, Demirkalem P, Celenk T, Soyletir G. A possible role of Bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. *Clin Microbiol Infect* 2006; **12**: 782-786 [PMID: 16842574 DOI: 10.1111/j.1469-0691.2006.01494.x]
28 **Housseau F**, Sears CL. Enterotoxigenic Bacteroides fragilis (ETBF)-mediated colitis in Min (Apc+/-) mice: a human commensal-based murine model of colon carcinogenesis. *Cell Cycle* 2010; **9**: 3-5 [PMID: 20009569]
29 **Moncrief JS**, Duncan AJ, Wright RL, Barroso LA, Wilkins TD. Molecular characterization of the fragilysin pathogenicity islet of enterotoxigenic Bacteroides fragilis. *Infect Immun* 1998; **66**: 1735-1739 [PMID: 9529104]
30 **Franco AA**, Cheng RK, Chung GT, Wu S, Oh HB, Sears CL. Molecular evolution of the pathogenicity island of enterotoxigenic Bacteroides fragilis strains. *J Bacteriol* 1999; **181**: 6623-6633 [PMID: 10542162]
31 **Franco AA**. The Bacteroides fragilis pathogenicity island is contained in a putative novel conjugative transposon. *J Bacteriol* 2004; **186**: 6077-6092 [PMID: 15342577 DOI: 10.1128/JB.186.18.6077-6092.2004]
32 **Buckwold SL**, Shoemaker NB, Sears CL, Franco AA. Identification and characterization of conjugative transposons CTn86 and CTn9343 in Bacteroides fragilis strains. *Appl Environ Microbiol* 2007; **73**: 53-63 [PMID: 17071793 DOI: 10.1128/AEM.01669-06]
33 **Sears CL**. Enterotoxigenic Bacteroides fragilis: a rogue among symbiotes. *Clin Microbiol Rev* 2009; **22**: 349-369, Table of Contents [PMID: 19366918 DOI: 10.1128/CMR.00053-08]
34 **Rasmussen BA**, Bush K, Tally FP. Antimicrobial resistance in anaerobes. *Clin Infect Dis* 1997; **24** Suppl 1: S110-S120 [PMID: 8994790 DOI: 10.1093/clinids/24.Supplement_1.S110]
35 **Hecht DW**. Anaerobes: antibiotic resistance, clinical significance, and the role of susceptibility testing. *Anaerobe* 2006; **12**: 115-121 [PMID: 16765857]
36 **Snydman DR**, Jacobus NV, McDermott LA, Golan Y, Hecht DW, Goldstein EJ, Harrell L, Jenkins S, Newton D, Pierson C, Rihs JD, Yu VL, Venezia R, Finegold SM, Rosenblatt JE, Gorbach SL. Lessons learned from the anaerobe survey: historical perspective and review of the most recent data (2005-2007). *Clin Infect Dis* 2010; **50** Suppl 1: S26-S33 [PMID: 20067390 DOI: 10.1086/647940]
37 **Nagy E**, Urbán E, Nord CE. Antimicrobial susceptibility of Bacteroides fragilis group isolates in Europe: 20 years of experience. *Clin Microbiol Infect* 2011; **17**: 371-379 [PMID: 20456453 DOI: 10.1111/j.1469-0691.2010.03256.x]
38 **Phillips I,** King A, Nord CE, Hoffstedt B. Antibiotic sensitivity of the Bacteroides fragilis group in Europe. European Study Group. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 292-304 [PMID: 1396748]
39 **Hedberg M**, Nord CE. Antimicrobial susceptibility of Bacteroides fragilis group isolates in Europe. *Clin Microbiol Infect* 2003; **9**: 475-488 [PMID: 12848722]
40 **Rogers MB**, Parker AC, Smith CJ. Cloning and characterization of the endogenous cephalosporinase gene, cepA, from Bacteroides fragilis reveals a new subgroup of Ambler class A beta-lactamases. *Antimicrob Agents Chemother* 1993; **37**: 2391-2400 [PMID: 8285623 DOI: 10.1128/AAC.37.11.2391]
41 **Snydman DR**, Jacobus NV, McDermott LA, Ruthazer R, Golan Y, Goldstein EJ, Finegold SM, Harrell LJ, Hecht DW, Jenkins SG, Pierson C, Venezia R, Yu V, Rihs J, Gorbach SL. National survey on the susceptibility of Bacteroides fragilis group: report and analysis of trends in the United States from 1997 to 2004. *Antimicrob Agents Chemother* 2007; **51**: 1649-1655 [PMID: 17283189 DOI: 10.1128/AAC.01435-06]
42 **Parker AC**, Smith CJ. Genetic and biochemical analysis of a novel Ambler class A beta-lactamase responsible for cefoxitin resistance in Bacteroides species. *Antimicrob Agents Chemother* 1993; **37**: 1028-1036 [PMID: 8517690 DOI: 10.1128/AAC.37.5.1028]
43 **Smith CJ**, Parker AC. Identification of a circular intermediate in the transfer and transposition of Tn4555, a mobilizable transposon from Bacteroides spp. *J Bacteriol* 1993; **175**: 2682-2691 [PMID: 8386723]
44 **García N**, Gutiérrez G, Lorenzo M, García JE, Píriz S, Quesada A. Genetic determinants for cfxA expression in Bacteroides strains isolated from human infections. *J Antimicrob Chemother* 2008; **62**: 942-947 [PMID: 18775891 DOI: 10.1093/jac/dkn347]
45 **Sóki J**, Gonzalez SM, Urbán E, Nagy E, Ayala JA. Molecular analysis of the effector mechanisms of cefoxitin resistance among Bacteroides strains. *J Antimicrob Chemother* 2011; **66**: 2492-2500 [PMID: 21873290 DOI: 10.1093/jac/dkr339]
46 **Thompson JS**, Malamy MH. Sequencing the gene for an imipenem-cefoxitin-hydrolyzing enzyme (CfiA) from Bacteroides fragilis TAL2480 reveals strong similarity between CfiA and Bacillus cereus beta-lactamase II. *J Bacteriol* 1990;

172: 2584-2593 [PMID: 2110145]
47 **Rasmussen BA**, Gluzman Y, Tally FP. Cloning and sequencing of the class B beta-lactamase gene (ccrA) from Bacteroides fragilis TAL3636. *Antimicrob Agents Chemother* 1990; **34**: 1590-1592 [PMID: 2121094 DOI: 10.1128/AAC.34.8.1590]
48 **Podglajen I**, Breuil J, Casin I, Collatz E. Genotypic identification of two groups within the species Bacteroides fragilis by ribotyping and by analysis of PCR-generated fragment patterns and insertion sequence content. *J Bacteriol* 1995; **177**: 5270-5275 [PMID: 7545155]
49 **Gutacker M**, Valsangiacomo C, Piffaretti JC. Identification of two genetic groups in Bacteroides fragilis by multilocus enzyme electrophoresis: distribution of antibiotic resistance (cfiA, cepA) and enterotoxin (bft) encoding genes. *Microbiology* 2000; **146** (Pt 5): 1241-1254 [PMID: 10832652]
50 **Johnson JL.** Taxonomy of Bacteroides I. Deoxyribonucleic acid homologies among Bacteroides fragilis and other saccharolytitic Bacteroides species. *International Journal of Systematic Bacteriology* 1978; **28**: 245-256
51 **Ruimy R**, Podglajen I, Breuil J, Christen R, Collatz E. A recent fixation of cfiA genes in a monophyletic cluster of Bacteroides fragilis is correlated with the presence of multiple insertion elements. *J Bacteriol* 1996; **178**: 1914-1918 [PMID: 8606165]
52 **Wybo I**, De Bel A, Soetens O, Echahidi F, Vandoorslaer K, Van Cauwenbergh M, Piérard D. Differentiation of cfiA-negative and cfiA-positive Bacteroides fragilis isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2011; **49**: 1961-1964 [PMID: 21346046 DOI: 10.1128/JCM.02321-10]
53 **Nagy E**, Becker S, Sóki J, Urbán E, Kostrzewa M. Differentiation of division I (cfiA-negative) and division II (cfiA-positive) Bacteroides fragilis strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Med Microbiol* 2011; **60**: 1584-1590 [PMID: 21680764 DOI: 10.1099/jmm.0.031336-0]
54 **Roberts MC**. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. *FEMS Microbiol Lett* 2008; **282**: 147-159 [PMID: 18399991 DOI: 10.1111/j.1574-6968.2008.01145.x]
55 **Wang Y**, Wang GR, Shelby A, Shoemaker NB, Salyers AA. A newly discovered Bacteroides conjugative transposon, CTnGERM1, contains genes also found in gram-positive bacteria. *Appl Environ Microbiol* 2003; **69**: 4595-4603 [PMID: 12902247 DOI: 10.1128/AEM.69.8.4595-4603.2003]
56 **Ikeda H**, Wakisaka A. [HLA typing and application of monoclonal antibodies]. *Nihon Rinsho* 1983; **41**: 841-845 [PMID: 6887551]
57 **Diniz CG**, Farias LM, Carvalho MA, Rocha ER, Smith CJ. Differential gene expression in a Bacteroides fragilis metronidazole-resistant mutant. *J Antimicrob Chemother* 2004; **54**: 100-108 [PMID: 15150173 DOI: 10.1093/jac/dkh256]
58 **Carlier JP**, Sellier N, Rager MN, Reysset G. Metabolism of a 5-nitroimidazole in susceptible and resistant isogenic strains of Bacteroides fragilis. *Antimicrob Agents Chemother* 1997; **41**: 1495-1499 [PMID: 9210672]
59 **Leiros HK**, Kozielski-Stuhrmann S, Kapp U, Terradot L, Leonard GA, McSweeney SM. Structural basis of 5-nitroimidazole antibiotic resistance: the crystal structure of NimA from Deinococcus radiodurans. *J Biol Chem* 2004; **279**: 55840-55849 [PMID: 15492014 DOI: 10.1074/jbc.M408044200]
60 **Reysset G.** Genetics of 5-nitroimidazole resistance in Bacteroides species. *Anaerobe* 1996; **2**: 59-69 [PMID: 16887551]
61 **Patel EH**, Paul LV, Casanueva AI, Patrick S, Abratt VR. Overexpression of the rhamnose catabolism regulatory protein, RhaR: a novel mechanism for metronidazole resistance in Bacteroides thetaiotaomicron. *J Antimicrob Chemother* 2009; **64**: 267-273 [PMID: 19525515 DOI: 10.1093/jac/dkp203]
62 **Stubbs SL**, Brazier JS, Talbot PR, Duerden BI. PCR-restriction fragment length polymorphism analysis for identification of Bacteroides spp. and characterization of nitroimidazole resistance genes. *J Clin Microbiol* 2000; **38**: 3209-3213 [PMID: 10970359]
63 **Gal M**, Brazier JS. Metronidazole resistance in Bacteroides spp. carrying nim genes and the selection of slow-growing metronidazole-resistant mutants. *J Antimicrob Chemother* 2004; **54**: 109-116 [PMID: 15190033 DOI: 10.1093/jac/dkh296]
64 **Alauzet C**, Mory F, Teyssier C, Hallage H, Carlier JP, Grollier G, Lozniewski A. Metronidazole resistance in Prevotella spp. and description of a new nim gene in Prevotella baroniae. *Antimicrob Agents Chemother* 2010; **54**: 60-64 [PMID: 19805556 DOI: 10.1128/AAC.01003-09]
65 **Katsandri A**, Avlamis A, Pantazatou A, Houhoula DP, Papaparaskevas J. Dissemination of nim-class genes, encoding nitroimidazole resistance, among different species of Gram-negative anaerobic bacteria isolated in Athens, Greece. *J Antimicrob Chemother* 2006; **58**: 705-706 [PMID: 16867996 DOI: 10.1093/jac/dkl285]
66 **Lubbe MM**, Stanley K, Chalkley LJ. Prevalence of nim genes in anaerobic/facultative anaerobic bacteria isolated in South Africa. *FEMS Microbiol Lett* 1999; **172**: 79-83 [PMID: 10079531]
67 **Theron MM**, Janse Van Rensburg MN, Chalkley LJ. Nitroimidazole resistance genes (nimB) in anaerobic Gram-positive cocci (previously Peptostreptococcus spp.). *J Antimicrob Chemother* 2004; **54**: 240-242 [PMID: 15150170 DOI: 10.1093/jac/dkh270]
68 **Marchandin H**, Jean-Pierre H, Campos J, Dubreuil L, Teyssier C, Jumas-Bilak E. nimE gene in a metronidazole-susceptible Veillonella sp. strain. *Antimicrob Agents Chemother* 2004; **48**: 3207-3208 [PMID: 15273153 DOI: 10.1128/AAC.48.8.3207-3208.2004]
69 **Pal D**, Banerjee S, Cui J, Schwartz A, Ghosh SK, Samuelson J. Giardia, Entamoeba, and Trichomonas enzymes activate metronidazole (nitroreductases) and inactivate metronidazole (nitroimidazole reductases). *Antimicrob Agents Chemother* 2009; **53**: 458-464 [PMID: 19015349 DOI: 10.1128/AAC.00909-08]
70 **Salyers AA**, Shoemaker NB, Li LY. In the driver's seat: the Bacteroides conjugative transposons and the elements they mobilize. *J Bacteriol* 1995; **177**: 5727-5731 [PMID: 7592315]
71 **Whittle G**, Hund BD, Shoemaker NB, Salyers AA. Characterization of the 13-kilobase ermF region of the Bacteroides conjugative transposon CTnDOT. *Appl Environ Microbiol* 2001; **67**: 3488-3495 [PMID: 11472924 DOI: 10.1128/AEM.67.8.3488-3495.2001]
72 **Yang W**, Moore IF, Koteva KP, Bareich DC, Hughes DW, Wright GD. TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. *J Biol Chem* 2004; **279**: 52346-52352 [PMID: 15452119 DOI: 10.1074/jbc.M409573200]
73 **Bartha NA**, Sóki J, Urbán E, Nagy E. Investigation of the prevalence of tetQ, tetX and tetX1 genes in Bacteroides strains with elevated tigecycline minimum inhibitory concentrations. *Int J Antimicrob Agents* 2011; **38**: 522-525 [PMID: 22014885]
74 **Nagy E**. Anaerobic infections: update on treatment considerations. *Drugs* 2010; **70**: 841-858 [PMID: 20426496 DOI: 10.2165/11534490]
75 **Mahillon J**, Chandler M. Insertion sequences. *Microbiol Mol Biol Rev* 1998; **62**: 725-774 [PMID: 9729608]
76 **Chandler M,** Mahillon J. Insertion sequences revisited. In: Craigh NL, Craigie R, Gellert M, Lambowitz AM, editors. Mobile DNA II. Washington, DC: ASM Press, 2002: 305-366
77 **Siguier P**, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006; **34**: D32-D36 [PMID: 16381877 DOI: 10.1093/nar/gkj014]

78 **Prentki P,** Teter B, Chandler M, Galas DJ. Functional promoters created by the insertion of transposable element IS1. *J Mol Biol* 1986;**191**:383-393. [PMID: 3029382 DOI: 10.1111/j.1574-6968.2006.00195.x]

79 **Turton JF**, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. *FEMS Microbiol Lett* 2006; **258**: 72-77 [PMID: 16630258]

80 **Evans JC**, Segal H. A novel insertion sequence, ISPA26, in oprD of Pseudomonas aeruginosa is associated with carbapenem resistance. *Antimicrob Agents Chemother* 2007; **51**: 3776-3777 [PMID: 17682099 DOI: 10.1128/AAC.00837-07]

81 **Umenhoffer K**, Fehér T, Balikó G, Ayaydin F, Pósfai J, Blattner FR, Pósfai G. Reduced evolvability of Escherichia coli MDS42, an IS-less cellular chassis for molecular and synthetic biology applications. *Microb Cell Fact* 2010; **9**: 38 [PMID: 20492662 DOI: 10.1186/1475-2859-9-38]

82 **Bouma JE**, Lenski RE. Evolution of a bacteria/plasmid association. *Nature* 1988; **335**: 351-352 [PMID: 3047585 DOI: 10.1038/335351a0]

83 **Tally FP**, Snydman DR, Gorbach SL, Malamy MH. Plasmid-mediated, transferable resistance to clindamycin and erythromycin in Bacteroides fragilis. *J Infect Dis* 1979; **139**: 83-88 [PMID: 438530 DOI: 10.1093/infdis/139.1.83]

84 **Smith CJ**, Tribble GD, Bayley DP. Genetic elements of Bacteroides species: a moving story. *Plasmid* 1998; **40**: 12-29 [PMID: 9657930]

85 **Shoemaker NB**, Guthrie EP, Salyers AA, Gardner JF. Evidence that the clindamycin-erythromycin resistance gene of Bacteroides plasmid pBF4 is on a transposable element. *J Bacteriol* 1985; **162**: 626-632 [PMID: 2985540]

86 **Smith CJ**. Characterization of Bacteroides ovatus plasmid pBI136 and structure of its clindamycin resistance region. *J Bacteriol* 1985; **161**: 1069-1073 [PMID: 2982783]

87 **Podglajen I**, Breuil J, Bordon F, Gutmann L, Collatz E. A silent carbapenemase gene in strains of Bacteroides fragilis can be expressed after a one-step mutation. *FEMS Microbiol Lett* 1992; **70**: 21-29 [PMID: 1577251]

88 **Podglajen I**, Breuil J, Collatz E. Insertion of a novel DNA sequence, 1S1186, upstream of the silent carbapenemase gene cfiA, promotes expression of carbapenem resistance in clinical isolates of Bacteroides fragilis. *Mol Microbiol* 1994; **12**: 105-114 [PMID: 8057831 DOI: 10.1111/j.1365-2958.1994.tb00999.x]

89 **Edwards R**, Hawkyard CV, Garvey MT, Greenwood D. Prevalence and degree of expression of the carbapenemase gene (cfiA) among clinical isolates of Bacteroides fragilis in Nottingham, UK. *J Antimicrob Chemother* 1999; **43**: 273-276 [PMID: 11252334 DOI: 10.1093/jac/43.2.273]

90 **Kato N**, Yamazoe K, Han CG, Ohtsubo E. New insertion sequence elements in the upstream region of cfiA in imipenem-resistant Bacteroides fragilis strains. *Antimicrob Agents Chemother* 2003; **47**: 979-985 [PMID: 12604530 DOI: 10.1128/AAC.47.3.979-985.2003]

91 **Sóki J**, Fodor E, Hecht DW, Edwards R, Rotimi VO, Kerekes I, Urbán E, Nagy E. Molecular characterization of imipenem-resistant, cfiA-positive Bacteroides fragilis isolates from the USA, Hungary and Kuwait. *J Med Microbiol* 2004; **53**: 413-419 [PMID: 15096551 DOI: 10.1099/jmm.0.05452-0]

92 **Sóki J**, Edwards R, Hedberg M, Fang H, Nagy E, Nord CE. Examination of cfiA-mediated carbapenem resistance in Bacteroides fragilis strains from a European antibiotic susceptibility survey. *Int J Antimicrob Agents* 2006; **28**: 497-502 [PMID: 17097857]

93 **Walsh TR**, Onken A, Haldorsen B, Toleman MA, Sundsfjord A. Characterization of a carbapenemase-producing clinical isolate of Bacteroides fragilis in Scandinavia: genetic analysis of a unique insertion sequence. *Scand J Infect Dis* 2005; **37**: 676-679 [PMID: 16126569 DOI: 10.1080/00365540510034482]

94 **Cascio GL**, Macaccaro L, Centonze AR, Sóki J, Fontana R, Mazzariol A. A new insertion sequence element containing a cfiA gene in the first imipenem-resistant Bacteroides fragilis strain isolated in Italy. *Int J Antimicrob Agents* 2009; **34**: 610-611 [PMID: 19744834]

95 **Roh KH**, Kim S, Kim CK, Yum JH, Kim MS, Yong D, Jeong SH, Lee K, Kim JM, Chong Y. New cfiA variant and novel insertion sequence elements in carbapenem-resistant Bacteroides fragilis isolates from Korea. *Diagn Microbiol Infect Dis* 2010; **66**: 343-348 [PMID: 20226324]

96 **Rogers MB**, Bennett TK, Payne CM, Smith CJ. Insertional activation of cepA leads to high-level beta-lactamase expression in Bacteroides fragilis clinical isolates. *J Bacteriol* 1994; **176**: 4376-4384 [PMID: 7517394]

97 **Tribble GD**, Parker AC, Smith CJ. Genetic structure and transcriptional analysis of a mobilizable, antibiotic resistance transposon from Bacteroides. *Plasmid* 1999; **42**: 1-12 [PMID: 10413660]

98 **De Palmenaer D**, Siguier P, Mahillon J. IS4 family goes genomic. *BMC Evol Biol* 2008; **8**: 18 [PMID: 18215304 DOI: 10.1186/1471-2148-8-18]

99 **Sóki J**, Edwards R, Urbán E, Fodor E, Beer Z, Nagy E. Screening of isolates from faeces for carbapenem-resistant Bacteroides strains; existence of strains with novel types of resistance mechanisms. *Int J Antimicrob Agents* 2004; **24**: 450-454 [PMID: 15519476]

100 **Sóki J**, Urban E, Szöke I, Fodor E, Nagy E. Prevalence of the carbapenemase gene (cfiA) among clinical and normal flora isolates of Bacteroides species in Hungary. *J Med Microbiol* 2000; **49**: 427-430 [PMID: 10798555]

101 **Yamazoe K**, Kato N, Kato H, Tanaka K, Katagiri Y, Watanabe K. Distribution of the cfiA gene among Bacteroides fragilis strains in Japan and relatedness of cfiA to imipenem resistance. *Antimicrob Agents Chemother* 1999; **43**: 2808-2810 [PMID: 10543771]

102 **Sóki J**, Gal M, Brazier JS, Rotimi VO, Urbán E, Nagy E, Duerden BI. Molecular investigation of genetic elements contributing to metronidazole resistance in Bacteroides strains. *J Antimicrob Chemother* 2006; **57**: 212-220 [PMID: 16338949 DOI: 10.1093/jac/dki443]

103 **Coque TM.** Mobilization of bla genes at the origin of ESBL and AmpC dissemination. In: 20th European Congress of Clinical Microbiology and Infectious Disease; 2010. Vienna: Wiley-Blackwell, 2010: S272

104 **Baquero F.** Modularization and evolvability in antibiotic resistance. In: Baquero F, Nombrella C, Cassel GH, Gutierrez-Fuentes JA, editors. Evolutionary Biology of Bacterial and Fungal Pathogens. Washington, DC: ASM Press, 2008: 233-247

105 **Turner P,** Edwards R, Weston V, Gazis A, Ispahani P, Greenwood D. Simultaneous resistance to metronidazole, co-amoxiclav, and imipenem in clinical isolate of Bacteroides fragilis. *Lancet* 1995; **345**: 1275-1277 [PMID: 7746059]

106 **Bayley DP**, Rocha ER, Smith CJ. Analysis of cepA and other Bacteroides fragilis genes reveals a unique promoter structure. *FEMS Microbiol Lett* 2000; **193**: 149-154 [PMID: 11094294]

107 **Vingadassalom D,** Kolb A, Mayer C, Rybkine T, Collatz E, Podglajen I. An unusual primary sigma factor in the Bacteroidetes phylum. *Mol Microbiology* 2005; **56**: 888-902 [PMID: 15853878]

108 **Podglajen I,** Breuil J, Rohaut A, Monsempes C, Collatz E. Multiple mobile promoter regions for the rare carbapenem resistance gene of Bacteroides fragilis. *J Bacteriol* 2001; **183**: 3531-3535 [PMID: 11344163]

109 **Bachoual R**, Dubreuil L, Soussy CJ, Tankovic J. Roles of gyrA mutations in resistance of clinical isolates and in vitro mutants of Bacteroides fragilis to the new fluoroquinolone trovafloxacin. *Antimicrob Agents Chemother* 2000; **44**: 1842-1845 [PMID: 10858340]

110 **Wang Y**, Rotman ER, Shoemaker NB, Salyers AA. Translational control of tetracycline resistance and conjugation in the Bacteroides conjugative transposon CTnDOT. *J Bac-*

teriol 2005; **187**: 2673-2680 [PMID: 15805513 DOI: 10.1128/JB.187.8.2673-2680.2005]
111 **Jeters RT**, Wang GR, Moon K, Shoemaker NB, Salyers AA. Tetracycline-associated transcriptional regulation of transfer genes of the Bacteroides conjugative transposon CTn-DOT. *J Bacteriol* 2009; **191**: 6374-6382 [PMID: 19700528 DOI: 10.1128/JB.00739-09]
112 **Miyamae S**, Ueda O, Yoshimura F, Hwang J, Tanaka Y, Nikaido H. A MATE family multidrug efflux transporter pumps out fluoroquinolones in Bacteroides thetaiotaomicron. *Antimicrob Agents Chemother* 2001; **45**: 3341-3346 [PMID: 11709306 DOI: 10.1128/AAC.45.12.3341-3346.2001]
113 **Pumbwe L**, Ueda O, Yoshimura F, Chang A, Smith RL, Wexler HM. Bacteroides fragilis BmeABC efflux systems additively confer intrinsic antimicrobial resistance. *J Antimicrob Chemother* 2006; **58**: 37-46 [PMID: 16757501 DOI: 10.1093/jac/dkl202]
114 **Sebald M,** Reysset G, Breuil J. What's new in 5-nitroimidazole resistance in the Bacteroides fragilis group? In: Borriello SP, editor. Clinical and Medical Aspects of Anaerobes: Wrightson Boimedical Publishing Ltd., 1990: 217-225
115 **Reysset G**, Haggoud A, Sebald M. Genetics of resistance of Bacteroides species to 5-nitroimidazole. *Clin Infect Dis* 1993; **16 Suppl 4**: S401-S403 [PMID: 8324155 DOI: 10.1093/clinids/16.Supplement_4.S401]
116 **Löfmark S**, Fang H, Hedberg M, Edlund C. Inducible metronidazole resistance and nim genes in clinical Bacteroides fragilis group isolates. *Antimicrob Agents Chemother* 2005; **49**: 1253-1256 [PMID: 15728943 DOI: 10.1128/AAC.49.3.1253-1256.2005]
117 **Trinh S**, Haggoud A, Reysset G, Sebald M. Plasmids pIP419 and pIP421 from Bacteroides: 5-nitroimidazole resistance genes and their upstream insertion sequence elements. *Microbiology* 1995; **141** (Pt 4): 927-935 [PMID: 7773395 DOI: 10.1099/13500872-141-4-927]
118 **Sherwood JE**, Fraser S, Citron DM, Wexler H, Blakely G, Jobling K, Patrick S. Multi-drug resistant Bacteroides fragilis recovered from blood and severe leg wounds caused by an improvised explosive device (IED) in Afghanistan. *Anaerobe* 2011; **17**: 152-155 [PMID: 21376821]

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