Chapter 20
Antimicrobial Resistance and Drug Efflux Pumps in *Bacteroides*

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Abstract *Bacteroides* spp. constitute an important part of the commensal intestinal microbiota, but some species such as *Bacteroides fragilis* are associated with human infections. There is an increasing occurrence of acquired antimicrobial resistance including multidrug resistance in *Bacteroides* spp., which, together with the limited availability of anti-anaerobe antimicrobials, raises a concern for effective therapy of *Bacteroides* infections. This chapter provides a current overview on antimicrobial susceptibility and resistance mechanisms of *Bacteroides* with detailed descriptions of the known drug efflux pumps, which contribute to both intrinsic and acquired resistance.

Keywords *Bacteroides* • Antimicrobial resistance • Multidrug resistance • Efflux • RND pumps

20.1 Introduction

In terms of bacterial classification and taxonomy, the genus *Bacteroides* is composed of >40 species. It includes the *Bacteroides fragilis* group comprising the most frequent clinically isolated species from human biological samples [1]. Bacteria of this genus are anaerobic, bile-resistant, non-spore forming, Gram-negative rods. They are part of the indigenous microbiota of the human and animal gastrointestinal tracts but can be found in other locations such as the mouth, the upper respiratory tract, and urogenital tract. The *Bacteroides* spp. of the *fragilis* group are the predominant microorganisms isolated by culture methods from feces. Metagenomic sequencing has confirmed that *Bacteroides* is a predominant genus of the gastrointestinal tract microbiota [2].

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Although *Bacteroides* are commensal microorganisms that play an important role in human health [2–4], some species are associated with human mixed infections such as intra-abdominal, obstetric-gynecologic, postoperative wound, complicated skin, and soft tissue infections. They are also causative agents of bacteremia [5]. Among the *B. fragilis* group, *B. fragilis* and *Bacteroides thetaiotaomicron* are the most frequently isolated species from clinical samples. *B. fragilis* may account for 40–78 % of the *Bacteroides* isolates recovered from intra-abdominal as well as other infections [1, 6]; *B. thetaiotaomicron* may account for 10–23 % of the isolates [1, 6]. However, while *B. fragilis* is the predominant species isolated from clinical samples, it is not the case in feces where other intestinal *Bacteroides* species are more frequently isolated [7].

Bacteroides spp. have been considered routinely susceptible to a number of broad-spectrum anti-anaerobic molecules. However, surveys following the long-term resistance trends of *Bacteroides* have reported an overall increase in resistance to classical and more modern antimicrobial agents [6, 8–12]. Although the numbers are still low, multidrug-resistant strains have been reported worldwide [13–18]. Moreover, a new multidrug-resistant species of *Bacteroides* was recently identified [19].

Among the different mechanisms for antimicrobial resistance, efflux transporters have been documented in *Bacteroides* spp. This chapter summarizes antimicrobial susceptibility and resistance mechanisms and subsequently provides an up-to-date description of efflux transporters among species of the *Bacteroides* and particularly the *B. fragilis* group. For more information about *Bacteroides* spp. and their commensal role, and their involvement in human disease or information about their physiology, metabolism, and clinical characteristics, several recent reviews are available [1, 5, 7].

20.2 Antimicrobial Susceptibility

Antimicrobial susceptibility of *Bacteroides* spp. has been monitored through national and regional surveys in different countries [6, 8–12, 18]. Even though there are geographic and institutional variations, resistance rates are dependent on the particular species and can therefore vary widely. For instance, *B. fragilis* is frequently more susceptible to many antimicrobial agents in comparison with other species of the *B. fragilis* group such as *Bacteroides vulgatus* and *B. thetaiotaomicron* [1, 5].

Briefly, based on the most recently published surveys [1, 6, 8–10], lower antimicrobial resistance rates of *B. fragilis* group were observed for: (i) carbapenems such as imipenem and meropenem (resistance rates are, respectively, from 0–1.2 % to 1–7.5 %), (ii) chloramphenicol (none reported), (iii) tigecycline (0–8 %), and (iv) metronidazole (0–1 %). Differences in β-lactam/β-lactamase inhibitor activity were observed when the susceptibility and resistance breakpoints from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used to evaluate *Bacteroides* resistance

rates. For example, piperacillin-tazobactam and amoxicillin/clavulanic acid show low antimicrobial resistance rates. All these antimicrobial agents are considered clinically relevant for the *B. fragilis* group infection therapy. However, anaerobic species identification and antimicrobial susceptibility testing are important in this regard [20]. Moreover, there are increasing reports of antimicrobial resistance including carbapenem and multidrug resistance in *Bacteroides* [16–18, 21–26], which is expected to affect the effective therapy regimens.

20.3 Antimicrobial Resistance Determinants

Currently, *B. fragilis* group species are considered today to be one of the most antimicrobial resistant among human pathogenic anaerobes. Indeed, as part of the commensal microbiota in the intestine, *Bacteroides* spp. exhibit high-level intrinsic resistance to bile salts [27] and inflammation-associated antimicrobial peptides [4] which are associated with *Bacteroides* fitness [4].

β-Lactamase production is the most common mechanism of resistance to β-lactam agents in the *B. fragilis* group. Cephalosporinase genes, *cepA* from *B. fragilis* [28], *cfxA* from *Bacteroides vulgatus* [29], and *cblA* from *Bacteroides uniformis* [30], have been identified. These enzymes are inhibited by the most commonly used β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam). For a minority of *B. fragilis* strains, carbapenem resistance is associated with a chromosomally encoded carbapenemase gene (*cfiA*) that may be "silent" or overexpressed [31]. There is evidence that resistance of *Bacteroides* spp. to β-lactams can be conferred by alteration of penicillin-binding proteins [32, 33]. Also, changes in outer membrane permeability barrier and particularly porin proteins seem to contribute to β-lactam resistance [34, 35]. However, the relative contributions of permeability changes, production of inactivating enzymes, and target modification to antimicrobial resistance needs to be clarified.

Resistance to macrolides (e.g., erythromycin), lincosamides (e.g., clindamycin), and streptogramins (e.g., pristinamycin and virginiamycin) is attributed to the macrolide-lincosamide-streptogramin B (MLS_B)-type *erm* genes encoding methylases that methylate the ribosome target and thus generate resistance by target modification [36]. Fluoroquinolone resistance has been primarily attributed to mutations in the DNA gyrase and topoisomerase genes and particularly in the quinolone resistance-determining region of the *gyrA* gene [37]. *Bacteroides* mechanism of chloramphenicol resistance has been associated with inactivation of the antibiotic by acetyltransferase [38]. The most common type of *Bacteroides* tetracycline resistance mechanisms is mediated by chromosomal genes encoding ribosomal protection proteins, such as TetQ [36]. Some strains were shown to harbor the *tetX* gene encoding flavin-dependent monooxygenase that inactivates tetracyclines in the presence of oxygen [39, 40].

Metronidazole, an important agent with anti-anaerobic activity is one of the mainstay drugs for the treatment of anaerobic infections. Its resistance is generally

attributed to the presence of *nim* genes that encode 5-nitroimidazole reductase enzymes which convert metronidazole to a nontoxic amine derivative [41]. Expression of a new *nim* gene (dubbed *nimJ*) was found in two multidrug-resistant clinical isolates, but the *nimJ* gene alone was considered to be unable to confer high-level resistance to metronidazole [21]. Of note, a recent study failed to confirm the protection of *B. fragilis* by Nim proteins from metronidazole [42]. Description of *nim*-negative strains resistant to metronidazole led to propose alternative resistance mechanisms [7]. Indeed, because of metronidazole mode of action, modification of bacterial metabolic or DNA repair activity may participate in resistance.

Bacteroides spp. possess a wide range of mobile genetic elements. These include plasmids, conjugative/mobilizable transposons, and bacteriophages [5]. Plasmids are common and can be found in 20–50 % of the *Bacteroides*; conjugative transposons are considered ubiquitous; they can be found in over 80 % of the *Bacteroides* spp. [5]. Many of these mobile genetic elements carry genes conferring resistance to the different classes of antimicrobials [1, 5, 7].

Metronidazole resistance *nim* genes have been identified on transferable plasmids [43, 44]. Transferable plasmid-linked chloramphenicol acetyltransferase conferring high-level resistance was documented for a clinical isolate of *B. uniformis* [45]. *Bacteroides* chromosomal genes encoding cephalosporinases can be transferred among species of the *B. fragilis* group. MLS_B-type *erm* genes are transferrable within and between *Bacteroides* spp. via conjugative plasmids or chromosomally located self-transmissible conjugative elements [1, 5, 7]. The ribosomal protection protein TetQ can be transferred by conjugative transposition among *Bacteroides* spp. but also between *Bacteroides* spp. and other bacteria [1, 5, 7]. Additionally, antimicrobial resistance expression has been correlated with activating insertion sequence (IS) elements located upstream of the *cfiA* gene [23], macrolide resistance genes [46], and metronidazole resistance *nim* genes [23]. Classically, anaerobes are naturally resistant to aminoglycosides as uptake of these antibiotics is likely dependent on oxygen- or nitrate-dependent electron transport chain which is lacking [47]. However, current recognition of multiple mechanisms including involvement of drug efflux mechanisms in aminoglycoside resistance requires more investigations of the intrinsic aminoglycoside resistance in *Bacteroides*. Moreover, a transposon carrying a streptomycin resistance gene (*addS*) was reported in *B. fragilis* [48].

Bacteroides antimicrobial resistance levels are linked to the distribution of the genetic determinants that are suspected or proven to be responsible for the resistance phenotypes. Based on the data of different surveys [12, 49–51], the most frequent resistance genes identified among resistant clinical isolates of *Bacteroides* spp. are *cepA*, *cfxA*, and *cfiA* for resistance to β-lactams, *ermF* (encoding 23S rRNA methyltransferase) for resistance to MLS_B group antibiotics, *nim* genes for metronidazole resistance, and *tet*Q for tetracycline resistance. Other less frequent resistance genes may also be found such as *tetX* for resistance to tetracyclines and glycylcyclines [39, 40], *mrs*(*SA*) for streptogramin resistance [52], or *bexA* for fluoroquinolone resistance [12, 49, 53].

To date, low numbers of multidrug-resistant strains have been reported worldwide for *Bacteroides* [13–18, 24]. Analysis of a multidrug-resistant clinical iso-

late of *B. fragilis* by whole genome sequencing revealed the presence of *nimF*, *cfiA*, and *erm* genes, respectively, related to metronidazole, carbapenem, and clindamycin resistance [24]. With the same approach, a metronidazole- and carbapenem-resistant *B. thetaiotaomicron* isolate was shown to contain *cat*, *ermF*, *nim*, *tetQ*, *tetX*, β-lactamase genes, and several efflux genes [25]. The multidrug resistance phenotype is attributed to several mechanisms including efflux transporters (see below).

20.4 Drug Efflux Pumps

Efflux systems in aerobic Gram-negative bacteria have been extensively studied [54] in contrast to anaerobes. With regard to *Bacteroides*, most of the available data concerns *B. fragilis* more than any other *Bacteroides* spp. The documented efflux pumps of the resistance-nodulation-cell division (RND) superfamily have been shown to transport β-lactams, fluoroquinolones, or metronidazole [15, 55–59]. These systems also transport other substrates such as ethidium bromide, triclosan, sodium dodecyl sulfate [57], bile salts [27], or quorum-sensing homoserine lactone autoinducers [60]. An exporter of the multidrug and toxic compound extrusion (MATE) family in *B. thetaiotaomicron* has been associated with norfloxacin, ciprofloxacin, and ethidium bromide efflux [53]. While numerous studies have been conducted to characterize the drug efflux systems of the RND superfamily (see below), macrolide and tetracycline transporters of the ATP-binding cassette (ABC) superfamily and the major facilitator superfamily (MFS) have also been reported [38, 52, 61] with some being found in conjugative transposons [62]. However, even though such efflux encoding genes were found, their substrate specificity has not yet been demonstrated.

20.4.1 RND Drug Efflux Pumps

Bioinformatic analysis of transporter proteins indicated that *B. fragilis* NCTC 9343 and YCH46 genomes has up to 18 putative RND-type proteins representing over 14 % of its total secondary transporters (TransportDB at http://www.membranetransport.org. Accessed on March 15, 2016). In *B. thetaiotaomicron* VPI 5482 and 19 RND-type putative efflux pumps (14 % of the total secondary transporters) are putatively identified but have not yet been characterized. Some of these RND pump homologs were also confirmed in the genome of a multidrug-resistant clinical isolate of *B. thetaiotaomicron* [25].

On the basis of homology with the *mexAB-oprM* efflux system genes of *Pseudomonas aeruginosa* [63, 64], Ueda et al. identified 16 chromosomal RND-type efflux pump genes, named *bmeABC1* to *bmeABC16* (for *B. fragilis* multidrug efflux) [65]. In terms of genetic organization, each operon encodes all genes for the

tripartite efflux components corresponding to the pump (*bmeB*), the membrane fusion protein (*bmeA*), and the outer membrane channel (*bmeC*). The arrangements of the different genes may vary within an operon. Two unusual features have been identified: the *bmeC10* outer membrane component gene may be fused with the *bmeB10* pump gene and two functional pump genes (*bmeB11* and *bmeB11*') are transcribed separately in *bme11*.

Functional characterization of the BmeABC RND efflux pumps in *B. fragilis* showed that 15 transcripts out of the 16 operons were detectable [57]. At least seven BmeB efflux pumps are considered functional in transporting antimicrobials and have overlapping broad substrate profiles, and four of them are involved in intrinsic resistance [65]. Deletion of the *bmeB3* gene resulted in the increased susceptibility of the mutant strain to β-lactams, fluoroquinolones, ethidium bromide, sodium dodecyl sulfate, and triclosan [66]. Expression of *bmeB3* pump in a hypersusceptible strain of *Escherichia coli* resulted in moderately higher minimal inhibitory concentrations (MICs) of several antimicrobial agents in this host [65]. The *bmeB5* gene was shown to be overexpressed in a metronidazole-resistant laboratory mutant of *B. fragilis*. Inactivation of BmeABC5 yielded a fourfold reduction in the metronidazole MIC and also increased susceptibility to other agents [65]. Single and multiple deletions of selected *bmeB* genes caused changes in MICs, which could be reduced by efflux pump inhibitors [57, 58, 65]. Interestingly, the deletion of more than two *bmeB* genes resulted in increased expression of other genes with corresponding MIC increase [57].

20.4.2 MATE Drug Efflux Pumps

In *B. fragilis* NCTC 9343 and YCH46 and *B. thetaiotaomicron* VPI 5482, 13 MATE-type putative efflux pumps (representing ca.10 % of the total transporters) were identified (TransportDB at http://www.membranetransport.org). To date, only one MATE-type efflux system, BexA, has been characterized in *B. thetaiotaomicron* [53]. This MATE pump is involved in the transport of norfloxacin, ciprofloxacin, and ethidium bromide.

20.4.3 Other Efflux Pumps

Based on a DNA microarray profiling of bacterial genes conferring resistance to macrolides, Cossone et al. identified in *B. fragilis* an ABC-type efflux gene homolog of the *msr*(*SA*) gene of *Staphylococcus aureus* [52]. Also, an MFS-type efflux pump homologous to the MefA transporter from *Streptococcus pyogenes* has been found on conjugative transposons in *Bacteroides* spp. [61]. More recently, three putative efflux pump genes coding for a MefA homolog, an ABC- and RND-type transporters were found in a conjugative transposon isolated from a multidrug-resistant clinical isolate of

B. fragilis [62]. A Mef homolog was also observed in *B. thetaiotaomicron* [25]. However, their contribution to and relevance in antimicrobial resistance remain unknown.

20.4.4 Regulation of Drug Efflux Pumps

Pumbwe and coworkers identified a putative TetR-family regulator gene (*bmeR5*) located upstream of the *bmeABC5* operon of a metronidazole-resistant *B. fragilis* laboratory mutant [58]. Experimental evidence demonstrated that BmeR5 is a local repressor of *bmeABC5* transcription and that mutations in the regulatory sequence intergenic region recognized by BmeR5 can lead to a depression and resistance to multiple antimicrobials. A multidrug-resistant clinical isolate of *B. fragilis* with increased *bmeABC5* expression was reported to show a point mutation in this specific region [58]. The same group reported that bile salts affected the transcription levels of 13 out of the 16 *bmeB* efflux pump genes of *B. fragilis* [27]: *bmeB5*, *bmeB6*, *bmeB15*, and *bmeB16* were overexpressed, and reduced expression was observed for *bmeB1* and *bmeB14*. Homoserine lactones were also revealed to modulate expression of *bmeB* efflux genes (*bmeB3*, *bmeB6*, *bmeB7*, and *bmeB10*) [60].

Since *B. fragilis* possesses a large number of RND efflux pumps and because of the documented emergence of isolates with high-level multidrug resistance phenotype, Pumbwe et al. [67] searched for the existence of putative *marA*-like global regulators. The authors showed that two putative AraC-type MarA homologs were induced by benzene and benzene-derived active compounds and suggested their role in a MarA-like system [59]. Like for other microorganisms [54], these data suggest that *Bacteroides* may turn on certain genes such as efflux pumps to extrude toxic compounds in addition to antimicrobial agents.

20.4.5 Efflux and Multidrug Resistance

Spontaneous resistant mutants relating to enhanced efflux pump activity have been reported in *Bacteroides* [55, 68]. The respective potentials of various antimicrobial agents to select for multidrug-resistant mutants of a wild-type *B. fragilis* strain and a quadruple RND efflux pump deletion have been investigated *in vitro* [66]. Out of 21 molecules tested, ampicillin, cefoxitin, doripenem, imipenem, levofloxacin, metronidazole, and sodium dodecyl sulfate selected mutants overexpressing one or more efflux pumps.

In *B. fragilis* clinical strains, the relationship between *bmeB* efflux pump overexpression and resistance to clinically relevant fluoroquinolones and β-lactams was investigated. The data suggested that low- to intermediate-level resistance to fluoroquinolone and high-level β-lactam resistance were correlated to *bmeB* efflux pump expression [56]. Such studies also confirmed a wide presence of resistance efflux gene overexpression in a number of clinical isolates [49, 56].

There have been reports of *B. fragilis* multidrug-resistant clinical isolates [13–18, 21, 24]. Although not all strains were genetically characterized, the multidrug resistance phenotype was potentially attributable to both chromosomal and plasmid-encoded resistance determinants. For one isolate, the RND-type efflux pump genes *bmeB9* and *bmeB15* were shown to be significantly overexpressed, and the addition of efflux pump inhibitors significantly increased susceptibility of the isolate to several structurally unrelated antimicrobials [15]. Thus, drug efflux likely contributes to resistance in multidrug-resistant isolates of *B. fragilis*. *Bacteroides* resistance to antimicrobial agents can potentially arise upon antimicrobial exposure, and particularly high-level resistance is obtained when efflux mechanisms are present in association with other mechanisms [37].

20.5 Concluding Remarks

Antimicrobial susceptibility surveillance reports highlight the increasing level of *Bacteroides* resistance to antimicrobial drugs commonly used for the treatment of anaerobic infections. In addition, some multidrug-resistant *B. fragilis* strains have been reported worldwide. The exposure of *Bacteroides* spp. to antimicrobial agents during infections or surgery prophylaxis therapies may indubitably select for multidrug-resistant strains. In this process, overexpression of efflux pumps participates to the development of the high level of antimicrobial resistance in *Bacteroides* spp. when associated in combination with other endogenous and/or exogenous resistance mechanisms. Among the putative transporters belonging to the different efflux transporter families, only several of them have been genetically and functionally characterized in *Bacteroides* spp. In terms of physiological functions, it is hypothesized that efflux systems may be relevant to *Bacteroides* adaptation for surviving in the gut and that this may be their primary function.

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