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MINIREVIEW

Time for Some Group Therapy: Update on Identification, Antimicrobial Resistance, Taxonomy, and Clinical Significance of the *Bacteroides fragilis* Group

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ABSTRACT *Bacteroides fragilis* group (BFG) species are common members of the human microbiota that provide several benefits to healthy hosts, yet BFG are also the most common anaerobes isolated from human infections, including intra-abdominal infections, abscesses, and bloodstream infection. Compared to many other anaerobes associated with disease, members of the BFG are more likely to be resistant to commonly used antimicrobials, including penicillin (>90% resistant), carbapenems (2 to 20% resistant), and metronidazole (0.2 to 4% resistant). As a result, infection with BFG bacteria can be associated with poor clinical outcomes. Here, we discuss the role of BFG in human health and disease, proposed taxonomic reclassifications within the BFG, and updates in methods for species-level identification. The increasing availability of whole-genome sequencing (WGS) supports recent proposals that the BFG now span two families (*Bacteroidaceae* and "Tannerellaceae") and multiple genera (*Bacteroides*, *Parabacteroides*, and *Phocaeicola*) within the phylum *Bacteroidota*. While members of the BFG are often reported to "group" rather than "species" level in many clinical settings, new reports of species-specific trends in antimicrobial resistance profiles and improved resolution of identification tools support routine species-level reporting in clinical practice. Empirical therapy may not be adequate for treatment of serious infections with BFG, warranting susceptibility testing for serious infections. We summarize methods for antimicrobial susceptibility testing and resistance prediction for BFG, including broth microdilution, agar dilution, WGS, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). We examine global trends in BFG antimicrobial resistance and review genomics of BFG, revealing insights into rapid activation and dissemination of numerous antimicrobial resistance mechanisms.

KEYWORDS *Bacteroides*, *Parabacteroides*, *Phocaeicola*, MALDI-TOF MS, whole-genome sequencing, antibiotic resistance, antimicrobial agents, taxonomy

Bacteroides, *Parabacteroides*, and *Phocaeicola* species are among the anaerobic organisms most frequently recovered from human infections (1). As notable members of the commensal microbiota residing in human mucosal sites, most notably the gut, these anaerobes can cause devastating infections when they gain access to

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normally sterile body compartments following trauma, surgery, or mucosal barrier disruption (for example, in the setting of cancer). In the absence of appropriate antimicrobial therapy, these infections can have mortality rates as high as 50% (2). Accordingly, empirical antimicrobial regimens in patients with increased risk target these organisms (3), and typically clinical microbiology laboratories will take steps to isolate and report these organisms from clinical specimens, even those that are grossly polymicrobial.

Often referred to collectively as the *Bacteroides fragilis* group (BFG), this collection of >20 distinct *Bacteroides*, *Parabacteroides*, and *Phocaeicola* species accounts for a majority of anaerobic clinical infections in humans (1). Historically, identification methods lacked resolution to differentiate members of the BFG; thus, when encountered clinically, these isolates were rarely reported to species level. However, implementation of new molecular technologies such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) in clinical laboratories may facilitate species-specific reporting (4). Growing evidence of species-related disease associations further advances the support for the clinical utility of routine species-level reporting.

BFG infections have become increasingly difficult to treat, with reports of increasing resistance to frontline anaerobic agents worldwide (5). Additionally, antimicrobial resistance (AMR) profiles vary across members of the BFG (4, 6). In the absence of anaerobic susceptibility testing in most clinical laboratories, species-level insights into predicted susceptibility patterns may help guide patient management and prevent treatment failures.

In light of these developments, clinical laboratories are armed with new tools to improve diagnosis and treatment of BFG infections. In this minireview, we describe the clinical significance of the BFG in human health and disease and highlight recent taxonomic reclassifications within the BFG and insights informed by comprehensive genomic analysis (7, 8). Additionally, we review methods for growth and recovery of BFG from clinical specimens and examine methods for species-level identification (9, 10). Trends in antimicrobial resistance are described, along with developments in phenotypic susceptibility testing and genomic insights into antimicrobial resistance (4, 11).

TAXONOMY, TAXONOMIC REVISIONS, AND STRAIN TYPING

Taxonomy and species-level identifications within the BFG hold great clinical value as this group of bacteria is phylogenetically diverse (Table 1 and Fig. 1A) and antimicrobial resistance (AMR) patterns vary between BFG species (4, 7). Historically, BFG taxonomic classifications were determined based on 16S rRNA gene sequencing (7), and several taxonomic revisions of the BFG have been proposed over the last few decades, including the reclassification of *Bacteroides distasonis*, *Bacteroides merdae*, and *Bacteroides goldsteinii* to a new genus, *Parabacteroides*, within the proposed "Tannerellaceae" family (at the time of writing, this family name is proposed but not fully validated) (8, 12). For discussion of these taxa, the genera and species deemed to be part of the BFG as defined in this review are outlined in Tables 1 and 2. In addition to *B. fragilis*, *Bacteroides thetaiotaomicron*, *Parabacteroides distasonis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, and *Bacteroides uniformis* are the most common species encountered clinically (1, 6, 13, 14). Despite divergence with *Bacteroides* at the family level, *Parabacteroides* spp. are commonly included with *Bacteroides* in BFG clinical surveys.

More recently, improvements in whole-genome sequencing (WGS) technology and the introduction of more comprehensive species delimitation methods, such as average nucleotide identity (ANI) (5) and genome BLAST distance phylogeny (GBDP) (7), have led to major restructuring of several species within the *Bacteroidota* phylum. A recent study has proposed reclassifying several *Bacteroides* species into the *Phocaeicola* genus including the notable BFG pathogens *B. vulgatus*, *Bacteroides dorei*, and *Bacteroides massiliensis*, based on a comprehensive study of over 1,000 *Bacteroides* genomes using GBDP to infer taxonomic relationships (7). We have also demonstrated the genetic distinction between strains derived from the *Bacteroides*, *Parabacteroides*, and *Phocaeicola* genera (4) (Fig. 1A). Although the proposed reclassifications have not yet been validated by the

TABLE 1 Taxonomy of members of the *Bacteroides fragilis* group and other *Bacteroides* spp.

Family	Genus	Species
Bacteroidaceae	*Bacteroides*	*B. acidifaciens*
		B. caccae
		B. cellulosilyticus
		B. eggerthii
		B. faecis
		B. fragilis division I
		B. fragilis division II
		B. helcogenes
		B. intestinalis
		B. nordii
		B. ovatus
		B. pyogenes
		B. salyersiae
		B. stercoris
		B. thetaiotaomicron
		B. uniformis
		B. xylanisolvens
	Phocaeicola	*P. coprocola*
		P. dorei
		P. massiliensis
		P. plebeius
		P. vulgatus
Tannerellaceae	*Parabacteroides*	*P. distasonis*
		P. goldsteinii
		P. gordonii
		P. johnsonii
		P. merdae

International Journal of Systemic and Evolutionary Microbiology (IJSEM) (Tables 1 and 2), the proposed nomenclature has been generally supported, is likely to be accepted and implemented, and is thus utilized throughout the remainder of the paper.

A surprising amount of genetic diversity is apparent for the namesake BFG species, *Bacteroides fragilis sensu stricto* (BFSS). Multiple WGS studies indicate that division I and division II BFSS bacteria are genetically divergent at the species level (4, 15). Division I BFSS harbors the cephalosporinase *cepA* and is generally susceptible to frontline β-lactam regimens, and the BFSS bacteria that colonize healthy human guts typically fall within this division (16). Division II BFSS isolates are capable of achieving high-level carbapenem resistance through insertion sequence (IS)-mediated activation of the chromosomal carbapenemase gene *cfiA*, which is absent in division I BFSS. Beyond the β-lactamase gene profile, division I and II BFSS bacteria have substantial genetic diversity in comparison of both the core and accessory genomes (4). With an ANI value of less than 0.95 between the two divisions, division I and II BFSS bacteria are distinct genomospecies, with notable differences in AMR and pathogenicity (4).

CLINICAL SIGNIFICANCE AND DISEASE ASSOCIATIONS

Like other anaerobic bacteria, BFG organisms colonize mucosal surfaces of their mammalian hosts. Comprising roughly 25% of the human intestinal microbiota, *Bacteroides* species contribute to carbohydrate metabolism and niche protection to facilitate a healthy gut (17). Breaches of these mucosal sites (for example, in the setting of trauma, cancer, or surgery) facilitate access to normally sterile body compartments where these commensal organisms can act as pathogens (1, 18).

Despite being outnumbered by other *Bacteroides* species 10- to 100-fold in the human colon, BFSS is the predominant anaerobic microorganism isolated from clinical specimens, regardless of the site of infection. A number of virulence factors contribute

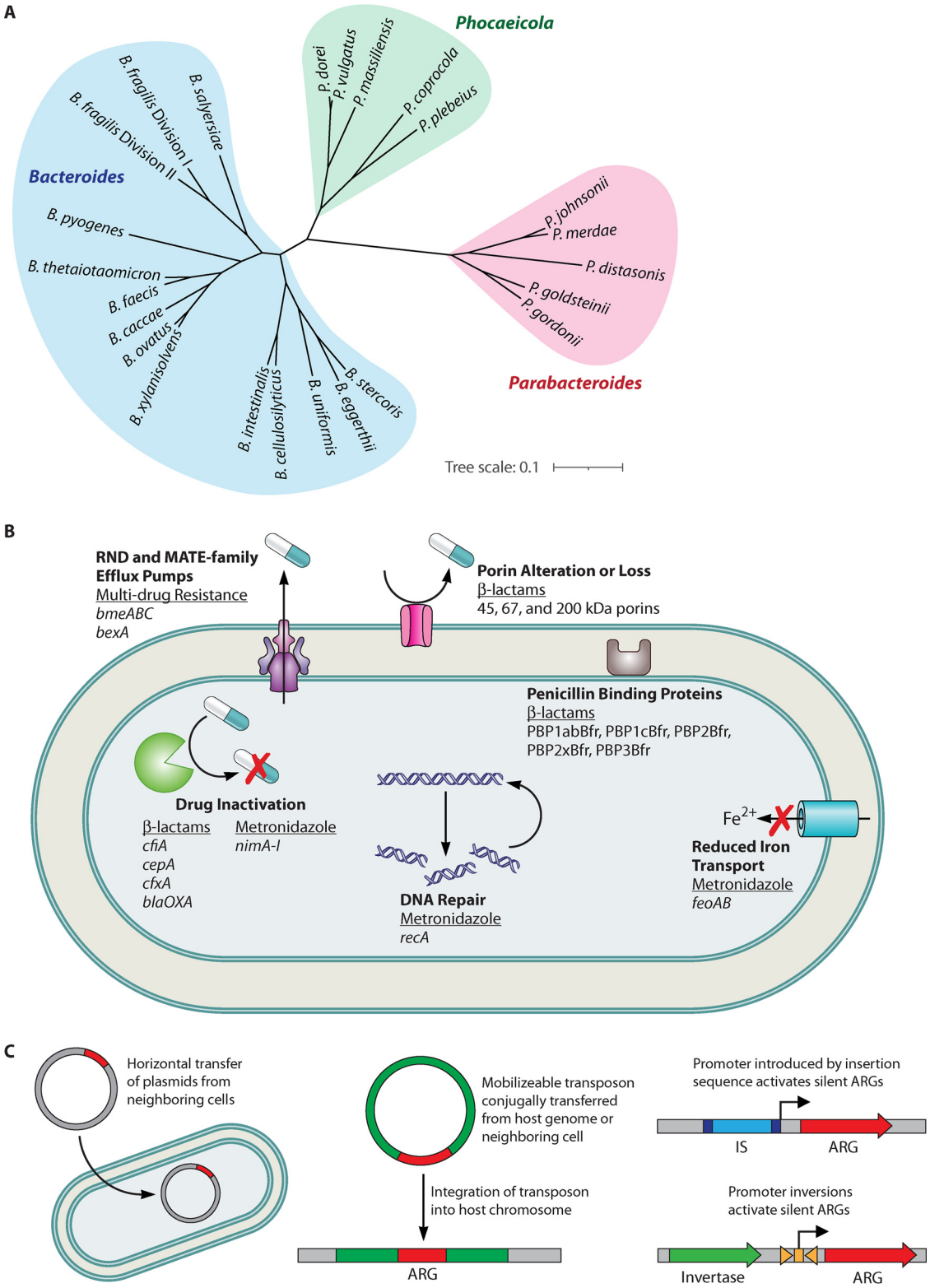

FIG 1 Phylogeny and antimicrobial resistance mechanisms in BFG bacteria. (A) Unrooted neighbor-joining phylogenetic tree (FastTree v2.1.7) generated from a core genome alignment (Roary v3.12.0, minimum BLASTP identity set to 70%) of major BFG type strain assemblies including *B. fragilis* division I (GCF_000025985.1), *B. fragilis* division II (GCA_021405735.1), *Bacteroides salyersiae* (GCF_000381365.1), *Bacteroides pyogenes* (GCF_000428105.1), *B. thetaiotaomicron* (GCF_016103195.1), *B. faecis* (GCF_000226135.1), *Bacteroides caccae* (GCF_002222615.2), *B. ovatus* (GCF_020149745.1), *Bacteroides xylanisolvens* (GCF_000210075.1), *B. intestinalis* (GCF_000172175.1), *Bacteroides cellulosilyticus* (GCF_000158035.1), *B. uniformis* (GCF_016117815.1), *Bacteroides eggerthii* (GCF_900445565.1), *Bacteroides stercoris* (GCF_000154525.1), *Phocaeicola massiliensis* (GCF_000373085.1), *P. vulgatus* (GCF_016766915.1), *P. dorei* (GCF_013009555.1), *Phocaeicola*

(Continued on next page)

TABLE 2 Revised and previous taxonomic classifications of members of the *Bacteroides fragilis* group and other *Bacteroides* spp.

Revised taxonomic designation	Previous designation	Member of BFG
Phocaeicola dorei	*Bacteroides dorei*	Yes
Phocaeicola vulgatus	*Bacteroides vulgatus*	Yes
Phocaeicola massiliensis	*Bacteroides massiliensis*	Yes
Parabacteroides gordonii	*Bacteroides gordonii*	Yes
Parabacteroides goldsteinii	*Bacteroides goldsteinii*	Yes
Parabacteroides distasonis	*Bacteroides distasonis*	Yes
Parabacteroides merdae	*Bacteroides merdae*	Yes
Pseudoflavonifractor capillosus	*Bacteroides capillosus*	No
Campylobacter ureolyticus	*Bacteroides ureolyticus*	No
Alistipes putredinis	*Bacteroides putredinis*	No

to the pathogenic potential of BFSS, including synthesis of adhesin and capsule, relative oxygen tolerance, and antigenic variation of surface structures. These are reviewed extensively elsewhere and will not be discussed in detail here (1, 19).

Intra-abdominal abscesses and bloodstream infection (BSI) are the most common infections caused by *Bacteroides* species in both adult and pediatric patients (1). BFG isolates can also be recovered from deep-seated abscesses, perforated appendicitis, necrotizing skin and soft tissue, and bone and joint infections (1). Although endocarditis with anaerobic organisms is rare, it does occasionally occur as a result of hematogenous spread in patients with gastrointestinal (GI) malignancy or other intra-abdominal infections; in those cases, BFG organisms are the most common anaerobic bacteria reported (1). Bloodstream infections (BSIs) are a common clinical presentation for BFG infection. Among anaerobic BSIs, *Bacteroides* spp. account for the majority of organisms isolated, with BFG accounting for nearly half of anaerobic BSIs (1). Infections with BFSS are associated with reported mortality up to 40%, but this can be mitigated with effective antimicrobial treatment (2, 20).

One virulence factor, the *Bacteroides fragilis* toxin (BFT), also known as fragilysin, has been implicated in a number of diseases (18). Enterotoxin-producing *B. fragilis* (ETBF) induces secretory diarrhea in animals and has also been isolated more frequently in human patients with diarrhea than in healthy individuals (18). Additionally, ETBF is thought to contribute to chronic colitis and colorectal cancer (CRC) by propagating the chronic inflammatory environment predisposing to disease (18, 21). However, the exact mechanisms underlying these disease correlations have not yet been fully elucidated. A number of host and additional microbial factors are also likely to contribute to these disease manifestations.

Among non-*fragilis Bacteroides* species, *B. thetaiotaomicron* has long been established as an important component of the healthy gut (17). The *B. thetaiotaomicron* genome is exquisitely adapted to fine-tune regulation of extensive carbohydrate utilization systems in concert with host nutrient sources, facilitating a mutualistic relationship between bacteria and host (1). Despite its known contributions to the healthy gut, *B. thetaiotaomicron* is often the most common non-*fragilis* BFG species reported from anaerobic infections and has been isolated from abdominal sites, blood, cerebrospinal fluid (CSF), and skin and soft tissue infections (1, 13). Another non-*fragilis* BFG species frequently isolated from similar sites is *B. ovatus*. One study has reported that non-*fragilis* BFG species are more likely to present clinically as bacteremia, especially when the patient outcome is death (22). However, we and others have previously reported no difference in the clinical sites from which *B. fragilis* and non-*fragilis* BFG species are recovered (4, 23). The role of other non-*fragilis* BFG species in health and disease is still being elucidated. Recent reports

FIG 1 Legend (Continued)

coprocola (GCF_000154845.1), *Phocaeicola plebeius* (GCF_000187895.1), *Parabacteroides johnsonii* (GCF_000156495.1), *Parabacteroides gordonii* (GCF_020297465.1), *Parabacteroides goldsteinii* (GCF_000969835.1), *P. distasonis* (GCF_020735945.1), and *Parabacteroides merdae* (GCF_900445495.1). (B) Molecular determinants of resistance in BFG bacteria. (C) Major genetic ARG mobilization and activation strategies in BFG.

have implicated *Parabacteroides distasonis* as both a potential probiotic and a pathogen with emerging AMR (24). Similarly, reports of invasive infection with *Phocaeicola dorei* may complicate purported beneficial uses (25).

METHODS FOR GROWTH IN CULTURE

A number of preanalytical factors impact the ability to recover BFG from clinical specimens. Cultures for anaerobic bacteria require specialized transport conditions, such as dedicated anaerobic transport medium (26). Surface swabs should not be sent for anaerobic culture; ideal specimens are tissue or fluid, especially that obtained during surgery (26).

BFG bacteria are recovered on general-purpose enriched media for anaerobic microorganisms, such as *Brucella* blood agar (BBA), CDC anaerobe agar, or Schaedler blood agar (26). Recovery of BFG from mixed cultures can be enhanced from potentially polymicrobial specimens by the addition of selective agar, such as *Bacteroides* bile esculin (BBE) agar, on which members of the BFG will grow and exhibit characteristic brown to black colonies; the growth of most other anaerobic bacteria is inhibited (26). *Phocaeicola vulgatus* does not always form black colonies (26).

A recent report describes a novel selective medium for the recovery of BFSS in clinical specimens (9); along with a brain heart infusion agar base, the medium is supplemented with yeast extract, cysteine, bile salts, vitamin K, hemin, glucose, esculin, ferric ammonium citrate, bromothymol blue, novobiocin, gentamicin, and kanamycin. BFSS isolates are large yellow colonies on the agar, with blackening of the medium after 48 h of incubation. The recovery of BFSS from clinical specimens is enhanced using this medium compared to conventional medium, and the growth of most other anaerobic bacteria is inhibited. While this medium has not been widely adopted, it may be useful for future surveillance studies.

BFG is detected efficiently in the anaerobic bottle of commercially available blood culture systems (27–29). The time to blood culture positivity for BFG is relatively short; for example, one report notes the mean time to blood culture positivity being 32 h and 99% of blood cultures with BFG signaling positive within 60 h (28).

METHODS FOR IDENTIFICATION

BFG bacteria are pleomorphic Gram-negative bacilli (size range 1.5 to 6 μM) that are nonpigmented, nonmotile, and encapsulated, and isolates are bile resistant (i.e., will grow in the presence of 20% bile) (11, 26). Catalase and indole activities vary by species and can be useful to aid identification to species level if phenotypic methods are used (11, 26). BFG bacteria are resistant to all three anaerobic special-potency disks (kanamycin [1,000 μg], vancomycin [5 μg], and colistin [10 μg]) which can be used for presumptive identification of BFG (11, 26).

Although historically BFG bacteria were often grouped together for clinical reporting, in the context of antimicrobial therapy implications, virulence profile differences, and clinical significance, laboratories should consider reporting members of the group to species level when possible. MALDI-TOF MS is being increasingly used for microorganism identification in clinical specimens, and overall, this method demonstrates excellent performance characteristics for the species-level identification of anaerobic bacteria, including BFG (30, 31). One recent study (10) interrogated 138 BFG isolates representing 8 *Bacteroides* spp. and 2 *Parabacteroides* spp. collected between 2010 and 2018 using three MALDI-TOF MS systems (Clin-ToF-II/Bioyong Explore v.3.2, Autof MS1000/Autof Acquirer v.1.0.123/Analyzer v.1.0.50, and Vitek MS/IVD database v.3.2), as well as the Vitek2 ANC card; 16S rRNA gene sequencing was the reference method for comparison. The investigators report that Autof MS identified 136 of the 138 isolates accurately to species level, Vitek MS identified 130 of the 138 isolates, Clin-ToF-II identified 130 of the 138 isolates, and Vitek2 ANC identified 131 of the 138 isolates. The biggest challenges were reported with accurate identification of some of the species that have recently undergone taxonomic revision, including *P. dorei* and

Bacteroides intestinalis. A recent evaluation of 174 isolates representing 9 species with Bruker Biotyper and Vitek MS (4) found 91% species agreement for Bruker Biotyper with the Research Use Only (RUO) database and 90% species agreement with the Vitek MS Knowledge Base v.3.0 compared to WGS, with challenging species being *Bacteroides faecis*, *B. ovatus*, and *P. vulgatus*. Similarly, another investigation noted 97% accuracy for *Bacteroides* species isolates representing 22 species with Bruker Biotyper and 96% with Vitek MS, with variable accuracy for *P. dorei*, *B. faecis*, and *Bacteroides nordii* (32).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

A number of methods are reported in the literature for the assessment of antimicrobial susceptibility of anaerobic organisms including BFG. Details of these methods and their performance with anaerobic organisms have been reviewed extensively elsewhere (33, 34). Here, we briefly review more established methods and focus discussion of recent developments in anaerobic antimicrobial susceptibility testing (AST) pertaining specifically to the BFG.

The agar dilution (AD) method, as described by CLSI-M11-A8 (35, 36), is largely recognized by the Clinical and Laboratory Standards Institute (CLSI) as the gold-standard method for anaerobic AST (33). Briefly, different concentrations of antimicrobial agents are incorporated into media on which standard suspensions of bacteria are spotted. Following (anaerobic) incubation at 36°C ± 1°C for 42 to 48 h, plates are observed to determine the lowest antibiotic concentration that inhibits growth (MIC). AD is more suited to batch testing of numerous isolates, and due to the labor, time, and expertise required to utilize this method, most clinical laboratories cannot adopt it for routine use.

Broth microdilution (BMD) is another method that allows for testing different concentrations of multiple antibiotics in a 96-well plate format. Following inoculation of microtiter plates with standard bacterial suspensions, plates are read after 46 to 48 h of incubation at 36°C ± 1°C to determine the MIC. While the availability of commercial panels and comparable performance of in-house panels facilitate use of this method worldwide (37), it is endorsed by CLSI only for *Bacteroides* spp. and *Parabacteroides* spp. (36). However, recent work from Europe suggests adequate performance of some commercial BMD methods compared to AD across Gram-positive and Gram-negative anaerobes more broadly (38).

Until recently, disk diffusion for anaerobic AST was not recommended by any international standards associations as a result of poor correlation with gold-standard AD (34). However, recent work out of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) developmental laboratory in Europe has demonstrated the feasibility of a disk diffusion method with high reproducibility and concordance with agar dilution with BFG (39). These findings were extended to other rapidly growing anaerobes and further validated across clinical laboratories in Europe (unpublished but available online at https://www.eucast.org/videos_and_online_seminars/online_seminars/). This work has made several changes compared to previously reported methods (40, 41) including (i) the use of fastidious anaerobe agar (FAA) as opposed to *Brucella* blood agar (BBA), (ii) species-specific zone diameter breakpoints that largely respect wild-type population distributions, and (iii) strict adherence to methodology (medium composition, incubation time, anaerobiosis conditions, etc.). EUCAST standards for testing include agar dilution and disk diffusion for *Bacteroides* spp. Testing is performed on FAA, and agar dilution is incubated at 35 to 37°C for 48 h; disk diffusion is incubated at 35 to 37°C for 16 to 20 h (42). However, FAA is not commercially available nor routinely used in the United States, potentially limiting use of EUCAST-based disk diffusion methods.

Gradient diffusion (GD) testing is the most widely utilized method for anaerobic AST among laboratories performing anaerobic susceptibility testing (33). Plastic strips impregnated with an increasing gradient concentration of an antimicrobial are placed onto *Brucella* blood agar with hemin and vitamin K preinoculated with a lawn of bacteria from a standardized suspension. Following at least 24 h of incubation (according to

the manufacturer's instructions), the MIC is read at the concentration at which the ellipse of inhibition crosses the test strip. GD testing is, in general, more expensive than other methods but is flexible and relatively easy to perform, allowing for the assessment of multiple antibiotics simultaneously and providing MIC values.

Overall, comparisons of BMD and GD are favorable with high categorical and essential agreement reported across methods for BFG (43). Notably, false metronidazole resistance has been reported when adequate anaerobiosis conditions are not met (44). Recently, concerns regarding detection of resistance to amoxicillin-clavulanate (AMC) among BFSS isolates by using gradient diffusion strips have been reported in Europe (45). These concerns arise from contrasting recommendations regarding the concentration of the β-lactamase inhibitor clavulanate. CLSI breakpoints are based on a testing methodology of AMC at a 2:1 ratio (36), while EUCAST breakpoints are based on testing AMC at a 2-mg/L fixed ratio of clavulanate (46). Commercial AMC GD strips are available at both a 2:1 ratio (Etest [bioMérieux] and MTS [Liofilchem]) and a fixed clavulanate concentration of 2 mg/L (MTS; M.I.C.E.; Oxoid Thermo Fisher Scientific). Interpretive criteria must follow the method used to avoid misclassification of resistant isolates as susceptible (45).

MALDI-TOF MS has been reported as a method for detection of carbapenem resistance in BFSS. Early reports demonstrate the ability of the Bruker Daltonics MALDI Biotyper 2.0 system to successfully differentiate main spectra of BFSS isolates harboring the chromosomal gene *cfiA* that confers resistance to all β-lactam antibiotics including carbapenems (division II) from *cfiA*-negative *B. fragilis* isolates (division I) (47, 48). However, detection of *cfiA* positivity alone in BFSS isolates does not always indicate phenotypic carbapenem resistance, as optimal CfiA expression requires upstream insertion sequence (IS) elements.

The MALDI Biotyper can also be used to assess carbapenem hydrolysis activity (49, 50). These methods detect a normalized ratio of the carbapenem-specific mass spectral peak intensities following incubation with a test isolate compared to that of the nonhydrolyzed carbapenem. Despite outperforming traditional phenotypic carbapenemase assays retrofitted for anaerobic bacteria, these methods have yet to be widely evaluated or adopted.

Other phenotypic methods for carbapenemase detection in BFSS have been utilized with various levels of success. One study reports that the Carba-NP assay detects carbapenemase activity with $>$90% sensitivity and specificity in BFSS isolates compared to the presence of upstream IS and *cfiA* via PCR (51). However, another study found that among 29 division II BFSS (*cfiA*-positive) isolates with a meropenem MIC range of 1 to $>$32 μg/mL, only 6 were carbapenemase positive by Carba-NP, of which 5 had a meropenem MIC of $\geq$16 μg/mL. Twenty-three (79%) isolates were carbapenemase positive by disk diffusion synergy, while all 29 isolates were carbapenemase positive by a MALDI-TOF-based method (STAR-Carba) (50). The authors suggest that carbapenemase detection by both Carba-NP and disk diffusion synergy tests is meropenem MIC dependent. More recently, an adaptation of the modified carbapenem inactivation method (mCIM) for use with BFSS named the "Ana-CIM" was proposed and clinically validated in a multicenter study (52). The Ana-CIM utilizes reagents readily available in clinical labs capable of performing anaerobic identification and requires 6 h of incubation of a test BFSS isolate with a meropenem disk in anaerobic media and under anaerobic conditions. The meropenem disk is then assessed for activity against a pan-susceptible *Escherichia coli* indicator strain following overnight incubation on Mueller-Hinton agar in air. BFSS isolates producing a carbapenemase result in a meropenem zone size of $\leq$8 mm against the indicator *E. coli* strain. Compared to ertapenem susceptibility tested by GD and interpreted with CLSI breakpoints, the Ana-CIM had 88% category agreement (CA) with 0 very major errors and 11% major error and 7% minor error rates and improved performance ($\geq$92% CA) when meropenem and/or EUCAST criteria were utilized as the reference method (52).

ANTIMICROBIAL RESISTANCE—SUSCEPTIBILITY PATTERNS AND GENETIC MECHANISMS OF RESISTANCE

BFG species demonstrate increasingly high rates of AMR and a wide variety of resistance mechanisms in comparison to other anaerobic pathogens (Fig. 1B) (1). Several BFG species are long-term residents of the commensal gut microbiota in healthy hosts, and the human gut can serve as a reservoir for antimicrobial resistance gene (ARG) acquisition and dissemination among BFG and non-BFG species (1, 53), which may limit effective treatment options for opportunistic infections. Despite the potential for inherent or acquired resistance mechanisms, historically, susceptibility testing for *Bacteroides* and *Parabacteroides* spp. was not routinely performed due to predictable susceptibility patterns. If anaerobic antimicrobial susceptibility testing (AST) was performed, it was limited to isolates recovered from invasive sites or complicated infections or in the setting of suspected treatment failure. Additionally, lack of consensus in interpretive criteria and of availability of methods that are both easy to use and cost-effective has limited widespread implementation of anaerobic AST across most clinical labs, hampering the ability to detect changing trends in susceptibility of anaerobic microorganisms. Despite these challenges, recent data suggest that resistance to some anti-anaerobic agents (such as clindamycin, β-lactam–β-lactamase inhibitor combinations, and carbapenems) is on the rise, supporting the need for routine AST in clinical practice or, at a minimum, regular and comprehensive susceptibility surveys of anaerobic organisms isolated from serious infections, particularly those with BFG (5, 54).

***β*-Lactam agents.** *Bacteroides* and *Parabacteroides* spp. are still considered intrinsically resistant to penicillin and ampicillin via β-lactamase production, with >90% resistance reported globally (Table 3). However, susceptibility to other β-lactam agents including cefotetan, cefoxitin, and ceftriaxone is variable (Table 3). A recent U.S. survey reports cefoxitin resistance rates of >5% across all BFG isolates tested but higher in non-*fragilis Bacteroides* spp. than in BFSS (9.1% versus 3.5%), increasing up to 15% in *B. ovatus* and *P. distasonis* (6). Similarly, reduced susceptibility to cefoxitin was observed more frequently in non-*fragilis Bacteroides* spp. in a Canadian survey from 2012 to 2019 (55). In parts of Europe, cefoxitin resistance rates ranging from 8% to 74% have been reported (56).

***β*-Lactam combination agents.** Globally, resistance to β-lactam combination agents remains low but may be elevated in some regions (Table 3). While several countries in Europe report rates of resistance to piperacillin-tazobactam to be <10%, recent surveys in China and Japan report 34% resistance to amoxicillin-clavulanate (AMC) and 11% to 12% resistance to piperacillin-tazobactam (57, 58). Similarly, some studies in Europe and the United States report higher rates of resistance to β-lactam combination agents in non-*fragilis Bacteroides* species than in BFSS (Table 3).

Resistance to β-lactam agents in BFG isolates is mediated by endogenous and acquired β-lactamases which vary by species (4). The CepA cephalosporinase is the most frequently detected β-lactamase, which is chromosomally encoded in division I BFSS and can be inhibited by β-lactamase inhibitors such as tazobactam, sulbactam, and clavulanic acid (59). Genes encoding another β-lactamase, Cfx are also commonly determined in clinical isolates (4, 60).

The known evidence for the role of penicillin-binding proteins (PBPs) and porins in β-lactam resistance in BFG is summarized in a previous review in 2007 (1), showing associations between reduced PBP affinity for various β-lactam agents and changes in porin structure as contributions toward β-lactam resistance in BFG. However, since then little evidence has emerged to further establish the role of PBPs and porins in resistance, and the available studies are largely associative. WGS has demonstrated the presence of at least 8 PBPs and 10 porins in a collection of 194 BFG isolates (4). With the aid of WGS data, complementation assays would show direct correlations between β-lactam resistance determinants (β-lactamases, PBPs, and outer membrane proteins) and phenotypic resistance and provide much-needed mechanistic insights on β-lactam resistance in BFG.

TABLE 3 Resistance profiles for BFG and commonly prescribed antimicrobials with anaerobic activity[n]

Continent, country, and yr(s) of isolate collection	*B. fragilis sensu stricto* (% resistant)								Other[a] *Bacteroides* spp. (% resistant)								Method	Interpretive guideline	Reference
	n	PEN	FOX	AMC	PTZ	Carba[b]	CLN	MTZ	*n*	PEN	FOX	AMC	PTZ	Carba[b]	CLN	MTZ			
North America																			
Canada,[c] 2012–2019	485	99	11.6	ND	2	M, 4	32	1	401	98	35.9	ND	5	M, 0.2	40	0.2	GD	CLSI M100 Ed30	55
USA,[d] 2010–2012	455	ND	3.7	ND	1.1	E, 2.4; I, 1.1; M, 0.9	24	0	324	ND	9.1	ND	0.9	E, 0.9; I, 0.3; M, 0.6	45	0	AD	CLSI M100 Ed23	6
USA,[e] 2018	83	ND	ND	ND	2	E, 2; M, 2	ND	0	91	ND	ND	ND	29.7	E, 3.3; M, 3.3	ND	1.1	GD	CLSI M100 Ed30	4
South America																			
Argentina,[f] 2006–2009	198	ND	6.1	ND	1.5	D, 1.9; E, 2.4; I, 1.5	23	0	165	ND	19.4	ND	1.2	D, 1.4; E, 2.4; I, 0.61	36	0	AD	CLSI M11-A7	63
Europe																			
Germany[g]	133	ND	ND	ND	6.2	M, 13	19	1.6	120	ND	ND	ND	M- 23	M, 7	49	9	GD	EUCAST v.10.0	80
Hungary,[h] 2014–2016	233	ND	3.4	1.7	ND	M, 9.9	26	0	167	ND	6.0	12.0	ND	M, 3.6	52.7	0.25	AD	EUCAST v.7.1	81
Poland,[i] 2013–2017	115	96	ND	1.7	ND	I, 0.87	18	0	135	96	ND	8.2	ND	I, 3.7	53	0	GD	EUCAST v.10.0	82
Spain,[j] 2006–2010	414	95	26.4	8.7	6.3	E, 2.2; I, 2.2	45	6.3	NR	NR	NR	NR	NR	NR	NR	NR	GD	CLSI M100 Ed20	83
UK,[k] 2016	130	ND	ND	5.4	0.8	M, 1.5	16.2	2.3	38	ND	ND	NR	5.3	M, 0	50	2.6	AD	EUCAST v.7.0	14
Asia																			
China,[l] 2017–2019	80	ND	3.8	34	11	I, 19; M, 19	87	7.5	35	ND	5.7	20	2.9	I, 2.9; M, 2.9	63	0	BMD	CLSI M100 Ed 28	57
Kuwait,[m] 2008–2012	906	100	ND	13	4.1	M, 1.8	46	2.7	347	85.6	ND	16.1	24.2	I, 0; M, 2.9	52.2	4.3	GD	CLSI M100 Ed 24	84

[a]See individual footnotes for the list of species included per study.
[b]Carbapenem agent tested is denoted with D (doripenem), E (ertapenem), I (imipenem), or M (meropenem).
[c]Percent not susceptible (includes intermediate and resistant) shown. Other non-*fragilis Bacteroides* species evaluated include *B. caccae*, *P. dorei*, *B. eggerthii*, *Bacteroides finegoldii*, *P. massiliensis*, *B. ovatus*, *B. stercoris*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*.
[d]Other non-*fragilis Bacteroides* species evaluated include *B. thetaiotaomicron*, *B. ovatus*, *P. vulgatus*, *P. distasonis*, *B. caccae*, *B. uniformis*, *B. eggerthii*, *B. stercoris*, *B. intestinalis*, *Bacteroides splanchnicus*, *P. dorei*, *P. goldsteinii*, *P. johnsonii*, and *P. merdae*.
[e]Percent not susceptible (includes intermediate and resistant) shown. Data for other non-*fragilis Bacteroides* species are calculated and include *B. ovatus*, *B. thetaiotaomicron*, *P. vulgatus*, *P. distasonis*, *B. faecis*, *B. xylanisolvens*, *B. pyogenes*, *P. merdae*, *B. caccae*, and *P. dorei*.
[f]Data for other non-*fragilis Bacteroides* species are calculated and include *B. thetaiotaomicron*, *B. caccae*, *P. vulgatus*, *P. distasonis*, *B. uniformis B. stercoris*, *P. merdae*, and *Bacteroides* spp. not further specified.
[g]Percent not susceptible (includes intermediate and resistant) shown. Data for other non-*fragilis Bacteroides* species are calculated, but species included were not further specified.
[h]Data for other non-*fragilis Bacteroides* species are calculated and include *B. thetaiotaomicron*, *B. ovatus*, *P. vulgatus*, *P. distasonis*, *B. caccae*, *B. uniformis*, *B. stercoris*, *B. cellulosilyticus*, *B. intestinalis*, *B. salyersiae*, *B. nordii*, and *P. goldsteinii*.
[i]Other non-*fragilis Bacteroides* species include *B. caccae*, *B. eggerthii*, *B. ovatus*, *B. ovatus/xylanisolvens*, *B. pyogenes*, *B. stercoris*, *B. thetaiotaomicron*, *B. uniformis*, and *P. vulgatus*.
[j]Percent not susceptible (intermediate and resistant) reported.
[k]Other non-*fragilis Bacteroides* species include *B. thetaiotaomicron*, *B. ovatus*, *P. vulgatus*, *B. uniformis*, *B. xylanisolvens*, *P. dorei*, *B. stercoris*, *B. caccae*, *B. faecis*, and *B. cellulosilyticus*.
[l]Other non-*fragilis Bacteroides* species include *B. thetaiotaomicron*, *B. ovatus*, *P. vulgatus*, *B. uniformis*, *Bacteroides novobacterioides*, and *P. distasonis*.
[m]Data for other non-*fragilis Bacteroides* species are calculated and include *B. ovatus*, *P. vulgatus*, and *Bacteroides* spp. not further specified.
[n]PEN, penicillin; FOX, cefoxitin; AMC, amoxicillin-clavulanate; PTZ, piperacillin-tazobactam; Carba, carbapenem; CLN, clindamycin; MTZ, metronidazole; ND, not done; NR, not reported; BMD, broth microdilution; AD, agar dilution; GD, gradient diffusion.

Carbapenems. Among β-lactam antibiotics, the carbapenems have the broadest coverage against anaerobic organisms. While carbapenem resistance rates among BFG isolates in the Americas have been <5%, resistance is higher in other regions, with intermediate/resistant isolate rates up to 20% reported across Europe and Asia (57, 58). Interestingly, carbapenem resistance appears to be higher in BFSS isolates than in non-*fragilis Bacteroides* species (Table 3).

Resistance to carbapenems in BFG is frequently associated with the *cfiA* gene encoding a metallo-β-lactamase that is activated by upstream IS elements (4). Division II BFSS harbors chromosomal *cfiA* and comprises the majority of carbapenem-resistant BFG isolates (4, 61). At least 28 variants of the *cfiA* gene paired with multiple classes of insertion sequences have been observed (62). Decreased carbapenem susceptibility has been noted in the absence of *cfiA* in BFSS and other BFG species, although the underlying mechanism is unknown (60, 63). We and others have identified previously uncharacterized β-lactamases in *cfiA*-negative BFG isolates (4). Overexpression of native efflux systems has been shown to contribute to reduced carbapenem susceptibility (57). Alterations in a BFSS penicillin-binding protein, PBP2Bfr, were shown to contribute to imipenem resistance in clinical isolates (64). Porin loss is another hypothetical mechanism of reduced carbapenem susceptibility but has not been studied extensively in the BFG.

Metronidazole. Metronidazole remains an effective agent for the treatment of most anaerobic bacteria, including BFG. Worldwide, susceptibility rates remain high (>90%) across all species (Table 3). However, in one report out of Pakistan, 20% metronidazole resistance was reported among BFG isolates collected in 2014 to 2017 (65). This is in stark contrast to the 3% of BFSS isolates testing metronidazole resistant reported from a survey in the same region conducted just 3 to 7 years prior (2010 to 2011), suggesting the potential for a rapid rise of metronidazole resistance (65, 66). Carbapenem and metronidazole co-resistance is rare but has been reported in multiple regions (6, 60, 66, 67).

Metronidazole resistance in BFG is most often associated with the presence of *nim* genes encoding a 5-nitroimidazole reductase, of which there are at least 10 isoforms reported so far (68). Similar to *cfiA*-mediated carbapenem resistance, *nim* genes can also be activated by ISs and are either plasmid based or chromosomal and commonly associated with mobilizable transposons. Overexpression of efflux machinery in BFG as well as *recA*, encoding a DNA repair protein, has been shown to confer metronidazole resistance (69, 70), which may explain observed metronidazole resistance in BFG isolates that lack *nim* genes.

Other agents and mechanisms of acquired resistance. Like other anaerobes, *Bacteroides* and *Parabacteroides* spp. are inherently resistant to aminoglycosides as antimicrobial activity of these agents require oxygen (1). Although they were initially widely used as empirical anti-anaerobic agents, high non-susceptibility rates (20 to 60%) have now been reported for clindamycin and moxifloxacin worldwide, limiting their clinical utility (Table 3) (3, 5, 54, 56). The *erm* genes, which are members of the macrolide-lincosamide-streptogramin B (MLS) resistance gene family, are associated with clindamycin and erythromycin resistance and are often carried with *tet* genes conferring tetracycline resistance through either ribosomal protection (*tetQ*) or tetracycline inactivation (*tetX*) (53). Carriage of both *tetQ* and *tetX* has been associated with elevated tetracycline and tigecycline resistance (71, 72). The chloramphenicol resistance gene, *cat*, encoding a chloramphenicol acetyltransferase, has been detected as plasmid-borne in rare cases (72).

Efflux. Efflux systems in BFG bacteria, including resistance-nodulation-division (RND) and multidrug and toxic compound extrusion (MATE) family efflux systems, contribute to resistance to multiple antibiotic classes (73). Overexpression of RND efflux systems encoded by the *bmeABC* operon and regulated by *bmeR* is induced by exposure to β-lactams, carbapenems, metronidazole, and quinolones (74). The *bexA* gene, encoding a MATE family efflux protein, has been implicated in resistance to fluoroquinolones (75).

AMR acquisition and activation. BFG bacteria have an incredibly "fluid" genome, capable of acquiring, activating, and repressing phenotypic functions such as AMR (Fig. 1C). The presence of ISs upstream of ARGs including *cfiA*, *cepA*, *cfxA*, *nim*, and *erm* genes is strongly associated with high-level AMR (4, 76). Mobilizable transposons also carry ARGs, and their mobilization can be stimulated by antibiotics, as is the case with CTnDOT, which carries *tetQ* and *ermF* and is mobilized by tetracycline (77). More recently discovered transposons include CTnHyb, which carries *nim* and *tet* genes as well as efflux determinants (78). Phase variation, a well-studied phenomenon in BFG typically associated with altering surface polysaccharides, can also selectively activate or deactivate ARGs following exposure to antibiotic stress by orienting invertible promoters upstream of the ARG in the "on" position (79). Additionally, plasmids in BFG bacteria can carry ARGs. A recent study identified 11 plasmids in six multidrug-resistant (MDR) BFSS isolates that carried ARGs, including pBFS01_2 carrying an IS-activated *nim* gene. Most of the plasmids were not yet identified as BFG associated (15). Furthermore, we have identified a plasmid carrying an uncharacterized *bla* gene activated by an IS in a carbapenem-resistant BFSS division I strain (4). Advances in the time and cost associated with long-read sequencing technologies, which enable identification of complete plasmid sequences and allow for uninterrupted assemblies that are confounded by repetitive IS elements in short-read assemblies, will improve the identification and characterization of mobilizable elements in BFG in the future.

SUMMARY AND FUTURE PERSPECTIVES

BFG are bona fide residents of healthy gut microbiomes worldwide (17), conferring a variety of commensal effects on the human host, yet they are the most frequently recovered isolates from anaerobic infections, confirming their role as a true pathobiont (1). Despite the clinical significance of BFG bacteria, several avenues of improvement in our understanding of BFG are warranted. The understanding of resistance mechanisms against key frontline agents such as β-lactams and β-lactam inhibitor combinations is lacking in comparison to other priority pathogens (4); however, we anticipate that this understanding will be improved by the increasing availability of WGS data for BFG as well as improved genome assembly and annotation methods. Susceptibility testing and species-level identifications of BFG are not always routinely performed in clinical settings, yet continual advancements are being made in technologies to rapidly predict phenotypic resistance and species identity (4, 10, 48). Within the BFG, phenotypic resistance profiles as well as specific resistance mechanisms have been shown to be species dependent across multiple studies (4, 6). WGS-based analysis supports extensive taxonomic revisions within the *Bacteroidota* phylum (7), improving the accuracy of BFG identifications and attributed AMR predictions (4). Although colonization with ETBF is associated with CRC (18, 21) and adaptive evolution of BFG members in healthy hosts has been investigated (16), the possibility of the gut microbiome as a reservoir for the myriad of infection types caused by BFG bacteria would be a useful avenue to explore through strain tracking between isolates and fecal metagenomes. With improved resolution of identification of members of the BFG in clinical specimens, our understanding of species-specific disease associations and antimicrobial resistance profiles will be further enhanced.

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