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Review Article

RECENT DEVELOPMENTS IN THE EXAMINATION OF THE BIOLOGY AND ANTIBIOTIC RESISTANCE MECHANISMS OF ANAEROBIC PATHOGENS

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ABSTRACT

Recent developments in the examination of the biology and antibiotic resistance mechanisms of anaerobic pathogens have significantly advanced our understanding of these organisms. Studies utilizing genomic and proteomic approaches have uncovered specific genetic determinants linked to virulence and resistance, revealing how pathogens like *Bacteroides fragilis* and *Clostridium difficile* adapt to antibiotic pressures. Mechanisms such as enzymatic degradation, efflux pumps, and biofilm formation have been identified as key contributors to their resilience against treatment. Additionally, the interplay between anaerobic pathogens and the human microbiome is being explored, highlighting how dysbiosis can lead to opportunistic infections. As antibiotic resistance continues to pose a major public health challenge, ongoing research is crucial for developing novel therapeutic strategies and improving diagnostic methods to effectively combat these resilient pathogens.

Keywords: *Bacteroides,* sub-inhibitory concentrations, resistance mechanism, carbapenems; genomics, proteomics.

Among anaerobic bacteria the *Bacteroides* species are nowadays the most significant since they are the most frequent opportunistic anaerobic pathogens and important members of the normal intestinal microbiota. Almost 30 % of the anaerobic infections are caused by *Bacteroides* strains and similarly 30-50 % of the bacterial strains from the faecal microbiota belong to the Bacteroidetes order being thus one of the major taxa there besides the Gram-positive Firmicutes. [1] The infections caused by *Bacteroides* are intra-abdominal and pelvic lung-, brain abscesses, upper respiratory tract and skin infections and bacteraemia, with rare diarrhoea manifested mainly among young mammals. While the most significant species *Bacteroides fragilis* accounts for ca. 60 % of the infections caused by *Bacteroides* it has only around 1 % prevalence in the colon which is explained by its high virulence properties.[1] It was among the earliest isolated anaerobe and now we can regard it as the type anaerobic pathogen, the anaerobic *Escherichia coli*, since its prevalence, member of the intestinal microbiota and the mostly investigated anaerobe species from there. The other intriguing property of the *Bacteroides* is that they belong to an ancient bacterial superphylum together with *Flavobacteria, Chlorobia* and other relatives with many specific microbiological properties. With time the *Bacteroides* genus was systematically reorganized separating among others *Prevotella* and *Porphyromonas*, and most recently the *Parabacteroides* and *Phocaeicola* genera.

Bacteroides are the most antibiotic resistant among all anaerobic pathogens regarding the number of resistance mechanisms and the resistance levels to each antibiotic used to treat them.[2] Here in this anniversary lecture concerning the most recent and important biological and antibiotic resistance issues will be discussed.

As concerns their presence in the normal microbiota of the gut they are not only commensals but well adapted to be exert useful functions there. These are the numerous glycan degrading activities, interaction with other microbiota members and thus capable of pathogen exclusion, vitamin and short-chain fatty acid production, bile conjugation and resistance, participation to the maturation of the gut, the immune system and the CNS.[1]

However, they have virulence mechanisms too, especially *B. fragilis* as an opportunistic pathogen. The most notable are the capsule and the capsular polysaccharides (CPS) which can induce abdominal abscesses and elicit cellular immunity, but iron uptake mechanisms, extracellular enzymes (hyaluronidase, chondroitin sulfatase, proteases, haemolysins), the enterotoxic fragilysisn, adhesins and aerotolerance (superoxide dismutase, catalase, alkyl hydroperoxide reductase) also play a role.[3]

The above-mentioned capsular polysaccharides, eight type CPS-A-H, have an interesting biology, may-be, mostly responsible to the useful and pathogenic nature of *B. fragilis*. CPS-A is composed of oligomers which have a negative and a positive charge and this 'zwitterionic' nature has been proven to mediate the abscess inducing and immune active functions. It has also been revealed that the operons of the eight CPS types, seven's promoters are invertible by a tyrosine recombinase. This feature is acting like an immune mimicry of *B. fragilis,* but other *Bacteroides* species are also capable to a lesser extent of doing this by similar mechanisms. The role of CPS-A is underlined that the health of gnotobiotic mice (correction of CD4+ T-cell deficiency, establishing a Th1/Th2 balance, prevention of inflammatory disease, expression of pro-inflammatory cytokines associated with the Th17 lineage) can be restored by CPS-A-producing *B. fragilis* strains whereas by CPS-A deficient *B. fragils* strain not.[4]

Concerning the antibiotic resistance mechanisms of *Bacteroides* the effector resistance genes in many cases are upregulated by insertion sequence (IS) elements causing the resistant phenotypes. It is also characteristic for *Bacteroides* that a variety of mobile genetic elements carry the resistance genes. [5] A summary of about the recent knowledge about these is shown in Table 1.

For β-lactams the *cepA* cephalosporinase, the *cfiA* carbapenemase and the *cfxA* extended-spectrum β-lactamase, capable of hydrolysing cephamycins, are the most important. The known examples of *cepA* activation are made by the CTn*86* conjugative transposon,[6] interestingly, the *cfiA* activation is made by numerous IS elements and the *cfxA* is usually activated by IS*Bf6* and resides on a mobilizable trans-

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Table 1. A summary on antibiotic resistance mechanisms of *Bacteroides*

Antibiotic group	Antibiotics	Resistence gene(s)	Resistence element	Insertion sequence
β-lactam	Penicillins/Cephalosporins	*cepA*	Chrormosomal (Division I)	(CTn*86*/IS*1224*)
	Cephamycins, β-lactamase /β-lactamase inhibitor combinations	*cfxA*	MTn*4555*	IS*Bf6*, IS*614*B
	Carbapenems	*cfiA* (*crxA*)	Chromosomal (Division II)	Several IS
MLS_B	Clindamycin	*ermF* (*ermB*, *ermG*, *msrSA*, *mefA*, *linA*)	Transposons (on large plasmids and chromosomes) or MTns/CTns	IS*4351* or no
5-nitroimidazoles	Metronidazole	*ldh, nimA-L*	Chromosomal or on small plasmids	Several IS
Fluoroquinolones	Moxifloxacin	gyrA, *bexA*	Chromosomal	No
Tetracyclines	Tetracycline	*tetQ*	CTns	No
Glycylcyclines	Tigecycline	?	?	?

poson (MTn*4555*) [7, 8]. Although it is hold that almost every *Bacteroides* isolate produces β-lactamase activity and hence there is the reason for their β-usually high ampicillin resistance, their ampicillin resistance do not correlate well with the CepA enzymatic activity. So, some other resistance mechanisms should be expected. It is also true for the cefoxitin resistance and *cfxA* carrier states. In this letter case, however, it turned out the low affinities of some penicillin binding proteins for cefoxitin is responsible for the remaining part of the resistance traits.[6] Additionally, this latter mechanism can act for the resistances of more β-lactamase susceptible β-lactams, e.g. ampicillin, too. The CfiA carbapenemase, in turn, can confer carbapenem resistance proportional to the resistance levels.

The wide spectrum carbapenems are often used and effective drugs for complicated *Bacteroides* infections so to investigate their resistance mechanisms contrary to the low rate, 1-5%, is important. The main carbapenem resistance mechanism of *Bacteroides* is the above-mentioned *B. fragilis* specific CfiA carbapenemase. 5-10% of the *B. fragilis* strains can harbour it but the resistance is only manifested if an IS element is inserted to the gene's upstream region or if the strain shows heterogeneous resistance phenotype thought to be regulated by the toxin-antitoxin system just upstream of *cfiA*.[9] From the respect of carbapenem resistance *B. fragilis* shows some more intriguing properties; it is separating into two genetically distinct divisions mainly defined by the carriage of the *cepA* (Division I) or the *cfiA* (Division II) genes. This separation was originally observed by DNA-DNA homology studies where the interdivision homology was lower than the intra-division measure. Since then PCR- and ribotyping, multi-locus enzyme electrophoresis, multi-locus sequence typing and MALDI-TOF MS typing also confirmed the existence of the two divisions.[10] Recently other distinctive genetic traits were also revealed from genomic sequencing or pangenome analyses.[11] Some authors also defined the Division II as a distinct species as *B. hominis*.[12, 13] Additionally, above *cfiA* another metallo-β-lactamase gene, *crxA*, has been described that is specific for certain strains of *B. xylanisolvens*.[14]

The many types of antibiotic resistance genes of *Bacteroides* were detected in isolates from infections or the normal microbiota. Comparison of the prevalences of the most frequent antibiotic resistance genes in these groups showed that genes on mobile genetic elements (*cfxA*, *tetQ*) are more prevalent among *Bacteroides* strains isolated from faecal samples. This observation suggests that *Bacteroides* have distinct horizontal gene transfer properties in the gut lumen and the mucosa which latter might serve as an origin for the infections of *Bacteroides* strains.[15]

Another interesting genetic trait of *Bacteroides* is that they can harbour small molecular weight, 'cryptic' plasmids which have three homology and molecular size types – 2.7 kb, 4.1 kb and 5.6 kb. Sequence analyses demonstrated that they usually code only for replication, mobilization and stability functions and their prevalence is quite high – about 50 % of strains from infections can carry at least one type. For the 2.7 kb one a recent study could demonstrate that it is more common in people in more industrialized countries, Hominidae-specific and can account for 0.1% of total metagenomic DNA. It has a replication and a mobilization gene only and has three very close molecular sequence types. These make it just for selfish DNA with no specific useful function. In parallel with the antibiotic resistance genes on MGEs, it might have high HGT transfer rates in the colon. But it is also interesting that its transfer can be increased in relation with inflammatory bowel diseases as its prevalence is higher in *Bacteroides* strains from the microbiota of patients with such maladies.[16].

CONCLUSION

In conclusion, we can say that *Bacteroides* are showing interesting behaviour in the normal microbiota and in infections, are representative members of a unique and ancient bacterial phylum with interesting biology and expectedly might serve with more intriguing novel research results in the future.

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