

Review article**Beta-lactamase production in anaerobic bacteria****Nwaokorie FO^{1*}, Ogunsola FT², Coker AO²**

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Email: franoby@yahoo.com**Abstract**

Anaerobes occur as major components of bacterial flora of the human skin and mucous membranes. They are responsible for a variety of serious and life-threatening infections. There is a great concern on the treatment of these infections due to the emergence of antibiotic resistance especially to beta-lactam drugs. Resistance to β -lactam drugs take place in anaerobic microbial species mainly by the production of beta-lactamase and this is frequently associated with therapeutic failures. In this review, the production, genetic determinant, ecological impact and the effects of β -lactamase on therapy is discussed.

Key words: Anaerobes, Antibiotic resistance, Beta-lactamase production**Introduction**

The involvement of anaerobes in human diseases and their increasing resistance to antibiotics especially beta-lactam drugs is well recognized. Of the agents used in therapy, beta-lactam drugs play an important role in the treatment of anaerobic infections. Beta-lactamase enzymes first described by Abraham and Chain in 1940 [1] are produced by many organisms and confer resistance to β -lactam antibiotics; penicillins, cephalosporins, cephamycins and carbapenems [2, 3]. It was believed that beta-lactamase enzyme was originally produced by soil bacteria to protect them against β -lactams produced by other soil organisms [4]. It was later that it became recognized that the presence of these enzymes at the site of infection could interfere with antibiotic therapy because of the resistance conferred on the organisms to these antimicrobial agents [5]. The enzymes are also found in many anaerobes of low virulence which protects them as well as other bacteria in their vicinity from β -lactam drugs [5,6]. Examples include the extended-spectrum β -lactamase (ESBL) ACI-1 from the Gram negative anaerobic cocci *Acidaminococcus fermentans* [7], cephalosporinase produced by *Bacteroides vulgatus*, *B. diastosonis*, *B. fragilis*, *B. uniformis*, *Prevotella intermedia* [8], as well as the penicillinase and oxacillinase found in *F. nucleatum* [9] and *Clostridium* species [10]. The presence of these β -lactamases is demonstrated by tests as simple as the nitrocefin to

complex molecular biology-based tests that detect the presence of genes encoding for the resistance.

Beta-lactamase resistance occurs as a result of indiscriminate use and overdose of antibiotics. The presence of these enzymes at the site of infection is associated with therapeutic failures and recurrent infections such as otitis media, tonsillitis sinusitis dental/lung abscess, which result in a longer duration of hospitalization [11]. This review discussed the production, significance of β -lactamase resistance and the genes responsible for resistance to β -lactam antibiotics in anaerobic bacteria.

Origin of β -lactamases production in anaerobes

The production of β -lactamase enzyme by anaerobes was first observed in *Bacteroides penicillinase* in late 1960s [12], but later in 1993, Roger and his co workers described the cephalosporins [13]. Since then, reports constantly acknowledge the presence of β -lactamase in anaerobic species and subsequent resistance to β -lactam drugs. The reasons for the emergence of resistant anaerobic strains differ considerably. While Nyfors et al. [14] showed that the production of β -lactamase among *Fusobacterium nucleatum* species correlates with constant exposure to antibiotics especially in children, others based their assertion on genetic transfer of resistant genes

among different bacteria in similar ecological environment [4,8,15,16].

In Gram negative bacteria, β -lactamase enzymes may be secreted into the periplasmic space and in Gram positive species, into the surrounding medium, however, in species like *B. vulgatus*, they are bound to the cell membrane [17] β -lactamase enzymes are classified based on the molecular structure as it regards to their nucleotide and amino acid sequences [18], or the function which they perform [19,20]. Different classes of β -lactamase are produced by anaerobes and the gene responsible maybe found in the chromosome or in plasmid.

Beta-lactamase producing anaerobes

Acidaminococcus fermentans

Acidaminococcus fermentans is strict anaerobic Gram negative cocci that occur as normal flora of the gastrointestinal tract in human and animals [21]. Infections involving these species include perianal abscesses, abdominal and pulmonary abscess and bacteremia [22,23]. Previously, they were described as one of the anaerobic species susceptible to β -lactam antibiotics, until an extended-spectrum β -lactamase (ESBL) producing strain capable of hydrolyzing extended-spectrum cephalosporins (cefotaxime, ceftriaxone, and ceftazidime, and the oxyimino-monobactam aztreonam) with ACI-1 gene resistant to amoxicillin and cefotaxime was recognized [21]. Sequencing of ACI-1 enzyme showed a close resemblance with CTXM-3, an extended-spectrum-lactamase, found in *Salmonella* enterica serovar Typhimurium belonging to Class A/group 2be [7, 19, 20, 22].

Bacteroides fragilis group

Bacteroides fragilis group are considered the most common anaerobes in human and animal infections. Except some strains of *B. diastons*, all species among the members of *B. fragilis* group, produce β -lactamase [24, 25]. These species include *B. fragilis*, *B. vulgatus*, *B. ovatus*, *B. uniformis*, *B. stercoris*, *B. splanhnicus*, *B. forsythus*, *B. thetaiotaomicron* and *B. ureolyticus* [26,27] and their resistance to penicillin and cephalosporins are mostly due to the production of chromosomally mediated class A β -lactamase [28]. Resistance pattern of these species shows that more than 95% of them are resistant to penicillin G and ampicillin [29], less than 50% are susceptible to ticarcillin and about 70% are susceptible to piperacillin [30,31]. In therapy, cefoxitin and piperacillin are commonly used for initial empirical treatment, however, resistance to these agents are found in areas such as North and South America, Europe Asia and South Africa [32,33, 34, 35, 36].

Different genes encode for variety of β -lactamases found in *Bacteroides* spp. Notable among them are the endogenous cephalosporinase encoded by *cepA* gene

[13], a *Cb1A* gene, which is a member of Class A/group 2e enzyme produced by *B. uniformis*, and cefoxitin resistance gene; *cfxA* assigned to Class A/group 2e found in *B. vulgatus* [37,38]. In addition, carbapenemase an extended spectrum zinc metallo β -lactamase genetically encoded by *CfiA* or *Cccr*, which belongs to Class B/group 3 is described among the *B. fragilis* species. [39,40,41]. Similarly, oxacillin hydrolyzing enzyme encoded OXA belonging to Class D is found in some *Bacteroides* group [20,42]. Reductions in the activities of penicillin or ampicillin against most *B. fragilis* due to β -lactamases is well known, however, the action of these enzymes are inhibited by β -lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) that acts against a wide spectrum of penicillins and cephalosporins. On the other hand, the metalloenzyme which hydrolyses carbapenems, imipenem, meropenem, ertapenem, as well as all β -lactam are likely to produce clinical resistance to β -lactam agents. The Class A β -lactamases; *CepA*, *Cfi A*, *Cccr A* and OXA are mediated by the chromosome and majority of these genes are not expressed [40,43]. Above all *cfxA* gene is the main determinant for enzyme expression found in *Bacteroides* species [44].

Bilophila wadsworthia

Bilophila wadsworthia is an anaerobic Gram negative bacterium that is a member of the normal flora in feces, saliva and vaginal fluids. These species have so far been isolated from patients with bacteraemia, brain and liver abscess, appendicitis and related clinical conditions [45, 46,47,48]. Initially, they were thought to be non-beta-lactamase producing because it was difficult to prove even when the antibiogram obtained in one study suggested resistance due to β -lactamase [47,49]. Following subsequent studies and modifications in identification methods, β -lactamase production was demonstrated in *B. wadsworthia* in the presence of pyruvate by Citron et al. in 1991 [50] and the enzyme characterized as cephalosporinase [51]. Presently, most strains of *B. wadsworthia* that produce β -lactamase enzyme are resistant to penicillin G and ampicillin. However, the gene responsible for β -lactamase production, the type, and class of β -lactamase produced is yet to be described.

Clostridium species

β -lactamases production in these Gram positive anaerobes are found among the species of *C. ramosium*, *C. butyricum* and *C. Clostridioforme* [52,53,54]. *C. ramosium* and *C. butyricum* reside in the lower intestinal tract of human as part of normal flora while *C. ramosium* is implicated in cases of bacteremia [55,56]. β -lactamase producing *Clostridium* species are frequently encountered in clinical specimens and their ability to produce β -lactamase is evident by their respective resistance to cephalosporins and other broad spectrum β -lactam drugs

[57,58,59,60]. Species of *Clostridium* produce inducible β -lactamase except *C. ramosium* that produces a plasmid mediated TEM-1 enzyme of Class A group 2, subgroup 2b [20]. Like the Gram negative *Bacteroides* species, they produce penicillinase and oxacillinase enzymes [61].

***Fusobacterium* species**

Certain species of Fusobacteria are capable of producing β -lactamases. Prominent among them, is *F. nucleatum*, the commonest species of fusobacteria found in the mouth, genital, gastrointestinal and upper respiratory tract. They are known to cause a variety of human infections ranging from periodontal diseases, tropical skin ulcers, peritonsillar abscesses, bacteraemia, liver abscesses, urinary tract infections, pericarditis, endocarditis, lung and pleuropulmonary infections to pre-eclampsia, preterm labor and low birth weight [62,63,64]. Other species include *F. varium* and *F. mortiferum* mainly encountered in patients with intrabdominal infections [63,64]. A multicenter survey showed that 4.5% of *F. nucleatum* produce β -lactamase and about 19% of them are resistant to penicillin due to β -lactamase production [65]. The enzyme β -lactamase FN1375 similarly found in *Clostridium* [66] and archaeal lactam utilization protein; Lam B involved in utilization of pyarolidinone is documented among these species [67]. Both enzymes are mainly found in *F. nucleatum* subsp. *vincentii* [67]. Furthermore, a class D β -lactamase FUS-1 (OXA-85) with oxacillinase activity is characterized among strains of *F. nucleatum* subsp. *Polymorphum* [68]. Although β -lactamase is found in *Fusobacterium* spp, majority of them are susceptible to penicillins and cephalosporins [67].

***Prevotella* species**

In the Genus *Prevotella*, β -lactamase is produced by *P. intermedia*, *P. oris*, *P. bivia*, *P. disiens*, *P. oralis*, *P. buccae* and *P. melaninogenica* species [2,69,70]. Most of them are implicated in neck, head and lower respiratory tract infections while species like *P. bivia* naturally occurs as vaginal flora in females [69,71]. β -lactamase production range from 31.9% in non pigmented to 68.4% in pigmented *Prevotella* species of dental origin [72,73,74]. Majority of the *Prevotella* species, produce broad spectrum cephalosporinase and penicillinase [75,76]. Like *Bacteroides*, *P. intermedia* produce cefoxitin resistance genes; CfxA Cccr, CfxA₁ and CfxA₂ and their presence is mostly related to the origin of the strains [77]. The gene CfxA, predominates in North America, CfxA₂ is mostly found in France [78,79], while In Japan, CfxA and CfxA₂ occurs respectively. 80]. In addition, Handal et al. demonstrated the occurrence of CfxA and CfxA₂ gene in 100% of β -lactam positive *Prevotella* strains from American and Norwegian patients with periodontal diseases [81]. When compared with other anaerobic organisms, *Prevotella* species remain the most prevalent

anaerobes producing β -lactamase (especially class A) and about 50% of them are resistant to penicillin and ampicillin [29,76].

***Porphyromonas* species**

Porphyromonas sps. are Gram negative anaerobes frequently described as one of the major pathogens in oral infection. The ability of *P. catoniae* to produce β -lactamase was demonstrated by Nyfors et al in 25% of infants following penicillin therapy [2]. A survey in Japan, showed that 8% of *Porphyromonas* species produces β -lactamase [82], in Italy a lower prevalence rate of 3% was recorded among periodontal isolates [83], while in USA, it was as high as 21% in samples obtained from intra-abdominal, Obstetric/gynaecology, and body fluid [30]. It is speculated that species of animal origin are often more β -lactamase produces than those derived from humans [64]. Like *Prevotella* and *Bacteroides* species, this brown or black pigmented anaerobe maintains a uniform susceptibility to carbapenems [76].

Ecological significance of beta-lactamase

The factors responsible for the origin and evolution of β -lactamase production is yet to be understood. According to literature, almost all Gram negative rods produce small amount of chromosomal β -lactamases with the primary aim of protecting themselves from β -lactams produced by Fungi, Actinomycetes, and other bacteria [84, 85, 86, 87,88,]. Anaerobes express constitutional β -lactamase activity, that are freely produced and secreted extracellular [89,90]. The enzyme diffuse within an abscess, surrounding tissue and secretions as well as other sites of infection where they protect the anaerobes producing them and other penicillin susceptible bacteria involved especially during synergistic infections [5,85,90,91,92]. This protective phenomenon often take place when the quantity of β -lactamase enzyme available in an environment is enough to break the beta-lactam ring present in penicillins before it can kill susceptible bacterial cells [5].

Detectable level of β -lactamase are present in abscesses, saliva, vagina, fecal specimen or other sites of infection that can be enough to bring about failure in therapy [84,90,92,93]. A typical example is the failure of penicillin to eradicate persistent tonsillitis in microbial mixed infection involving aerobic beta-hemolytic streptococcus species and β -lactamase producing anaerobes [94]. This finding correlates with a similar report in which "free" β -lactamase were found in 86% of acute and chronic sinusitis aspirates recovered from a mixed infection involving *S. aureus*, *B. fragilis*, *Prevotella* and *Fusobacterium* species resulting into a failure in amoxicillin therapy [5, 95]. Furthermore, It is possible to acquire certain species of β -lactamase producing anaerobes as a microbial flora from birth, however, the prevalence of β -lactamase production increases when

these organisms are exposed to β -lactam agents during therapy especially in children [14,96,97].

Intestinal micro floras are potential reservoir of resistant organisms and bacteria from this area predominately cause postoperative infections. About 70% of hospitalized patient harbor resistant anaerobic strains in their intestinal tracts, mainly belonging to the *Bacteroides fragilis* group [93]. These resistant strains, offer a reservoir for resistant genes that can be transfer to pathogenic species [98].

Anaerobic infections are usually polymicrobial and this provide conducive environment for the exchange of genetic elements between aerobes and anaerobes species thereby increasing the chances of acquiring potentials for virulence and antimicrobial resistance. There is an understanding that β -lactamases are encoded by genes extra-chromosomally transferable [2]. Species like *Bacteroides* possesses mobile elements, such as plasmids, conjugative and mobilizable transposons. The contribution of these elements in promoting the transfer of antibiotic resistance was shown in a study by Ferreira et al. that demonstrated the involvement of mobilizable transposon Tn4555 in spreading the *cfxA* gene in *Bacteroides* species [8]. Similarly, the conjugal transfer of genes encoding β -lactamase from *P. intermedia* to other *Prevotella* and *Bacteroides* is believed to be responsible for the increasing amount of β -lactamase production which constantly correlates with the continuous emergence of penicillin resistance among these species [74].

The presence of bacteria species resistance to antimicrobial agents enhances continuous existence of a pathogen in an infected host [99]. This can lead to prolonged infections that may become life threatening and at the same time support rapid and unrestricted spread of pathogens within a community.

Conclusion

Anaerobic infections are reported regularly and there are variations in their resistance patterns to β -lactam antibiotics among different geographic locations indicating the need for an urgent review of the existing treatment guidelines. Anaerobes contribute significantly in maintaining the human microbial ecology. An alteration in this balance and subsequent prevalence of resistance to routine antimicrobial agents may have an impact on the general health of an individual. There is a need therefore, to identify and monitor the prevalence of beta-lactamase species, and evaluate new antimicrobial agents in order to enhance empirical treatment.

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