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Oral Gram-negative anaerobic bacilli as a reservoir of β-lactam resistance genes facilitating infections with multiresistant bacteria

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ABSTRACT

Many β-lactamases have been described in various Gram-negative bacilli (*Capnocytophaga*, *Prevotella*, *Fusobacterium*, etc.) of the oral cavity, belonging to class A of the Ambler classification (CepA, CblA, CfxA, CSP-1 and TEM), class B (CfiA) or class D in *Fusobacterium nucleatum* (FUS-1). The minimum inhibitory concentrations of β-lactams are variable and this variation is often related to the presence of plasmids or other mobile genetic elements (MGEs) that modulate the expression of resistance genes. DNA persistence and bacterial promiscuity in oral biofilms also contribute to genetic transformation and conjugation in this particular microcosm. Overexpression of efflux pumps is facilitated because the encoding genes are located on MGEs, in some multidrug-resistant clinical isolates, similar to conjugative transposons harbouring genes encoding β-lactamases. All these facts lead us to consider the oral cavity as an important reservoir of β-lactam resistance genes and a privileged place for genetic exchange, especially in commensal strictly anaerobic Gram-negative bacilli.

1. Introduction

The oral cavity is colonised by >700 commensal or resident bacterial species, often organised as a biofilm; one-half of these micro-organisms have not been cultivated [1,2]. They survive, evolve, communicate and interact [3]. The resident [4] and transient (mucosal, pharyngeal, pulmonary, gastrointestinal, nasal, sinus and ear mucosa) bacteria can co-exist. The different niches of the oral cavity (tongue, tooth, gingival sulcus, mucosa) [5] can promote co-aggregation and persistence of certain species. The oral cavity constitutes a special environment in which bacteria may store and exchange their genetic material.

Two of the most common human diseases (caries and inflammatory periodontal disease) result from the accumulation of bacterial biofilms (plaques) on tooth surfaces. Oral health is the result of a balance between the resident flora and defence systems of the host. When this balance is disturbed, commensal and transient bacteria will be responsible for various local infections (gingival, periodontal, endodontic, etc.) [6–8] or systemic diseases [9,10], such as cardiovascular diseases, respiratory infections, diabetes [11] and preterm birth [12]. Treatment of these infections is primarily probabilistic, favouring β-lactam, macrolide–lincosamide–streptogramin and nitroimidazole antibiotic families. β-Lactams (especially amoxicillin) are used as the first-line treatment against infections of the oral cavity [13]. Intensive or inadequate use of β-lactam antibiotics in medicine and dentistry favours the selection of bacteria that have acquired resistance to other antibiotics [14,15]. Antibiotic resistance genes gradually spread among other pathogenic bacterial species by horizontal gene transfer in resident or transient bacterial populations [10,16–18]. Some species of the oral cavity can carry, in addition to antimicrobial resistance genes, genes encoding

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virulence factors, which facilitate their persistence, the expression of virulence, and survival in this complex environment.

The first aim of this review was to describe the main mechanisms of resistance to β-lactam antibiotics and their current prevalence in Gram-negative oral bacilli. The second objective was to relate them to mechanisms of gene transfer observed in the oral cavity. This close relationship between species favours: (i) the dissemination of genetic factors for antimicrobial resistance; (ii) the co-transfer of factors promoting bacterial survival and persistence in the environment; and (iii) interspecies bacterial co-operation.

2. Prevalence of resistance to β-lactam antibiotics in Gram-negative bacilli from the oral cavity

Oral bacilli such as *Treponema*, *Leptotrichia*, *Campylobacter rectus* and *Porphyromonas* are usually susceptible to β-lactam antibiotics [19]. Veloo et al. [20] found two isolates of the capnophilic *Aggregatibacter actinomycetemcomitans* that appeared to be amoxicillin-resistant according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria but not according to Clinical and Laboratory Standards Institute (CLSI) criteria, and failed to show β-lactamase activity. Resistance is often linked to the production of β-lactamases, increasingly common in the oral cavity [13,21,22].

Bacteria of the genus *Haemophilus* can carry a plasmidic β-lactamase of the TEM group. This can affect 30% of the isolates in France. Ioannidis et al. [23] investigated the high prevalence of bla_{TEM} antimicrobial resistance genes in subgingival and tongue samples of Greek subjects. The DNA sequence of this bla_{TEM} gene corresponds to the whole mature TEM β-lactamase that is widespread in Gram-negative bacteria.

Along with *Prevotella*, the *Capnocytophaga* genus constitutes the main source of β-lactamases in the oral microbiota [13,24–26]. Oral carriage of *Capnocytophaga* spp. in adults and children is estimated to be 100%, of which 16–82% are β-lactamase-producing isolates (respectively in healthy and periodontitis patient groups) [24]. The more prominent producing species are *Capnocytophaga sputigena* (64% of isolates), followed by *Capnocytophaga ochracea* (11% of isolates). β-Lactamase-positive isolates were also found among the more rarely identified species of *Capnocytophaga leadbetteri*, *Capnocytophaga gingivalis* and *Capnocytophaga granulosa* [24]. For some authors, the high prevalence of resistant *Capnocytophaga* spp. is a threat as it is very frequently isolated from the oral cavity [24,27,28]. In the literature, minimum inhibitory concentrations (MICs) to the different β-lactam antibiotics were reported to vary between strains of *Capnocytophaga* [29–32]. β-Lactamase-producing *Capnocytophaga* spp. isolates (including third-generation cephalosporins) usually remained susceptible to imipenem, cefoxitin and to the combination amoxicillin/clavulanic acid [24,30].

Two-thirds of clinical isolates of *Prevotella* produce a cefuroximase of CfxA class. Prominent β-lactamase-positive species are *Prevotella buccae* and *Prevotella intermedia*, followed by *Prevotella bivia*, *Prevotella disiens* and *Prevotella denticola* [33]. Some very rare strains of *P. bivia*, which produce another β-lactamase of class A that hydrolyses cefoxitin, have also been reported [34]. A strain resistant to imipenem (MIC = 16 μg/mL) has recently been described [35].

Rare acquired penicillinases have also been described in *Fusobacterium nucleatum* (<10% of isolates) [20,33,36].

Resistance to β-lactams in endodontic infections (infections of the inner portion of the teeth) is frequently related to anaerobic Gram-negative bacteria [14], particularly *Bacteroides* spp. [37].

3. Mechanisms of resistance to β-lactam antibiotics in Gram-negative bacilli in the oral cavity

3.1. Production of β-lactamases

β-Lactamases hydrolyse the amide linkage of the β-lactam ring to give an acyl–enzyme, which is then degraded to inactive acid. These enzymes, which protect bacteria, are classified according to the criteria of Ambler [38] or Bush [39,40]. β-Lactamases produced by oral Gram-negative bacilli belong to Ambler class A (CepA, CblA, CfxA, CSP-1 and TEM), class B (CfiA) or class D (FUS-1) (Table 1).

3.1.1. PEN-Y type β-lactamase

Only one report has described an *F. nucleatum* subsp. *polymorphum* producing PEN-Y β-lactamase (group 2a) isolated from blood cultures [43].

3.1.2. CepA β-lactamase

CepA, a chromosomal cephalosporinase belonging to Ambler class A, is an endogenous cephalosporinase found in *Bacteroides fragilis*. This β-lactamase is ubiquitous, yet it is frequently inactive. It is encoded by the *cepA* gene that appears to be only vertically transferred [44]. Most often, *Bacteroides* spp. isolates overproduce the CepA β-lactamase or have permeability defects. The presence of the *cepA* gene leads to inactivation of the aminopenicillins, carboxypenicillins and ureidopenicillins, maintaining the activity of inhibitors of β-lactamases.

3.1.3. CblA β-lactamase

CblA, a chromosomal β-lactamase belonging to Ambler class A, is a specific endogenous cephalosporinase of *Bacteroides uniformis*, susceptible to clavulanic acid. The homology between sequences of CblA and CepA is 43% for the proteins (51% for nucleotide sequences). A comparison of the protein sequence alignment of CepA with other β-lactamases reveals the conservation of at least four common elements of Ambler class A [45].

3.1.4. CfxA-type β-lactamases (CfxA, CfxA2 and CfxA3)

The study of clinical isolates of *Bacteroides vulgatus* resistant to antibiotics of the β-lactam family [46] revealed the presence of a new broad-spectrum β-lactamase named CfxA (active on cefoxitin), belonging to Ambler class A [25,47]. β-Lactamases of the CfxA group (CfxA, CfxA2 and CfxA3) belong to the 2e group of the Bush classification. This cefuroximase includes enzymes with significant activity against cephalosporins and monobactams, rather than penicillins (resistance to aminopenicillins, first-generation cephalosporins, cefuroxime and oral third-generation cephalosporins and inhibition by low concentrations of clavulanic acid). These serine enzymes are found in Gram-positive and Gram-negative micro-organisms, encoded by genes localised on chromosomes and/or plasmids [38]. β-Lactamases in this class have four preserved elements according to Ambler et al. [38]: a consensus active site Ser–Lys–XX; a helix at position 130; a glutamate at position 166; and a Lys–Thr–Gly sequence at position 234. The isoelectric point for CfxA (*B. vulgatus*) is 5.8. This 321-amino-acid protein with a molecular weight of 35.375 Da is encoded by the genes *cfxA* [47,48], *cfxA2* [17,49] and *cfxA3* [30]. The *cfxA2* gene shows 98% homology with *cfxA* described in *B. vulgatus* [50]. The protein sequence contains 321 amino acids and has a characteristic E272 K substitution [25,51]. The *cfxA3* gene presents 99% identity to *cfxA* of *B. vulgatus* and *cfxA2* of *P. intermedia* [51]. Analysis of the 966-bp nucleotide sequence of *cfxA3* indicates that this gene differs from *cfxA* of *B. vulgatus* by two amino acid substitutions (K272E and Y239D) and from *cfxA2* of *P. intermedia* by substitution of one amino acid (Y239D). Indeed, CfxA3 is different from CfxA2 because of an