Genetic determinants associated with cfxA-positive clinical Capnocytophaga isolates

To cite this version:

HAL Id: hal-01166301
https://hal-univ-rennes1.archives-ouvertes.fr/hal-01166301
Submitted on 19 Nov 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Genetic determinants associated with cfxA-positive clinical Capnocytophaga isolates

Zohreh Tamanai-Shacoori\textsuperscript{a}, Anais Dupont\textsuperscript{a}, Manon Auffret\textsuperscript{a}, Vincent Peton\textsuperscript{a}, Frédérique Barloy-Hubler\textsuperscript{b}, Elodie Ehrmann\textsuperscript{c}, Martine Ropert\textsuperscript{d,e}, Martine Bonnaure-Mallet \textsuperscript{a}, Anne Jolivet-Gougeon\textsuperscript{a,d,e}anne.gougeon@univ-rennes1.fr

\textsuperscript{a}Equipe de Microbiologie, EA 1254, Université de Rennes 1, Université Européenne de Bretagne, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France
\textsuperscript{b}Plateforme Amadeus, Rennes, France
\textsuperscript{c}Pôle Odontologie–CHU Nice, Faculté d’Odontologie, Université de Nice Sophia-Antipolis, Nice, France
\textsuperscript{d}Pôle Biologie–CHU Rennes, 2 rue Henri Le Guilloux, 35033 Rennes, France
\textsuperscript{e}INSERM U991, 35000 Rennes, France

Sir,

\textit{Capnocytophaga} spp. have a role in the pathogenesis of various forms of periodontal disease and systemic infections, particularly severe in neutropenic cancer patients. The prevalence of β-lactam-resistant oral bacteria is increasing in clinical isolates [1]. All of the reported β-lactam-resistant \textit{Capnocytophaga} isolates are β-lactamase-producers, but minimum inhibitory concentrations (MICs) for the different β-lactams are variable [2]. The objective of the current study was therefore...
to explain the variability in β-lactam MIC profiles in 31 cfxA gene-positive oral 
Capnocytophaga spp. clinical isolates with various antibiotypes. This study 
investigated: (i) the presence of other β-lactamase genes in addition to cfxA (blaCSP-1, 
cepA/cblA and cfiA); (ii) the expression level of cfxA in representative isolates with 
different antibiotic phenotypes; and (iii) the potential causes of cfxA expression 
variability, including mutation(s) in cfxA genes, location of the cfxA gene on a 
plasmid or the chromosome, and detection of the prevalence of mobile genetic 
determinants [mobA, oriT, repA, IScoc1 and transposons (Tn)] described as being 
involved in cfxA mobilisation and dissemination in Capnocytophaga spp. strains. 

All 31 isolates were clearly identified as Capnocytophaga gingivalis (n = 1), 
Capnocytophaga spp. (n = 1), Capnocytophaga ochracea (n = 2), Capnocytophaga 
granulosa (n = 3), Capnocytophaga leadbetteri (n = 3), Capnocytophaga 
AHN9576/AHN9798/AHN8471/ChDc/ChDCOS43 (n = 4) and Capnocytophaga 
sputigena (n = 17) by 16S rRNA gene sequencing. MIC₉₀ and MIC₅₀ values (MICs 
that inhibit 50% and 90% of the isolates, respectively) were all >256 mg/L for 
amoxicillin and first-and second-generation cephalosporins but were variable for 
third-generation cephalosporins. This variation in MICs for β-lactams was not due to 
the concomitant presence of other resistance genes: the cepA/cblA and cfiA genes 
were never detected, and the blaCSP-1 gene [3] was amplified in 11/31 (35%) of cfxA-
positive Capnocytophaga isolates (Fig. 1). The presence of blaCSP-1 was not 
significantly associated with higher MICs of cefotaxime [MIC > 16 mg/L according to 
Clinical and Laboratory Standards Institute (CLSI) breakpoints (http://clsi.org/)] 
compared with the presence of cfxA only (P > 0.1). In four isolates, MICs of β-
lactams were low (range, <0.016–2 mg/L) with a negative nitrocefin test, despite a
positive cfxA PCR. In 29/31 isolates, the presence of 966 bp corresponding to the complete sequence of cfxA was detected (27 isolates were β-lactam-resistant but 2 were β-lactam-susceptible). PCR assay, sequencing and in silico analysis showed that the CfxA COOH-terminal region (C-ter) in two susceptible isolates was replaced by a glycosyltransferase (96% homology) for one and with a partial hypothetical efflux pump (98% homology) for the other, with 16-bp and 82-bp overlapping gene sequences, respectively. Replacement of the whole C-ter region of cfxA was linked to β-lactamase gene inactivation, despite a positive cfxA PCR in the 5’ region. Of note, the C-ter region of cfxA appeared to be a preferentially targeted area or ‘hotspot’ for the acquisition of foreign genetic material.

Among the clinical isolates, 52% harboured plasmids of 3.5, 5 and/or 9 kb. PCR amplified Tn (77.4%), ISCoc1 (61.3%), repA (54.8%) and mobA (74.2%) that was related to plasmid detection ($P < 0.05$). This was not the case for the oriT gene (16.1%) ($P > 0.1$) (Supplementary Table S1). In the cfxA-positive Capnocytophaga isolates, mobA and repA genes were mainly detected (100% and 94%, respectively, among plasmid-positive isolates) and related to plasmid detection ($P < 0.05$). In 74% of mobA-positive isolates, the mobA and cfxA region were linked by a 96-bp intergenic sequence mainly found in plasmid-positive strains ($P < 0.01$). Higher MICs of cefotaxime (MIC > 4 mg/L) [Comité de l’Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2013; http://www.sfm-microbiologie.org/page/page/showpage/page_id/90.html] were significantly related to the presence of mobA ($P = 0.0002$), mobA–cfxA junction ($P = 0.0015$), repA ($P = 0.0003$) and at least one plasmid in bacterial strains ($P < 0.0001$). The presence of bla_CSP-1, oriT, ISCoc1 or Tn did not significantly influence the MICs of cefotaxime.
bla\textsubscript{CSP-1} was detected on the chromosome or a plasmid and in different species of \textit{Capnocytophaga}, indicating a large diffusion of this novel extended-spectrum \(\beta\)-lactamase among oral \textit{Capnocytophaga} spp.

An unchanged complete \textit{cfxA} sequence in two \(\beta\)-lactam-susceptible isolates indicated a possible misexpression of this gene under the influence of the \textit{cfxA} genetic environment. Gene copy number as the only explanation of the expression variability of \textit{cfxA} has already been observed [4].

The presence of the 96-bp junction \textit{mobA–cfxA} sequence could be important for optimal expression of the \textit{cfxA} gene. Higher MICs of \(\beta\)-lactams were related to \textit{cfxA} overexpression arising from IS integration upstream of the coding sequence [5]. The diversity of plasmid profiles of \textit{Capnocytophaga} isolates did not favour dissemination of several different \(\beta\)-lactam resistance genes via a single plasmid. In the current study, the \textit{cfxA} gene could be located on a plasmid or and the chromosome, whatever the species or MIC of \(\beta\)-lactams.

The numerous genetic rearrangements in the \textit{cfxA} gene and the presence of various genetic mobile elements in oral \textit{Capnocytophaga} suggest that this genetic site might be a reservoir for antibiotic resistance genes and their spread among different species. Furthermore, maintenance of this gene, even in an inactive form, might contribute to the persistence of antibiotic resistance genes in the oral flora.

\textbf{Acknowledgments:} The authors thank Jeremy Violette, Nolwenn Oliviero and Hélène Solhi for technical assistance; Philippe Gautier for help with the sequence
analysis; and Adina Pascu for editorial assistance. This work was supported by the Conseil régional de Bretagne (France).

**Funding:** None.

**Competing interests:** None declared.

**Ethical approval:** Not required.

**References**


Fig. 1. Detection of \textit{blaCSP-1}, \textit{mobA}, \textit{cfxA–mobA} (junction), \textit{oriT}, \textit{repA}, transposons (Tn), \text{ISCoc1} and plasmids (presence of at least one plasmid) according to different species in clinical \textit{cfxA}-positive \textit{Capnocytophaga} isolates.
FIG 1. The $ bla_{CSP-1}$, $mobA$, $cfxA-mobA$ (junction), $oriT$, $repA$, $Tn$, $ISCoc1$ genes and plasmid detection (presence of at least one plasmid), according to different species in clinical $cfxA$-positive *Capnocytophaga* isolates.