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Detection of FUS-1 (OXA-85), a Class D Beta-lactamase from *Fusobacterium nucleatum* Subspecies *Polymorphum* in Nigeria

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Authors' contributions

This work was carried out in collaboration with all authors. Author FON designed the study, collected the data and wrote draft of the manuscript, authors KOS and POA recruited and examined the patients, author MAF performed some of the experiments, author FTO contributed to the study design and was involved in writing the manuscript. Author AOC provided technical advice and revised the manuscript. All authors read and approved the final manuscript.

Short Communication

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ABSTRACT

Aims: Beta-lactamase production and subsequent resistance to β-lactam drugs has been a global concern in the treatment of Gram negative anaerobes. The aim of this study was to identify *F. nucleatum* strains producing Class D β-lactamase through the detection of FUS-1 (OXA-85) resistance gene.
Place and Duration of Study: Department of Preventive Dentistry, Lagos University Teaching Hospital, Idi-Araba, between February 2010 and November 2010.
Methodology: Twenty two oral clinical samples were obtained from patients with chronic periodontitis who admitted to previous use of amoxicillin. Antibacterial susceptibility of the bacterial isolates was determined by E-test on Brucella Blood agar. Amplification of

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the bacterial DNA was carried out by PCR using *F. nucleatum* species-specific primer, FUS-1 specific for *bla*FUS-1 and strain-specific primers for subspecies *nucleatum,' fusiforme, polymorphum* and *vincentii.*
Results: From the 19 samples collected, *F. nucleatum* was isolated, and the identity of the isolates was confirmed by PCR. Four of the isolates produced similar bands with the control strain, 3 (15.7%) strains were able to produce amplication with FUS-1 primer specific for *bla*FUS-1 gene found in β-lactamase producing *F. nucleatum* subsp. *polymorphum.*
Conclusion: This study shows the presence of class D β-lactamase producing *F. nucleatum* species in Nigeria.

Keywords: Beta-lactamase; resistance genes; Fusobacterium nucleatum; subspecies polymorphum.

1. INTRODUCTION

Infections caused by resistant microbial species fail to respond to treatment resulting to prolonged illness and in some cases may lead to death. Close contact with patients harboring resistant strains puts an entire population at the risk of spreading resistant strains and possibilities of severe illness. Beta-lactamase production and subsequent resistance to β-lactam drugs is a global concern. Several classes of this enzyme are produced by a variety of microorganisms however, oxacillinase (OXA-type) enzymes are widespread and mostly described in Gram negative microorganisms [1]. Oxacillinase are class D beta-lactamase that hydrolyses oxacillin drugs. So far, over 150 variants of OXAs are known to exist [2,3]. Like aerobes, β-lactamase production correlates with the emergence of penicillin resistance among anaerobes [4,5].

Resistant species of *F. nucleatum* are present in the oral cavity of humans especially those with history of previous antimicrobial therapy [1,6]. So far five subspecies are recognized and a newly proposed *F. nucleatum* ChDC F128 [7] of which three subspecies are known to produce β-lactamases. Possession of β-lactamase resistance gene and production of β–lactamase enzyme may be important in defining the pathogenic potentials of the different subspecies. FUS-1 is a type of OXA-85 narrow-spectrum class D β-lactamase that hydrolyses benzylpenicillin and oxacillin found in *F. nucleatum* subs. p*olymorphum* [8]. *bla*FUS-1 gene encoding for this enzyme is located in the chromosome of *F. nucleatum* species [8]. The enzyme shares about 25 to 44% identity with other class D β-lactamases, but differs slightly because it is not inhibited by sodium chloride and clavulanate [7].

Fusobacterium infection is prevalent in colon cancer, cavernous sinus thrombosis, cerebral infarction and pregnancy complications [9,10,11]. Furthermore, *F. nucleatum* species are frequently implicated in polymicrobial infections like abscesses, soft tissue infections, and diabetic foot infections in association with oxacillin resistant species [12,13,14] hence, the concern on its management and control. The development of antibiotic resistance in anaerobic bacteria has a huge impact on the selection of antimicrobial agents for empirical therapy. The contribution of FUS-1 to β-lactamase resistance in clinical isolates of *Fusobacterium* spp. might represent a serious therapeutic problem. Inadequate treatment may result in the formation and spread of antibiotic resistant species as well as therapeutic failure. Therefore, there is a need for appropriate selection of antimicrobial regimen in mixed microbial infections. In addition, accurate species identification and detection of

resistant genes would reduce cases of mis-diagnosis and alleviate subsequent treatment failure. This study investigated the presence of class D β-lactamase producing *F. nucleatum* subsp. *polymorphum* in Nigeria.

2. MATERIAL AND METHODS

2.1 Study Design/Patients

Twenty two (22) patients with chronic periodontitis attending the Lagos University Teaching Hospital, Idi-Araba, Nigeria who had used amoxicillin in the last three month were recruited. The study was approved by the Research and Ethics Committee of the Lagos University Teaching Hospital (LUTH) Idi-Araba Proc. No. ADM/DCST/221/VOL.10.

2.1.1 Bacterial isolation and identification

Isolation and identification of *Fusobacterium nucleatum* was performed as previously described [15,16]. DNA was obtained from the isolates by boiling 300µl of broth culture in sterile ultrapure water for 10 min. The solution was centrifuged at 14,000g for 10min and the supernatant (DNA) was transferred into a new sterile tube and used as template for PCR analysis [17]. DNA concentrations were determined by nano-spectrophotometry at 260 and 280 nm (Model ND 1000, Thermo Scientific Inc.). The identity of the isolates was confirmed by PCR using *F. nucleatum* species-specific primer [17]. They were further identified to their sub-species level using primers specific for subsps. *nucleatum, fusiforme, polymorphum,* and *vincentii* as previously reported [18,19,20] (Table 1). In addition, FUS-1 primer was used to determine the presence of *bla*FUS-1 gene [7].

2.2 Antimicrobial Susceptibility Testing

Antibacterial susceptibility to amoxicillin was determined by E-test (AB Biodisk, Solna, Sweden) on Brucella Blood agar incubated under anaerobiosis at 37°C for 48 h. The results of susceptibility testing were interpreted according to the CLSI guidelines [21]. *F. nucleatum* subsp. *polymorphum* ATCC 10953 obtained as a kind donation from Prof. Mario Julio Avila-Campos of Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo-USP, Brazil was used as a positive control.

Table 1. Oligonucleotide primers used for identification

Species/Subspecies/Gene	Primer/Primer Sequence (5'to 3')	Expected Product Size (bp)	Annealing Temp. (ºC)	Reference
Fusobacterium nucleatum	FN-F: AGAGTTTGATCCTGGCTCAG FN-R:GTCATCGTGCACACAGAATTGCTG	360	60	Tomazinho and Avila-Campos, 2007
Fusobacterium nucleatum subsp. *nucleatum*	Fu12-F2: CCTGCAGGAACAATAAGAC Fu12-R2: TGA AAG GCA AGG TGA AG	328	57	Kim et al., 2005
Fusobacterium nucleatum subsp. *fusiforme*	Fs17-F14: GATGAGGATGAAAAG AAACAAAGTA Fs17-R14: CCATTGAGAAGGGCTATTGAC	393	55	Shin et al., 2010
Fusobacterium nucleatum subsp. *polymorphum*	FnpF: CCAGGAGGAATAGGGGTAGG FnpR: GCCATTTCAGCTTCAACTCC	280	50	Machuca et al., 2010
Fusobacterium nucleatum subsp. *vincentii*	Fv35-F1: ATAATGTGGGTGAAATAA Fv35-R1: CCCAAGGAAAATACTAA	208	50	Shin et al., 2010
*bla*FUS-1 gene	FUS-1-F: GCCATATGTTATTATTTA TGTTCTCGAT FUS-1-R: GCGGATCCTTATTTTATA ACATTTATATTTTTG	778	62	Voha et al., 2006

3. RESULTS AND DISCUSSION

Twenty eight (28) oral clinical samples were obtained from 22 patients with chronic periodontitis recruited for the study. This study demonstrated that male patients were the most predominant group with chronic periodontitis which is in agreement with previous findings [16,22,23] (Table 2). *F. nucleatum* species were isolated from 19 (67.9%) samples. Fifteen of these isolates were further identified as *F. nucleatum* subsp. *nucleatum* and four as *F. nucleatum* subsp. *polymorphum*.

Table 2. Age and sex distribution of patients with chronic periodontitis

Age Range (Years)	Number of cases (n=22)	Male (%)	Female (%)
11-20	1	1 (4.5%)	0 (0%)
21-30	2	1 (4.5%)	1 (4.5%)
31-40	7	4 (18.2%)	3 (13.6%)
41-50	9	6 (27.3%)	3 (13.6%)
51-60	2	0 (0%)	2 (9.1%)
61-70	1	1 (4.5%)	0 (0%)

Mean age= 40.5 years

Susceptibility pattern to amoxicillin was easily determined on the 19 isolates by E-test. The results showed that five of the isolates were resistant to amoxicillin of which three were F nucleatum subspecies nucleatum and two were F. nucleatum subsp. polymorphum (MICs, >128 µg/ml). (Table 3). BlaFUS-1 gene was detected in three (3; 15.7%) isolates identified as F. nucleatum subsp. polymorphum (Fig. 1), showing the presence of FUS-1 (OXA-85) a Class D Beta-lactamase from F. nucleatum subsp. polymorphum. One of the isolates FNp5 possessing BlaFUS-1 gene was resistant to amoxicillin (MIC, >256 µg/ml).

Table 3. Relationship between the presence of *bla*FUS-1 and amoxicillin MICs

Species	Isolate	*Amoxicillin MIC (µg/ml)	*bla*FUS-1 gene
Fusobacterium nucleatum subsp. *nucleatum*	FNn1	>256	-
	FNn9	>256	-
	FNn16	>128	-
Fusobacterium nucleatum subsp. *polymorphum*	FNp3	>256	+
	FNp5	>128	-
	FNp19	2	+
	FNp25	2	+

**All MICs were interpreted using CLSI clinical breakpoints*

This study showed that subsp. *polymorphum* may or may not have *bla*FUS-1 gene. In addition, two of the isolate having the gene were not resistant to amoxicillin supporting the

idea that the presence of a resistance gene in the genome of a microorganism is not always related to the resistance phenotype because some of these strains remain susceptible to β-lactams [8]. Isolating species resistant to amoxicillin was not surprising because the recruited patients were already on amoxicillin therapy. Although *F. nucleatum* subsp *nucleatum* is among the species of *F. nucleatum* producing β–lactamase enzymes, FUS-1 (OXA-85) has not been described in this subspecie. Furthermore, our inability to detect *bla*FUS-1 gene in subsp. *fusiforme* and *vincentii* may be due to the fact that β-lactamase production is rare in these subspecies. Moreover, *F. nucleatum* subsp. *vincentii* does not possess oxacillin gene [24].

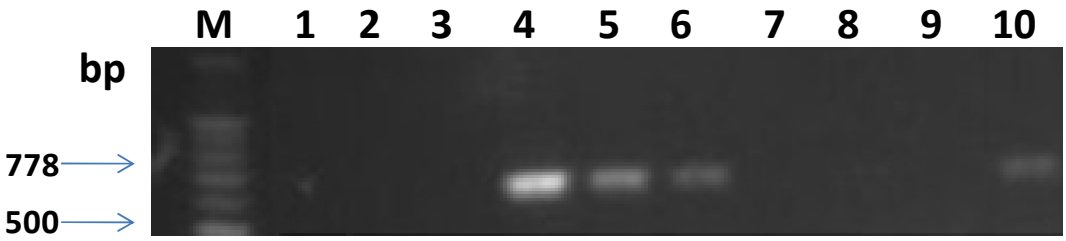

Fig. 1. Image from gel electrophoresis of amplicons obtained after PCR analysis. Lane M: 100 bp DNA marker, lane 4 *F. nucleatum* subsp. *polymorphum* ATCC 10953, lanes 5, 6 &10 showed visible amplification of BlaFUS-1 gene detected in *Fusobacterium nucleatum* subsp. *polymorphum*

In mixed microbial infections *F. nucleatum* is found in association with species like *Staphylococcus*, *Pneumococcus*, and *Streptococcus*, sensitive to oxacillin. Such cases are likened to infections occurring on the skin, subcutaneous cell tissue, upper and lower respiratory tract, urogenital tract, as well as septicemia, acute and sub-acute endocarditis, and osteomyelitis [9,10,11]. *Fusobacterium* species are moderately sensitive to penicillin antibiotics. However studies have shown the presence of β-lactamase producing strains and subsequent resistance to beta-lactam drugs [1,25]. Oxacillin is not a drug of choice in the treatment of anaerobic infections [26] however; our observation of the presence of *F. nucleatum* strain producing OXA-85 may be a problem in mixed microbial infections because they could transfer this gene to species of same or different genus in similar ecology. Furthermore, they may tend to protect other pathogenic species originally susceptible to oxacillin resulting into recurrent infections, prolonged hospital stay, therapeutic failure and even death.

Defining methods for rapid and easy detection of phenotypic characteristics possessed by class D β-lactamase producers is quite challenging. This has hindered the possibility of monitoring the presence of microorganisms capable of producing Class D β–lactamase enzyme. By using PCR, the primers used in this study produced visible and corresponding bands as previously described [8] showing the presence of *bla*FUS-1 gene in *F. nucleatum* subsp. *polymorphum*

4. CONCLUSION

This study has shown the presence of class D β-lactamase producing *F. nucleatum* subsp. *polymorphum* in Nigeria. Since detection of FUS-1 gene is an important indication of the

spreading of this gene, there is need to focus on the spread of genes responsible for drug resistance in our population.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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