Antibiotic Resistance in *Bacteroides* and *Prevotella* to β-Lactams, Lincosamide and Nitroimidazole: A 20 Year Survey in Lagos, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author LOE designed the study, interpreted the results and prepared the final manuscript. Author NNN led the investigation team and produced the first draft of the manuscript. Authors FAB, AMK and WWE collated and analyzed the data and managed the literature searches. Author OOO carried out the clinical diagnosis. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The propensity to develop resistance to antibiotics has accounted for the predominance of *Bacteroides* and *Prevotella* in infections due to anaerobic bacteria. The observed differences in resistance pattern across geographical boundaries underscored the timeliness for this study to review data on antibiotic susceptibility and resistance markers amongst *Bacteroides* and *Prevotella* to β-lactams, lincosamide and nitroimidazole from selected hospitals in Lagos, Nigeria from 1992-

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2011. *Bacteroides*, mostly *B. fragilis* were the sole anaerobic gram negative bacilli in peritonitis following lower abdominal surgery and bloodstream infections, while *Prevotella bivia* and *P. melaninogenica* were more frequent in periodontal abscess, pelvic inflammatory disease and chronic suppurative otitis media. The MIC range and MIC$_{90}$ of the anaerobes for the antibiotics does not indicate changes in group resistance to antibiotics though isolated cases were recorded. While clear cut patterns were not established for other species, *B. fragilis* resistance increased for amoxicillin-clavulanic acid from 18.2% in 1992-1996 to 31.4% in 2006-2011 and from 27.3% in 1992-1996 to 34.3% in 2006-2011 for cefoxitin. In contrast, decrease susceptibility was obtained against clindamycin (54.5% in 1992-1996 to 22.9% in 2006-2011). Similarly, 22.7% of *B. fragilis* strains have the *cep*A and/or *cfx*A gene in 1992-1996 compared to 32.1% in 2006-2011. While not all isolates with *cep*A and/or *cfx*A genes were resistant to the β-lactams, all isolates carrying the *erm*F or *nim* genes were resistant to the lincosamide (clindamycin) or nitroimidazole (metronidazole) respectively. The presence of antibiotics resistance genes can be used in tracking resistance amongst species of *Bacteroides* and *Prevotella*. With emerging new technologies, gene screening may prove more effective and reliable for therapeutic decisions for anaerobic bacterial infections especially since the genes can be screened from the infective exudates. This will eliminate the need for culture and antibiotic susceptibility testing for this group of fastidious microorganisms.

1. INTRODUCTION

Bacteroides, Fusobacteria and *Prevotella* are probably the most frequently isolated Gram negative anaerobes in clinical infections; peritonitis, Gram negative septicemia, chronic suppurative otitis media, pelvic inflammatory diseases and puerperal sepsis, cystic fibrosis, periodontitis, dentoalveolar, cutaneous and soft tissue abscesses including anorectal and hiradenitis suppurativa [1-8]. The burden of these infections on patients' healthcare and the understanding of their polymicrobial nature require constant review of therapeutic regimen. Knowledge on the involvement of anaerobic bacteria in human infections is growing. Once thought to be relatively harmless with pathogenic potential only attributed to the toxigenic clostridia and *Bacteroides fragilis*, have emerged in major life-threatening infections including bacteremia, puerperal breast abscess and non-puerperal breast abscesses where they account from between 1-17% cases [9-11]. Boucher et al [10] reported *P. bivia* pure culture in a patient with non-puerperal breast abscess after flucloxacillin empiric therapy 500mg/6 hours for 2 days. AST results showed resistance to clindamycin but susceptible to amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, and metronidazole. Anaerobic gram negative bacilli (AGNB) are more frequently encountered in bacteremia with *B. fragilis* accounting for more than 50% of cases and *Fusobacterium* spp 5% and infection is mostly defined by polymicrobial etiology [12-14]. Surgical procedures involving the GIT and dental structures have predisposing role to Gram negative bacteremia [15]. Data from our environment have implicated AGNB in infections of the Head, Neck and Throat. In one study on otitis media, AGNB accounted for 147 of the 242 anaerobes isolated [1]. Other studies both in Nigeria and elsewhere have implicated species of *Bacteroides*, *Prevotella*, *Porphyromonas* and *Fusobacterium* in periodontal diseases, dentoalveolar abscess, post-surgical sepsis and female genital tract infections [2,4,7].

Therapeutic considerations for anaerobes are broadening as more species are identified to play active role in human infections. This is compounded by emergence of antibiotic resistant strains that have made treatment more difficult [16-18]. Adding to this dilemma is the relative lack of rapid and cost effective methods for routine AST for anaerobes. The implication is to rely on data from reference laboratory for empiric therapy; but this may not be representative as susceptibility pattern varies from country to country. Jamal et al [19] reported resistance of less than 5% in AGNB to imipenem and metronidazole in Kuwait hospital for the period 2002 to 2007. Wide spread resistance to clindamycin up to 20% in anaerobes, and rising resistance to β-lactam antibiotic in *Bacteroides*, *Prevotella* and *Fusobacterium* have been reported [20-25]. Resistance to metronidazole a mainstay drug for anaerobic infections have been reported from many countries at MIC > 32mg/L to be 1.5% in UK in 1995, 3.8% in 1997 and

7.5% in 1998 [21], and the pattern vary occasionally slightly from one country to another [22,24]. The emerging pattern from different reports and countries will definitely define the future prescription pattern of antibiotics for anaerobic infections.

Resistance by *Bacteroides* species to mainstay antibiotics such as metronidazole, clindamycin, cephalosporins and other β-lactam antibiotics, macrolides and the fluoroquinolones are reported with increasing frequency from different countries [25-28]. Other studies have also revealed steady increase in resistance by non-bacteroides Gram negative anaerobic bacilli to some antibiotics [29-31]. The role of resistance genes in mediating resistance to antibiotics in Gram negative anaerobic bacteria is well delineated in the literature. For instance *cep*A, *cfx*A and *cfi*A encode for resistance to cephalosporins and penicillins, cefoxitin, and carbapenem respectively [32,33], while resistance to metronidazole have been associated with the *nim*A-H, J genes and for the macrolide-lincosamide are a variety of genes including *erm*B, *erm*F, *erm*G, *msr*SA, *mef*A, and *lin*A [34,35].

It is of major concern to observe in the literature the absence of data from Nigeria on antibiotic resistance of anaerobic bacteria especially with reference to molecular mechanisms. In the present study we reviewed retrospectively resistant trends in *Bacteroides* and *Prevotella* to three classes of antibiotics commonly prescribed for mixed aerobic-anaerobic infections in Nigeria; β-lactams, lincosamides and nitroimidazole and attempt to correlate the presence of resistant genes with the emerging pattern. In addition, we discussed our findings in relation with susceptibility patterns reported from other geographical regions. This may represent a preliminary report from Nigeria on trends and patterns of antibiotic susceptibility by anaerobes and consequently may add to the global picture on antibiotic resistance in anaerobes and in devising strategies for effective antibiotic stewardship.

2. MATERIALS AND METHODS

2.1 Isolates and Sources

The isolates used for this study were from patients with peritonitis following lower abdominal surgery (PLAS), periodontal abscess (PDA), pelvic inflammatory disease (PID), chronic suppurative otitis media (CSOM), septic abortion (SAB), dentoalveolar abscess (DVA) and bloodstream infections (BSI) seen over a 20 year period (1992-2011) in four specialist hospitals (Lagos University Teaching Hospital, Idi-Araba; Lagos State University Teaching Hospital, Ikeja; General Hospital, Gbagada; Military Hospital, Yaba) in Lagos, Nigeria. Specimens collected include peritoneal fluid/abscess (needle aspirates) for PLAS, subgingival pocket plague/aspirate (paper points/needle aspirate) for PDA, endometrial material and purulent secretion (endometrial suction and swab obtained from cervix uteri) for PID, purulent ear exudate were obtained by aspiration or with swab for CSOM, peritoneal fluid and tissue biopsy for SAB, needle aspirate for DVA, and blood (10 ml venous blood) for BSI. All specimens were transported in anaerobic vials or modified Cary-Blair transport medium. Anaerobic cultures were done on both non-selective and selective media (Brucella agar supplemented with 5% sheep blood and vitamin K_1 (1 µg/ml) and hemin (5 µg/ml); Bacteroides bile esculin agar for selective isolation of *B. fragilis* group; kanamycin vancomycin laked blood agar for pigmented and non-pigmented *Prevotella* species and *Bacteroides*. Inoculated plates were incubated anaerobically at 37°C in 10% CO_2 generated by GasPak system (bioMerieux, Marcy L' Etiole, France) for 24-72 h. The isolates were identified by Gram's morphology, motility and other phenotypic characteristics for anaerobic gram negative bacilli. The isolates were identified to species level using the scheme as given in (Table 1) [36]. The isolates were from specific research studies carried at different time over the period reported, since there were no routine laboratory screening for anaerobes at these Centres. This study only considered the *Bacteroides* and *Prevotella* and reported on the antibiotic profile of frequently isolated species. Species studied included *B. fragilis* (110 isolates), *B. ovatus* (38 isolates), *P. melaninogenica* (57 isolates) and *P. bivia* (42 isolates). The distribution of the species across the study period is indicated in Table 3. Isolates were maintained in glycerol Brain Heart Infusion at -70°C.

2.2 Antibiotic Susceptibility Testing

The MICs of the isolates from 1992 to 2001 for the antibiotics were determined by agar dilution as described in Clinical and Laboratory Standards Institute (CLSI) [37] while E test was used for isolates from 2002 to 2011. However, the agar dilution method was used to test amoxicillin-clavulanic acid from 1992 to 2011.

Between 1992 and 2001 the E-test antibiotic strips were not available in Nigeria and the agar dilution method was conventional. In the agar dilution method, serial two-fold dilutions of the antibiotics were prepared in Brain heart infusion agar (BHIA) supplemented with hemin (5µg/ml) and vitamin K_1 (0.5µg/ml). Antibiotic concentration in agar ranged from 0.125 to 128µg/ml for cefoxitin, clindamycin and metronidazole, while for amoxicillin-clavulanic acid the agar concentrations were from 0.25/0.125 to 128/64. Controls consisted of agar plates with no antibiotics to test for the viability of the test organisms and sterility of the medium. Bacterial inoculum was prepared from pure culture by transferring between 3-5 colonies on BHIA agar supplemented into pre-reduced thioglycollate broth supplemented with hemin (5µg/ml) and vitamin K_1 (0.5µg/ml) (Oxoid Ltd., Wade Road, Basingstoke, Hants, UK) and incubated at 37° C in 10% CO_2 generated by GasPak system (bioMerieux, Marcy L' Etiole, France) for 24 h. The broth culture was diluted to a density of 10^5 cfu/ml standardized against a 0.5 MacFarland standard. Freshly prepared BHIA plates supplemented containing different concentrations of the antibiotics were then inoculated with a Steers replicator by spotting. Plates were incubated at 37° C for 48 h in an anaerobic jar using the GasPak anaerobic gas generating envelop (bioMerieux, Marcy L´ Etiole, France). The MIC was defined as the lowest concentration of the antibiotic that inhibited growth or produced a faint haze of growth. The E-test was performed according to the Manufacturer's instructions (bioMerieux, Marcy L' Etiole, France) on Brucella base agar plates supplemented with 5% v/v horse blood, vitamin K_1 (10µg/ml) and haemin (5µg/ml). *Bacteroides fragilis* ATCC 25285 obtained from the American Type Culture Collection (ATCC, Manassas, VA) was used as the reference strain. MIC endpoint for E-test was read as the point of interception of the zone of inhibition on the strip in accordance with defined guidelines. Beta-lactamase production was tested in all strains using the chromogenic cephalosporin disk (nitrocefin; ABBiodisk, Solna, Sweden). Antibiotics minimum inhibitory concentrations (MICs) for the anaerobic gram negative bacilli (AGNB) was interpreted based on the CLSI breakpoints guidelines [37] as susceptible or resistance as given in Table 2.

2.3 DNA Extraction and Amplification

Total genomic DNA extraction was by chemical lysis and physical disruption [38,39]. DNA was purified with a Qiamp DNA Mini Kit ("tissue protocol", Qiagen, Hilden, Germany) as described by the Manufacturer. The DNA concentration and purity were determined by spectrophotometer (Model, D-37520, Thermo Scientific, Waltham, Massachusetts, MA, USA) and nanodrop (Model, 2000, Thermo Scientific, Waltham, Massachusetts, MA, USA). The OD 260/280 reading of the purified DNA was $\geq$1.8. The concentration of DNA used was 50 ng/µl. The DNA was stored at -20° C for further use.

PCR amplifications were performed in a total volume of 25µl made up of the genomic DNA, PCR H_2O, primers and Red Taq Mastermix (Sigma-Aldrich, USA). The Red Taq mastermix contained Taq DNA polymerase, antibodies to the Taq DNA polymerase, 32mM $(NH_4)_2SO_4$, 130mM Tris HCl, 0.02% Tween 20, 2mM $MgCl_2$ and dNTPs (dATP, dCTP, dGTP, dTTP). The protocol for amplification was set in the PCR thermocycler (Bio-Rad, Germany) depending on the primers used: {5´-TTTCTGCTATGTCCTGCCC-3´, *cep*A1; 5´-ATCTTTCACGAAGACGGC-3´, *cep*A2 at 52° C and 35 cycles [40]; 5´-ATGTTCAGAGAAATGGGGCGTAAGCG-3´, *nim*3 and 5´GCTTCCTTGCCTGTCATGTGCTC-3´, *nim*5 at 57° C and 25 cycles [41]; 5´-ATCGTAGTTTTGAGTATAGCT-3´, *cfx*A1 and 5´-TAAAGCACTCCGATAACGAT-3´ *cfx*A2 at 57° C and 30 cycles [42]; 5´-CCTTATGGCATTACTTCCGA-3´, *erm*F1 and 5´-GGACCTACCTCATAGACAAG-3´ *erm*F2 at 55° C and 30 cycles [43]}.

A water blank with no template was included in the run as a negative control. *B. fragilis* 638R containing *nim* genes was used as positive control while *B. fragilis* NCTC 11295 was included as a *nim* gene negative control [44]. After the amplification, the PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide and visualized by UV transillumination (302 nm; UVP, Inc.).

2.3.1 Beta-lactamase and blaZ gene detection

Beta-lactamase production was tested in all strains using the chromogenic cephalosporin disk (nitrocefin; ABBiodisk, Solna, Sweden). Results were read after 15, 30, and 60 min. Determination of the *blaZ* gene which codes for resistance to β-lactam drugs was carried out as described [38,21]. Primers for amplification of

Table 1. Group identification of some anaerobic gram negative bacilli based on phenotypes

	Van (5µg)	Kan (1000µg)	Col (10µg)	Growth in 20% bile	Catalase	Indole	Lipase	Pigment	Brick-red fluorescence
B. fragilis group	R	R	R	+	V	V	-	-	-
Other *Bacteroides*	R	R	V	-$^+$	-$^+$	V	-	-	-
Prevotella spp.#	R	R^S	V	-	-	V	V	+$^-$	+$^-$
P. intermedia	R	R^S	S	-	-	+	+$^-$	+	+
P. loescheii	R	R	V	-	-	-	V	+	+
Other *Prevotella*	R	R	V	-	-+	-+	-+	+	+

Agar pitting bacteria (*Campylobacter* spp and *Bacteroides ureolyticus*)

	Van (5µg)	Kan (1000µg)	Col (10µg)	Growth in 20% bile	GSFF	Nitrate reduction	Urease	Motility	Pitting of agar
B. ureolyticus	R	S	S	-	+	+	+	-	V
Campylobacter spp.	R	S	S	-	+	+	-	+$^-$	V

Rapid identification of non-pigmented *Prevotella* spp.

	Van (5µg)	Kan (1000µg)	Col (10µg)	Indole	Esculin hydrolysis	β-NAG	α-fucosidase	β-xylosidase	Colistin
P. enoeca	R	R	R/S	-	V	+	+	-	R
P. bivia	R	R	R/S	-	-	+	+	-	R/S
P. disiens	R	R	R/S	-	-	-	-	-	

Van vancomycin; Kan kanamycin; Col colistin; # Prevotella melaninogenica; GSFF Growth stimulated by formate-fumarate

Table 2. CLSI antibiotic breakpoints for anaerobic gram negative bacteria

Antibiotics	MIC breakpoints (µg/ml)	
	Susceptible	**Resistance**
Amoxicillin + clavulanic acid	≤ 4/12	≥ 16/8
Cefoxitin	≤ 16	≥ 64
Clindamycin	≤ 2	≥ 8

blaZ were used, stau-*blaZ*-fwd: (5'-CAAAGATGATATAGTTGCTTATTCTCC-3') and stau-*blaZ*-rev: (5'-TGCTTGACCATTTTATCAGC-3') (Eurofins, Germany).

PCRs were performed in 25 µl volume containing 1.5 µl each of primer, 8.5 µl of PCR H_2O, 12.5 µl RedTaq Mastermix and 1 µl of genomic DNA. Cycling conditions comprised 94^oC for 5 min, followed by 35 cycles of 94^oC for 30 s, 50^oC for 30 s and 72^oC for 30 s, with a final extension at $72^o C$ for 7 min. The amplification products were analyzed on 1.5% (w/v) agarose gels stained with ethidium bromide and visualized under UV light.

3. RESULTS

Table 3 is a surveillance data from the four referral hospitals of clinical conditions from which anaerobic bacteria were isolated. *Bacteroides* spp were the sole pathogen in peritonitis following lower abdominal surgery and bloodstream infections. *Bacteroides* and *Prevotella* were predominant in septic abortion and pelvic inflammatory disease, while from periodontitis, dentoalveolar abscess and chronic suppurative otitis media were isolated *Prevotella*.

Table 4 gives the resistance of the species within the periods. *B. fragilis* has a more established pattern, showing increasing resistance to amoxicillin-clavulanic acid and cefoxitin and decreasing resistance to clindamycin. Against metronidazole decreasing resistance occurred from 1992 (22.7%) to 2006 (14.8%) and then increased to 20% in 2011. For the *Prevotella* spp, though the trend was not consistent, significant is that 2 of the isolates sensitive to other antibiotics were resistant to cefoxitin. Across antibiotics for corresponding period, amoxicillin-clavulanic acid showed better activity than cefoxitin. Correspondingly the MIC values showed *B. fragilis* as the most resistant of the species tested. Against amoxicillin-clavulanic acid the MIC_{90} for *B. fragilis* increased from 16/8µg/ml in 1996 (upper range) to 64/32µg/ml in 2011 with a narrower MIC range (0.5/0.25-128/64µg/ml). The MIC_{90} of *P. bivia* increased from 2/1µg/ml in 1996 to 4/2µg/ml in 2011, however, the MIC_{90} in 2001 was 16/8µg/ml (the 2 resistant strains in that year had MIC of 16/8µg/ml and fall within 90% of the isolates). Apart for *B. fragilis* that had increase in MIC_{90} from 1992 to 2011 against cefoxitin, other species have relatively constant MIC_{90} for the antibiotic, though minor differences may be observed in the MIC range. There was no change in MIC_{90} values of clindamycin for the *Bacteroides* from 1992 to 2011 though decreasing resistance was evident from the lowering of the MIC range values.

The number of isolates with *cep*A and/or *cfx*A genes and the percentage resistance mediated as a result is given in Table 5. All the isolates produced β-lactamase and were therefore not indicated in Table. Percentage resistance associated with these resistance markers increased from 22.7% in 1996 for *B. fragilis* to 32.1% in 2011, 9.1% in 1996 for *P. bivia* to 43.8 % in 2011 and 23.5% in 1996 for *P. melaninogenica* to 37.5% in 2011. The distribution of resistance genes to clindamycin (*erm*F) and metronidazole (*nim*) are shown in Table 5.

4. DISCUSSION

The four species reported on in this study were the most frequently isolated anaerobic Gram negative bacilli in the clinical conditions studied. Other species of Gram negative anaerobic bacilli isolated were not included in this report because they were encountered less frequently and their inclusion may not be representative of the susceptibility profile of the species. The prevalence of these species is a reflection of the location of infection as defined by Papaparaskevas et al. [2]; *Bacteroides* predominated in bacteremia and intra-abdominal/pelvic infections while *Prevotella* sites of infection were the respiratory tract, skin and soft tissue. The predominance of *B. fragilis* in clinical infections over other anaerobic Gram negative bacilli as evident in the present study is associated with its superior pathogenic properties [28,29].

Table 3. Occurrence of *Bacteroides* and *Prevotella* species in the clinical conditions studied

Source of specimen	AGNB isolated	Distribution 1992-'96 1997-2001 2002-'06 2007-'11	Total isolation (%)
PLAS	*B. fragilis*	11 8 15 12	46 (63.01)
	B. ovatus	3 5 9 10	27 (36.99)
BSI	*B. fragilis*	6 10 12 14	42 (84.00)
	B. ovatus	2 1 2 3	8 (16.00)
SAB	*B. fragilis*	5 3 6 2	16 (36.36)
	B. ovatus	0 1 0 2	3 (6.82)
	P. bivia	5 8 9 3	25 (56.82)
PID	*B. fragilis*	0 2 2 1	5 (22.73)
	P. bivia	6 5 5 1	17 (77.27)
PDA	*B. fragilis*	0 0 1 0	1 (3.45)
	P. melaninogenica	7 14 5 2	28 (96.55)
DVA	*P. melaninogenica*	5 3 4 0	12 (100)
CSOM	*P. melaninogenica*	5 7 3 2	17 (100)

AGNB, anaerobic gram negative bacilli

Table 4. Antibiotic resistance in *Bacteroides* and *Prevotella* (1992-2011)

Bacterial species (number tested)	Percentage resistance (MIC_{90} µg/ml)			
	AMC[1]	CEFXT	CLIN	MET
1992-06[1]				
B. fragilis (22)	18.2 (16/8)	27.3 (32)	54.5 (64)	22.7 (8)
B. ovatus (6)	16.7 (4/2)	33.3 (16)	50 (16)	0 (8)
P. bivia (11)	9.1 (2/1)	9.1 (4)	9.1 (16)	0 (4)
P. melaninogenica (17)	23.5 (16/4)	23.5 (16)	5.9 (2)	0 (4)
1997-01[1]	22.1 (16/8)			
B. fragilis (26)	0 (2/1)	19.2 (16)	42.3 (64)	15.4 (8)
B. ovatus (5)	15.4 (16/8)	20 (16)	80 (16)	0 (8)
P. bivia (13)	8.3 (2/1)	23.1 (16)	7.7 (4)	4.2 (4)
P. melaninogenica (24)	29.6 (32/16)	16.7 (16)	8.3 (4)	4.2 (4)
2002-06[2]	20 (16/8)			
B. fragilis (27)	7.1 (4/2)	29.6 (32)	25.9 (64)	14.8 (16)
B. ovatus (10)	8.3 (4/2)	20 (16)	30 (16)	10 (4)
P. bivia (14)	31.4 (64/32)	28.6 (16)	14.3 (8)	8.3 (8)
	17.7 (64/32)	33.3 (16)	25 (8)	0 (2)
P. melaninogenica (12)	0 (4/2)			
	0 (4/2)			
2007-11[2]				
B. fragilis (35)		34.3 (64)	22.9 (64)	20 (16)
B. ovatus (17)		23.5 (16)	23.5 (16)	5.9 (8)
P. bivia (4)		50 (16)	0 (4)	0 (4)
P. melaninogenica (4)		50 (16)	0 (4)	0 (4)

[1]; MIC determined by agar dilution method, [2]; MIC determined by E-test; AMC, amoxicillin-clavulanic acid; CEFXT, cefoxitin; CLIN, clindamycin; MET, metronidazole

The results on the antibiotic susceptibility pattern from the present study did not show major deviation from those reported elsewhere. From Kuwait [19], *B. fragilis* was reported to show increasing resistance to most of the antibiotics tested while with most other anaerobes the trend over the period 2002 to 2007 was relatively stable. For instance highest resistance of *B. fragilis* to amoxicillin-clavulanic acid was 15% over the period with MIC_{90} of 8µg/ml, 50.8% to clindamycin at MIC_{90} >256µg/ml and to metronidazole 1.5% with a wide MIC range of 12->256µg/ml. We observed 13.2% rise in resistance to amoxicillin-clavulanic acid and 7% to cefoxitin in *B. fragilis* isolates from Nigeria. In contrast to the Kuwait study, a decrease in resistance of 31.6% was recorded against clindamycin; these differences from the two

countries may be due to prescription pattern for clindamycin. Clindamycin is a reserved therapeutic option for life threatening Gram positive bacterial infections in many hospitals in Nigeria. This restricted use may account for the decreasing resistance by *B. fragilis* to clindamycin.

In Croatia [30] resistance levels were comparatively lower compared to those from Kuwait and Nigeria. This study showed that *Bacteroides* spp was less resistant compared with other Gram negative anaerobic bacilli to amoxicillin-clavulanic acid (5.7% against 8%) and metronidazole (2.9% against 8%). The Moscow study [45] further highlights the geographical differences in strains susceptibility to antibiotics. *B. fragilis* group resistances were 3% metronidazole (MIC > 4µg/ml), 4.5% amoxicillin-clavulanic acid (MIC > 4µg/ml), and 22.4% clindamycin (MIC > 4µg/ml). No resistance was reported in *Prevotella* to imipenem and amoxicillin-clavulanic acid, but to metronidazole and clindamycin resistance was 7% and 12% respectively. This signals the emergence of

multi-drug resistant strains in Moscow. Though percentage resistance seems to vary from country to country the pattern across genera or group is identical with the *Bacteroides* especially the *B. fragilis* showing leading resistance profile in most studies. In most cases, all *B. fragilis* were resistant to penicillin and ampicillin which is mediated by chromosomal β-lactamases (*cfiA* and *cepA* genes) [18]. The resistance to carbapenems is mediated by *cfiA* gene which codes a metallo β- lactamase. The presence of IS elements in the upstream of the *cfiA* gene is required for its expression [16,25]. The most common mechanism of *B. fragilis* resistance to metronidazole is the expression of nitroreductases (*nim* genes dependent mechanism), which reduces the nitroimidazoles drug's nitro group to an inactive amino group [24]. Typical variations likely to occur from one location to another inform on the pattern of use of antibiotics necessitates that local antibiotic susceptibility pattern be considered from time to time in treatment prescription and in formulation of antibiotic policy especially for the treatment of infections involving anaerobes.

Table 5. Presence of antibiotic resistance genes in the anaerobic Gram negative bacilli

Resistance marker	*B. fragilis*	*B. ovatus*	*P. bivia*	*P. melaninogenica*
1992-06[1]				
cepA*	6 (27.3)	2 (33.3)	3 (27.3)	6 (35.3)
cfxA	5 (22.7)	1 (16.7)	1 (9.1)	4 (23.5)
ermF	12 (54.6)	3 (50)	1 (9.1)	1 (5.9)
Nim	5 (22.7)	0 (0)	0 (0)	0 (0)
1997-01[1]				
cepA*	6 (3.1)	1 (20)	3 (23.1)	4 (16.7)
cfxA	5 (19.2)	1 (20)	2 (15.4)	4 (16.7)
ermF	11 (42.3)	4 (80)	1 (7.7)	2 (8.3)
Nim	4 (15.4)	0 (0)	0 (0)	1 (4.2)
2002-06[2]				
cepA*	8 (29.6)	2 (20)	4 (28.6)	4 (33.3)
cfxA	8 (29.6)	2 (20)	3 (21.4)	4 (33.3)
ermF	7 (25.9)	3 (30)	2 (14.3)	3 (25)
Nim	4 (14.8)	1 (10)	1 (7.1)	0 (0)
2007-11[2]				
cepA*	12 (34.3)	4 (23.5)	1 (25)	2 (50)
cfxA	12 (34.3)	4 (23.5)	1 (25)	2 (50)
ermF	8 (22.9)	4 (23.5)	0 (0)	0 (0)
Nim	7 (20)	1 (5.9)	0(0)	0 (0)

*All cepA positive isolates produced β-lactamase; a; erythromycin and clindamycin resistance pooled
Values in Table represent number of isolates carrying resistance genes; Values in parenthesis represent
percentage of isolates with genes

In the present study we establish corresponding correlations between presence of antibiotic resistance genes in our isolates and the level of resistance observed to all the antibiotics tested. The spread of these resistance genes over time may account for the buildup in resistance from 1996 to 2011 as indicated in this study. Since all the isolates tested positive for β-lactamase (*cep*A positive), all *cfx*A positive isolates carry the *cep*A gene. While not all isolates with *cep*A and/or *cfx*A genes were resistant to the β-lactams, all isolates carrying the *erm*F or *nim* genes were resistant to the lincosamide (clindamycin) or nitroimidazole (metronidazole) respectively. It is important to state that in this study, only the presence of a few of these resistance genes was screened. In future studies we shall expand the scope of resistance genes screened to be able to associate their contribution to the mechanisms of resistance in Gram negative anaerobes. However, other studies [16,18,24,25,31] have reported on the roles of resistance genes and from the various reports it is clear that more needs to be known as to how resistance develops and spread within the anaerobic Gram negative bacilli. Therefore as knowledge increase on the role of resistance genes in mediating antibiotic resistance, detection of their presence can be used in tracking resistance amongst species of *Bacteroides* and *Prevotella*.

5. CONCLUSION

Prescription pattern for the anaerobic component of mixed infections is usually empiric since routine culture and sensitivity test for anaerobes are seldom carried out in many hospital laboratories in Nigeria. Some physician will include metronidazole to the prescription or give broad spectrum beta-lactam antibiotics usually a β-lactam-β lactamase stable antibiotic. The data presented here were from series of research studies from 1992 to 2011 and may serve as a guide in predicting the emerging pattern of antibiotic resistance in these anaerobes in Nigeria. Typical variations in antibiotic resistance patterns likely to occur from one location to another will require that local antibiotic susceptibility pattern be considered from time to time in treatment prescription for infections involving anaerobes. It is envisaged that as knowledge is expanding and with introduction of new diagnostic technologies changes in the susceptibility reported here might have occurred. With these advances, anaerobic studies especially as it involves treatment of anaerobic infection may in the near future depend on direct screening of resistance genes. This will be a rapid diagnostic method that is likely to bypass culture and antibiotic susceptibility testing which usually take between 3-5 days or more before results are available. The wide spread acceptance and incorporation of resistance gene screening into routine diagnostic practices will be dependent on cost.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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