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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

CURRENT TRENDS IN ANTIMICROBIAL RESISTANCE AMONG ANAEROBES: RESULTS FROM U.S. NATIONAL SURVEY

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Since recognition of transferable clindamycin and tetracycline resistance in *Bacteroides* in 1980, we have coordinated a US national survey on the susceptibility *of B. fragilis* to assess trends in resistance and to provide guidance on susceptibility, since these species are not routinely tested in clinical laboratories.

Methods: Agar dilution MICs were determined for 1356 isolates from 2006-2008 for *B. fragilis* and related species from 7 geographically diverse centers in the US. Antibiotics included 4 carbapenems, 2 β-lactam/ β-lactamase inhibitors, 2 quinolones, 1 glycylcycline, clindamycin, linezolid, metronidazole, and chloramphenicol. Isolate identity was confirmed by API 20ATM. 20 isolates with elevated MICs to the carbapenems were analyzed for the presence of cfia resistance genes by PCR using primers described by Kato et al.

Results: Analysis of resistance trends from 2006-2008 for *Bacteroides fragilis* showed an increase in resistance to the carbapenems, with resistance rates rising from 2006 to 2008 from 1.5% to 5.4% for doripenem, 2 to 4.5% for ertapenem, 0.9 to 2.7% for imipenem, and 2 to 5.4% for meropenem. Cefoxitin resistance rose from 5.1% to 9.4%. Clindamycin resistance remained unchanged with rates ~ 29%. Moxifloxacin resistance rose from 29.8% to 34.8% in 2008. Tigecycline resistance was 3% in 2006 compared to 5.3% in 2008. For the β-lactamase inhibitors, piperacillin-tazobactam resistance rose from 1% to 2.7%, while ampicillin-sulbactam remained unchanged (~3.5%).

For non-*fragilis* species such as *B. ovatus, B. thetaiotaomicron,* and *B. vulgatus* moxifloxacin resistance rose from approximately 35% to over 60% in 2008. Clindamycin resistance was over 40%. Chloramphenicol remained 100% active; however, isolates from geographically diverse areas had MIC's to chloramphenicol of 16 μg/ml. No metronidazole resistance was observed in 2008 although 4 isolates had an MIC of 8 μg/ml.

Genetic analysis of β-lactamases showed that 13 of 20 (65%) isolates with high MICs to carbapenems were *cfi* positive. 3 had an IS element. However, the presence of the *cfi* metallo-b-lactamase did not account for all the carbapenem-resistance, since 7 isolates that were *cfi*-negative had carbapenem MICs of 2 – 32 μg/ml.

Conclusion: In 2008 resistance of *B. fragilis* to the carbapenems increased dramatically. However, even with the rise in resistance, the rates are still low and represent only a handful of isolates from geographically diverse areas. The β-lactamase inhibitors also remain active. Clindamycin and moxifloxacin resistance is very high, and these agents cannot be regarded as useful as monotherapy for seriously ill patients with intraabdominal mixed infections. Metronidazole and chloramphenicol resistance, although not detected, shows a few strains with higher MIC's. Whether these trends will continue need to be assessed in future surveillance studies. These results underscore the need for continuous surveillance and the importance of detecting resistance factors to monitor the dissemination of these genetic elements.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

ANTIMICROBIAL RESISTANCE IN *CLOSTRIDIUM DIFFICILE:* THE EUROPEAN PERSPECTIVE

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Clostridium difficile is the leading cause of hospital-acquired diarrhea and the number of outbreaks has risen markedly since 2003. The emergence and spread of resistance in *C. difficile* is complicating treatment and prevention. Most isolates are still susceptible to vancomycin and metronidazole, however transient and heteroresistance to metronidazole have been reported. The prevalence of resistance to other antimicrobial agents such as tetracyclines, lincosamides, quinolones and rifamycins is highly variable in different European countries, ranging from 0% to 100%. Isolates of common polymerase chain reaction ribotypes are more resistant than uncommon ribotypes. Most of the resistance mechanisms that have been identified in *C. difficile* are similar to those in other Gram-positive bacteria, including mutation, selection and acquisition of the genetic information that encodes resistance. Better antimicrobial stewardship and infection control are needed to prevent further spread of resistance in *C. difficile*.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

THE LESSON LEARNED FROM THREE EUROPE-WIDE ANTIBIOTIC RESISTANCE STUDIES ON *BACTEROIDES*

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Members of the *Bacteroides* genus (number of the species changing over the time), are the most widely isolated anaerobic bacteria from different infections and are considered the most resistant group of anaerobic bacteria as well. During the past twenty years three studies were organised in Europe involving 15, 19 and 13 countries, respectively and altogether 3397 clinical isolates, belonging into the *Bacteroides* genus, were evaluated. To follow the development and spread of the resistance among these strains is difficult, as antibiotic susceptibility testing of clinically relevant anaerobes in different routine laboratories in Europe is less and less frequently performed due to budgetary constraints and as clinicians treat many presumed anaerobic infections empirically.

During all three studies clinically relevant non-duplicate isolates were involved with great attention on the correct species determination and the origin of the isolates. The same methodology was used during the past 20 years such as agar dilution determination of the MICs. The tested antibiotics changed with the time. To be able to compare the changes in the resistance actual break-points accepted by CLSI were used. As EUCAST started to define break-points for anaerobic bacteria during the 3rd study EUCAST break-points were also considered where available.

Through out the twenty years the chromosomally mediated beta-lactamase production was the most prevalent among *Bacteroides* strains in Europe. Clindamycin resistance in *Bacteroides* is mediated by a macrolide-lincomycin-streptogramin (MLS) mechanism and its frequency differs in different countries in Europe. Much higher resistance can be observed in southern countries than in northern countries. Resistance to beta-lactam-beta-lactamase inhibitor combinations was studied using amoxicillin-clavulanic acid and/or piperacillin-tazobactam. Increase in resistance was observed to both combinations throughout the years. The same is true for cefoxitine and in the third study several hetero-resistant isolates were found. The occurrence of resistance to imipenem and metronidazole among *Bacteroides* strains merit special clinical importance. The presence of the *cfi*A gene is much more prevalent than the expression of the imipenem resistance; however the spread of the *cfi*A gene among species other than *B. fragilis* is still very rare. The molecular genetic methods looking for the resistance genes among strains with elevated MICs against these antibiotics draw the attention that resistance break-points should be reconsidered. The resistance to moxifloxacin showed great differences in different countries with higher resistance rates in the northern countries than in the southern countries. Very low resistance rate was observed in the third study to tigecyclin.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

MICROBIOLOGICAL APPROACH TO EVALUATION OF ANTIBIOTICS FOR THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* ASSOCIATED DISEASE (CDAD)

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Clostridium difficile was first described in 1935 as part of the intestinal microflora of neonates. While the severe form of the disease caused by the organism (pseudomembranous colitis) was described in 1893 the correlation of the organism with the disease we know today as *Clostridium difficile* associated diarrhea (CDAD) was not made until 1978. Today it is estimated that there are better then 300,000 cases in the United States alone with the rate almost doubling in the years from 1996 to 2008. CDAD is responsible for more deaths than all other intestinal infections combined. *C. difficile* produces two enterotoxins: toxin A and toxin B. Toxin A being responsible for diarrhea and toxin B being responsible for cytopathic changes to enterocytes. Today a new strain of *C. difficile* B1/NAP1/027 is capable of producing 20 times the amount of toxins (TcdA and TcdB) then other strains and produces a binary toxin. A wide variety of treatment and control modalities are used today for CDAD. CDAD treatment generally involves cessation of the inciting antibiotic, *C. difficile*-targeted antibiotic therapy and other supportive measures. Antibiotic therapy generally involves the use of vancomycin or metronidazole with both antibiotics having potent in vitro activity against the vegetative form of *C. difficile*. Because of increasing treatment failure rates with metronidazole vancomycin is more widely used for treatment of CDAD. Both antibiotic treatments have a high incidence of relapse or reinfection with a different stain of *C. difficile*. The need for additional antibiotics for the treatment of CDAD is well recognized. Microbiology considerations in the evaluation of antibiotics for treatment of primary CDAD will be discussed. Topics to be discussed are pharmacokinetics, in vitro microbiology data, surveillance data, animal model data, clinical trial design, clinical trial evaluation, and post marketing studies.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

RESISTANCE OF *CLOSTRIDIUM DIFFICILE* TO CATIONIC ANTIMICROBIAL PEPTIDES

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Clostridium difficile causes a potentially fatal intestinal disease that is increasing in incidence and severity. These infections are often chronic and incredibly difficult to eradicate. Though this organism presents an enormous public health burden, little is understood about how *C. difficile* colonizes the human intestine. In order to persist in the intestinal environment, the bacteria must cope with a continuous onslaught by host defenses. Cationic anti-microbial peptides, or CAMPs, are small positively charged molecules that have microbicidal activities. Humans produce a variety of CAMPs that are concentrated in areas of the body that routinely encounter microorganisms, such as the intestines. These peptides play a critical role in innate host defenses, preventing the growth and spread of both gram-positive and gram-negative bacteria. Naturally, many organisms have evolved mechanisms to circumvent the killing effects of CAMPs. Using model CAMPs, we have established that *C. difficile* is not only sensitive to these compounds, but also responds to low levels of CAMPs by expressing genes that lead to CAMP resistance. By plating the bacterium on medium containing inhibitory concentrations of the CAMP Nisin, we isolated a mutant capable of growth at wild-type rates in 3 times the inhibitory concentration of CAMPs. This mutant also showed markedly increased resistance to the CAMPs Gallidermin and Polymyxin B, demonstrating tolerance to a wide variety of antimicrobial peptides. Using whole-genome resequencing techniques (Illumina), we were able to identify the mutated gene responsible for the high-level resistance phenotype as CD1352. This gene encodes an orphan histidine kinase (putative sensor protein) that lies adjacent to an ABC-transporter operon. Transcriptional analysis of the ABC-transport genes reveals that this operon is up-regulated in the presence of Nisin in wild-type cells, and much more highly expressed in the CD1352 mutant. Additional analysis of this putative CAMP-transport system will provide insight into the mechanisms *C. difficile* uses to subvert the effects of antimicrobial peptides.
Conclusion: These results provide the first evidence of a *C. difficile* gene associated with antimicrobial peptide resistance. Furthermore, investigating mechanisms of antimicrobial peptide resistance presents an additional direction for exploring treatments of *C. difficile* infections.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

BACTEROIDES FRAGILIS RECA PROTEIN OVEREXPRESSION CAUSES RESISTANCE TO METRONIDAZOLE

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Bacteroides fragilis is a human gut commensal and an opportunistic pathogen causing anaerobic abscesses and bacteraemias which are treated with metronidazole, a DNA damaging agent. This study examined the role of the DNA repair protein, RecA, in maintaining endogenous DNA stability and its contribution to resistance to metronidazole and other DNA damaging agents.

RT-PCR of *B. fragilis* genomic DNA showed that the *recA* gene was co-transcribed as an operon together with two upstream genes, putatively involved in repairing oxygen damage. A *B. fragilis recA* mutant was generated using targeted gene inactivation. Fluorescence microscopy using DAPI staining revealed increased numbers of mutant cells with reduced intact double stranded DNA. Alkaline gel electrophoresis of the *recA* mutant DNA showed increased amounts of strand breaks under normal growth conditions, and the *recA* mutant also showed less spontaneous mutagenesis relative to the wildtype strain. The *recA* mutant was sensitive to metronidazole, ultraviolet light and hydrogen peroxide. A *B. fragilis* strain over-expressing the RecA protein exhibited increased resistance to metronidazole compared to the wild type.

This is the first study to show that over-expression of a DNA repair protein in *B. fragilis* increases metronidazole resistance. This represents a novel drug resistance mechanism in this bacterium.

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EVALUATION OF EFFLUX PUMPS ROLE IN CLINDAMYCIN RESISTANCE IN *BACTEROIDES FRAGILIS*

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Clindamycin is one of the first-choice antimicrobial agents for treating anaerobic infections. However, resistance to this drug, especially in strains belonging to *Bacteroides* spp genus, has limited use in empiric therapy. The *erm*F gene, responsible for clindamycin resistance in *Bacteroides fragilis* is rarely detected in resistant strains, and apparently it is not spread in Brazil. Recently, it was identified 16 three-component RND-family efflux pumps systems homologues to *Pseudomonas aeruginosa* MexAB-OprM system in *B. fragilis* (*bme*ABC1-16). It has been suggested that overexpression of these efflux pumps could contribute to clinically relevant antimicrobial resistance. Thus, the aim of this study was to determine correlation between bmeB efflux pumps and resistance in the absence of *erm*F and related genes. Based on this data, PCR was used to detect the *bme*B3 gene encoding the transporter protein of the *bme*ABC3 operon in resistant (MIC range 8 to >256 mg/mL) and sensitive strains (MIC range 0.5 to 4 mg/mL). Thirty-three strains were tested and the gene was found in 88% and 75% of the sensitive and resistant strains respectively. To determine the number of copies of the gene in the strains which tested positive, Southern Blotting was used with a probe designed from the *B. fragilis* ATCC 25285 type strain that carries the *bme*B3 gene. Our results show that there is only one copy of the gene in all strains tested so far. Although most of the studies relate the presence of the *bme*B3 gene with antimicrobial resistance, in our case it was not possible to elucidate its role concerning clindamycin resistance. To measure the expression of efflux pumps genes in the presence and absence of clindamycin, we adapted an RNA extraction protocol for RT-PCR assays. This work may be helpful to explain the *B. fragilis* intrinsic resistance to this antimicrobial class.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *BACTEROIDES* SP. AND *PARABACTEROIDES DISTASONIS* ISOLATED FROM AN INTENSIVE CARE UNIT IN BRAZIL

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Bacteroides fragilis is a minor component gastrointestinal tract and it is the principal anaerobic species associated with infectious processes, such as intra-abdominal and bloodstream infections. Two of the major commensal species found in the gut, *Bacteroides thetaiotaomicron* and *Parabacteroides distasonis*, have been raised as potential pathogens and reservoir for antibiotic resistance genes. Since increasing resistance to various antimicrobial agents is a significant problem in choosing appropriate antimicrobial agents to treat anaerobic infections, the aim of this study was to investigate the susceptibility profiles of the anaerobic bacteria isolated from an intensive care unit (ICU). The tests were carried out with 537 bacterial samples collected from 193 different patients from an ICU of Clementino Fraga Filho University Hospital, in Rio de Janeiro, during a period of one year. One hundred and twenty three strains were isolated from 65 different patients and 26.01% *B. fragilis*, 25.2% *B. thetaiotaomicron*, 20.33% *P. distasonis*, 13.83% *B. vulgatus* and 14.63% other *Bacteroides* sp were recovered. Antimicrobial profiles were obtained by using agar dilution and E-test techniques based on CLSI recommendations. All strains presented susceptibility to imipenem, but 4 strains showed an intermediate profile to amoxicillin/clavulanate (MIC 8 mg/L) and 3 strains presented a decrease susceptibility to metronidazole (MIC 4 mg/L). The overall resistance rates were 49.59% for clindamycin, 75.6% for tetracycline, 21.13% for cefoxitin, 19.51% for cefotaxime, and 26.01% for moxifloxacin. Twenty two (70.96%) *B. thetaiotaomicron* strains showed resistance to at least three different antimicrobials. Conversely, three (9.37%) *B. fragilis* strains presented resistance to three antimicrobials. All strains were also investigated for the presence of resistance genes by using PCR and the following results were found *cep*A (32.52%), *cfi*A (4.87%), *tet*Q (85.36%), *erm*F (12.19%) and *nim* (1.62%). Altogether, our results reflect the importance of surveys of antimicrobial susceptibility profiles and the relevance of detecting major genetic determinants to monitor the dissemination of these genes in Brazil.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

CHARACTERIZATION OF *BACTEROIDES FRAGILIS* PUTATIVE PLASMINOGEN-BINDING PROTEIN BFP60 MUTANTS

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The Gram-negative anaerobic bacterium *B. fragilis* is a member of the commensal flora of the human intestine, but is also frequently found in severe intra-abdominal infections. Recently, a putative plasminogen-binding protein, Bfp60 located in the outer membrane, was identified in *B. fragilis*. This molecule was assayed and showed to recognize and convert Plasminogen (Plg) into Plasmin. Indiscriminate activation plasmin can cause tissue damage and can also transform a nonproteolytic bacterium into a proteolytic one. Thus, the aim of this work was to construct insertion mutants using the clinical strain 638R, to demonstrate that Bfp60 is important to the virulence of the species in *in vitro* and *in vivo* assays. Briefly, oligosaccharide primers were designed to amplify an internal region of the gene *bfp*60 (512 bp) and the *Sph*I/*Pst*I bfp60 fragment of pGEM T easy was cloned into *Sph*I/*Pst*I -digested pFD516 and the new construct mobilized from *Escherichia coli* DH10B in *B. fragilis* strain (BE1) by aerobic triparental filter mating. To construct a BE1 strain that could constitutively express the Bfp60 protein, the DNA containing the entire gene (1692 pb) was used. *Sac*I/*Xba*I *bfp*60 fragment was cloned into *Sac*I/*Xba*I–digested pFD340 and transformed into *E.coli* DH10B cells and the construct mobilized by aerobic triparental filter mating into the 638R strain. All mutants were selected on BHIS agar plates containing 100μg/mL Gentamicin, 10 μg/mL Erythromycin and 50 μg/mL Rifampicin. Mutants were analyzed for Plg adhesion; and conversion of Plg into plasmin. In peritonitis the coagulation and fibrinolytic cascade are up regulated, with the formation of fibrin in the abdominal cavity leading to the intra-abdominal abscess. Thus, mutants for the putative plasminogen-binding protein might help to understand if the Plg recognition and activation can contribute for the abscess formation in *B. fragilis*.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

CHARACTERIZATION OF THE MULTIPLE DRUG RESISTANCE REGULATOR MARR FROM *BACTEROIDES FRAGILIS*

Ferreira, L.Q.;[1] Teixeira, F.L.;[1] Silva D.N.S.;[1] Ferreira, E.O.;[1] Rocha, E.R.;[2] Lobo, L.A.;[1] Domingues, R.M.C.P.*[1]
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The MarR belongs to family of proteins known to bind directly to DNA and regulate the expression of numerous virulence and resistance genes. MarR regulators are widely distributed among bacteria and archea and share a structural similarity but can present divergence at amino acid level. This characteristic may actually contribute to an increased diversity and species-specific regulation with MarR proteins being able to recognize different signaling molecules and DNA targets. MarR regulators homologues have been shown to control the expression of virulence and resistance genes in a number of pathogens. The anaerobic bacteria *Bacteroides fragilis* is a common member of the intestinal microflora and participates in several beneficial processes in the gut. Despite this fact, it is also the anaerobe most commonly isolated from endogenous infections. Several virulence factor have been proposed to explain this ambiguous behavior, among these the most important are the expression of a polysaccharide capsule, an enterotoxin (ETBF), the expression of adhesins, its resistance to antimicrobial drugs and finally its aerotolerance. *B. fragilis* is a highly metabolic versatile bacteria and regulation of these virulence factors is critical in the transition from the commensal to the pathogenic scenario. *B. fragilis* strain YCH46 harbors three copies of homologues of the *MarR* gene, but so far none of these genes could be correlated to a phenotype in *B. fragilis*. In this work we are evaluating the role of the MarR homologues in resistance to antimicrobial drugs. To access the expression of the three homologues, bacteria were grown to exponential phase at 37°C in BHI broth and total RNA was extracted. RT-PCR was used to generate cDNA and PCR was performed with specific primers for each of the *MarR* homologue. All three genes were expressed under this conditions. One of the homologues (GeneID: 3081554) was deleted from the parental strain by gene disruption for phenotypic characterization. Furthermore, the three homologues were cloned into a commercial expression plasmid (pET26b) and successfully expressed and purified from *E. coli.* We believe that a better understanding of the regulation of resistance genes can provide important insight and possibly new strategies for infection control.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

DETECTION OF FUS-1 (OXA-85), A CLASS D BETA-LACTAMASE FROM *FUSOBACTERIUM NUCLEATUM* SUBSP. *POLYMORPHUM* IN NIGERIA

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The isolation of β-lactamase-mediated resistant species of *F. nucleatum* subsp. *polymorphum* from the oral cavity of children or patients with previous antimicrobial therapy has been reported. Analysis has shown that FUS-1 a narrow-spectrum class D β-lactamase hydrolysing benzylpenicillin and oxacillin is found in *F. nucleatum* subs. *polymorphum.* The objective of this study was to identify *F. nucleatum* strains producing a β-lactamase by detecting the FUS-1 (OXA-85) resistance gene by PCR. Twenty eight oral clinical samples were obtained from 22 patients with chronic periodontitis attending at Lagos University Teaching Hospital, Idi-Araba, Nigeria. From 19 (67.9%) of the collected samples, *F. nucleatum* was isolated. Bacterial DNA was obtained from the clinical samples and from the isolates by boiling. Results showed that 3 strains were able to produce a specific amplicon with FUS-1 primer specific for bla_{FUS}-$_1$ gene found in β-lactamase producing *F. nucleatum* subsp. *polymorphum.* The strain-specific primers for *F. nucleatum* subsp. were not able to produce any amplicon with *fusiforme*, *nucleatum* and *vicenttii* respectively. This study shows the presence of class D β-lactamase producing *F. nucleatum* subsp. *polymorphum* in Nigeria.

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ANTIBIOTICS: RESISTANCE & SUSCEPTIBILITY

MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *PREVOTELLA INTERMEDIA* TO METRONIDAZOLE AND AMOXICILLIN IN NIGERIA

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Prevotella species are frequently implicated in periodontal infections and recent reports have linked them with coronary heart disease. Limited literatures on the species and their antimicrobial patterns are available in Nigeria. Similarly, anaerobic cultures or periodic antimicrobial testing on anaerobes are not routine in our respective laboratories. The aim of this study was to identify *P. intermedia* isolates obtained from patients with chronic periodontitis by PCR and determine their susceptibility pattern to metronidazole and amoxicillin. Subgingival samples from 53 patients with chronic periodontitis obtained by sterilized paper point were cultured on Fastidious Anaerobe Agar supplemented with 5% sheep blood and incubated in anaerobiosis. Presumptive analysis was done based on colonial morphology, Gram's reaction biochemical analysis, bile susceptibility, and susceptibility profile to colistin, kanamycin and vancomycin. The isolates were identified by PCR using specific primers to *P. intermedia* and *P. nigrescens.* Their antimicrobial susceptibility to metronidazole and amoxicillin were also determined by E-test. Of the 53 samples cultured, 52 isolates were recovered. Among them, 16 (30.8%) were black pigmented, 19 (36.5%) were brown pigmented, 10 (19.2%) showed brown/black pigmentation, while 7 (13.5%) were none pigmented. By PCR, 5 black pigmented isolates were identified as *P. intermedia* (9.6%) and none was identified as *P. nigrescens*. On antimicrobial susceptibility of the 16 black pigmented isolates to metronidazole, 12 (75%) were susceptible while 4 (25%) were resistant. Comparatively, 13 (81.3%) were sensitive to amoxicillin while (18.8%) were resistant. PCR gave a confirmatory identity of *P. intermedia*. The presence of strains resistant to antibiotics commonly used for empirical therapy in our dental clinics, suggests the need for more study on antimicrobial susceptibility of anaerobes in our Nigerian population.

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LOW PREVALENCE OF MOBILE *ERM* GENES AMONG CLINDAMYCIN-RESISTANT BACTERIA FROM THE *BACTEROIDES FRAGILIS* GROUP ISOLATED IN COSTA RICA

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Costa Rican physicians treat infections by *Bacteroides* sp. with clindamycin despite the fact that resistance to this drug is increasing among anaerobic bacteria from other latitudes.

Bearing in mind that mobile rRNA methylases encoded by *erm* genes can jeopardize the therapeutic effectiveness of clindamycin, we used a PCR-based approach to determine the prevalence of the *ermA*, *ermB*, *ermF*, and *ermG* genes in 37 clindamycin-resistant bacteria from the *Bacteroides fragilis* group and biparental filter matings to assess whether their resistance traits were susceptible of lateral transfer via conjugation. We also investigated the prevalence of *tetQ* and the susceptibility of the strains to tetracycline because *erm* genes are commonly linked to *tetQ* in conjugative transposons.

Minimum inhibitory concentrations of clindamycin ranged from 8 ug ml^{-1} to 64 ug ml^{-1} with a MIC_{50} of 16 ug ml^{-1}. In contrast to our working hypothesis, only 6 strains had *erm* genes (16%): 1 had the *ermA* gene, 2 had the *ermG* gene, and 3 the *ermF* gene. Half the erm^{+} isolates transferred their resistance to clindamycin laterally. Resistance to tetracycline was common (n=96%) and a large number of isolates had the *tetQ* gene (n=90%).

Our results reveal that mobile *erm* genes are scarce in the collection analyzed.

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EFFECT OF FLUOROQUINOLONE RESISTANCE SELECTION ON PRODUCTION OF BACTERIOCIN IN A STRAIN OF *CLOSTRIDIUM PERFRINGENS*

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To evaluate the effect of fluoroquinolone resistance selection on the production of bacteriocins in *Clostridium perfringens,* bacteriocin production in the gatifloxacin-resistant *C. perfringens* mutant NCTR-10G, which has higher alpha and theta toxin production than the wild-type strain NCTR, was compared with that of the parent strain. Fluoroquinolone-resistant mutant and wild-type strains were grown under anaerobic conditions. Filter-sterilized culture supernatants, used to assay for bacteriocin production, were spotted on lawns of the bacteriocin indicator strain *C. perfringens* 13124 and 15 chicken isolates of *C. perfringens* on blood agar plates and were incubated anaerobically at 37°C for 24 hours. The appearance of a zone of inhibition in the bacterial lawn at the site of inoculation with the sterile supernatant indicated the presence of a bacteriocin to which this isolate was sensitive. The supernatant from *C. perfringens* NCTR inhibited the growth of *C. perfringens* strain 13124 and 13 out of the 15 chicken isolates. The supernatant from the gatifloxacin-resistant strain NCTR-10G did not inhibit *C. perfringens* strain 13124, but the bacteriocin activities of the norfloxacin and ciprofloxacin-resistant strains NCTR-50N and NCTR-50C were the same as those of the wild type. Primers designed from published sequences of *C. perfringens* strain SM101 and other bacteriocin producers did not amplify the bacteriocin gene from strain NCTR**,** nor did the primers generated from the putative positive regulator (UviA) of the bacteriocin gene of *C. perfringens* SM101. This indicates that the sequences of this bacteriocin and its regulator are different from those of known *C. perfringens* bacteriocins. Low-stringency Southern blot hybridization, using an amplified bacteriocin gene fragment from the strain SM101 bacteriocin as a probe, resulted in low-level hybridization of this fragment to the plasmid isolated from *C. perfringens* NCTR and confirmed variation of this bacteriocin from that of strain SM101. The bacteriocin from *C. perfringens* strain NCTR appears to be different from known *C. perfringens* bacteriocins, and gatifloxacin resistance selection in this strain was associated with decreased production of the bacteriocin.

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INVESTIGATION OF THE PREVALENCE OF THE MAJOR ANTIBIOTIC RESISTANCE AND FRAGILYSIN GENES OF *BACTEROIDES* STRAINS

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We set out to examine the prevalence of the known common antibiotic resistance genes of the *Bacteroides* on a large collection of strains (n=693), and to gain information on the possible existance of combinations of genes and their carrying genetic elements with regard to taxonomic units, e.g. *B. fragilis* Divisions I and II, and non-fragilis *Bacteroides* (NFB).

Antibiotic susceptibility measurements were previously carried out by agar dilution; species identification was performed by MALDI-TOF mass spectrometry. Genotypes for the *cepA*, *cfxA*, *cfiA*, *ermF*, *nim*, *tetQ* and *bft* genes were examined by RealTime PCR, using 96-well plate platform and fluorescent dye (SybrGreen) detection.

The most common gene was the tetracycline resistance, *tetQ* gene, with prevalences of 78.5% and 85% among the *B. fragilis* and NFB strains, respectively. High frequencies were also found for the *cepA* gene: 74.3% among the *B. fragilis* and 57.5% among the NFB strains. The incidence of *cepA* and *ermF* genes was in the same range as the phenotypic resistance for cefoxitin and clindamycin, respectively, but they were differentially distributed between the *B. fragilis* and the NFB strains. The differences in prevalence of these genes between the two taxa in question were statistically significant. The frequencies of the *cfiA* and *bft* genes among the *B. fragilis* strains were 8.7% and 14.2%, respectively. A somewhat unexpected phenomenon, coincident occurrence of the *cfiA* and *bft* genes, was found in 4 of the *B. fragilis* strains. As regards the four other genes examined, a similar analysis demonstrated that the occurrence of the *cepA* and *cfiA* genes correlated negatively with a high level of significance among the *B. fragilis* strains. For *cfiA* vs. *cfxA* and *cfiA* vs. *ermF*, a clear conclusion could not be drawn because of the low number of strains involved, and for *tetQ* the test was not significant. Of the 693 *Bacteroides* strains examined, only 2 harbored *nim* genes.

The analysis of a high number of *Bacteroides* strains for common antibiotic and virulence genes revealed an unexpected rate of interactions between these genes and between the genes and the harboring taxa. The alarming co-occurrence of *bft* and *cfiA* genes, may-be explained by the independent occurrence, which might arise from the *bft* genes harbored on mobile conjugative transposons.