Effect of metronidazole and amoxicillin on *Bacteroides* species isolated from surgical wounds of patients attending Christiana specialist hospital Owerri and Braithwaite memorial specialist hospital Port Harcourt, Nigeria

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Abstract:
This study was carried out to determine the effect of metronidazole and amoxicillin antibiotics on *Bacteroides* species. One hundred surgical wound swabs were collected from patients attending Christiana Specialist Hospital Owerri (CSHO) and Braithwaite Memorial Specialist Hospital Port Harcourt (BMSHP). The specimens were studied by standard microbiological analysis. Antibiotic susceptibility test was carried out by disk diffusion method. The data obtained were subjected to statistical analysis of variance (ANOVA). The result showed 32.4% of *Bacteroides fragilis* and 25.4% of *Bacteroides ureolyticus* were isolated from patients attending BMSHP while 25.4% of *B. fragilis* and 16.9% of *B. ureolyticus* from CSHO. Both *B. fragilis* and *B. ureolyticus* had the highest percentage occurrence of (32.4%) and (25.4%) respectively for BMSHP and lowest percentage of (25.4%) and (16.9%) respectively for CSHO. However, there is a significant difference ($p<0.05$) in frequency occurrence of *Bacteroides* species isolated from both locations. Both metronidazole and amoxicillin were effective against *B. fragilis* with average percentage effectiveness of 68.2% and 56.6% respectively for the two locations while metronidazole with (66.7%) was only effective against *B. ureolyticus* for both locations. The minimum inhibitory concentration showed the efficacy of the two antibiotics in the treatment of *Bacteroides* infection; however, metronidazole proved more potent. The similarities between the susceptibility patterns of the antibiotics observed emphasize the need to continue monitoring the emergence of resistance of most frequently used antibiotics against anaerobes.

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Introduction

Bacteroides is a genus of gram-negative, bacillus bacteria. They are rod shaped, non-endospre forming anaerobes, and may be either motile or non-motile, depending on the species. They are bile resistant. They have an outer membrane, a pepitidoglycan layer and a cytoplasmic membrane (Hannah and Wexler, 2005). They are normally mutualistic, making up the most substantial portion of the mammalian gastrointestinal flora, where they play a fundamental role in processing of complex molecules to simpler ones in the host intestine. They maintain a complex and generally beneficial relationship with the host when retained in the gut, but when they escape this environment, they can cause significant pathology including bacteremia and abscess formation in multiple body sites including the CNS, the head, the neck, the chest, the abdomen, the pelvis, the skin, and the soft tissues (Armour *et al*., 2007). The species include *Bacteroides caccae, Bacteroides coagulans, Bacteroides coprocola, Bacteroides eggerthii, Bacteroides fragilis, Bacteroides massiliensis, Bacteroides nordii, Bacteroides ovatus, Bacteroides plebeius, Bacteroides pyogenes, Bacteroides salyersiae, Bacteroides stercoris, Bacteroides tectus, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgates, Bacteroides ureolyticus* etc (Hannah and Wexler, 2005).

Wound infections are recognized as potentially serious complications in patients subjected to surgical operations. Outbreaks of postoperative sepsis are common and such infections significantly lead to severity of patients' illness. The development of postoperative wound infections depends on complex interplay of many factors. When there is a decrease in the integrity and protective function of the skin, large number of different pathogens will enter into the wound and initiate an inflammatory response characterized by the classic signs of redness, pains, swelling, raised temperature and fever. This process aims to restore homeostasis. Bacterial infection in surgical wound and burn patients that often lead to sepsis are common and difficult to control in hospitals (Armour *et al*., 2007).

Hospital acquired infections remain a cause of morbidity, extended hospital stay and death of patients (Holizheimer *et al*., 1990). The surgical wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin. *Bacteroides* present a huge problem as a source of infection during gastrointestinal surgeries, thus proper measures and efficient surgical draining must take place in other to lower the risk of infection (Shittu *et al*., 2004).

The recent advances in medicines such as the advent of elaborate surgery, intensive care, use of board spectrum antibiotics and immunosuppressive drugs, the availability of invasive procedures or instrumentations and the increase in the number of immune compromised patients – oncology patients on cytotoxic therapy, patients with organ transplant and patients with HIV/AIDS (Human Immune Virus/Acquired Immune Deficiency Syndrome) and a direct consequence of this is that there is a rise in patients with impaired immune defences thereby leading to an increase in nosocominal infections especially by Gram negative organisms. Such organisms may be found in the patients' own flora, damp environmental sites or hospital equipments (Olayinka *et al*., 2004; Alvarez-Lerma *et al*., 2006; De Bus *et al*., 2014).They exhibit natural resistance to many antibiotics and antiseptics in which they survive for long periods and may even multiply in the presence of minimal nutrients and have ability to colonize traumatized skin (Armour *et al*., 2007). The knowledge of the causative agents of wound infections has proved to be helpful in the selection of empiric antimicrobial therapy and on infection control measures in the hospital (Shittu *et al*., 2004). Although *Bacteroides* are important, their negative effect outweighs their importance.

Most surgical wounds are contaminated by both pathogens and body commensals (Anguzu and Olila, 2007), but the development of infection in the site greatly depends on the complex interplay of many factors which include virulence of the infecting organism, (Bowler *et al*., 2001). Patient risk factors such as diabetes, cigarette smoking, obesity and coincident remote site infections or colonization (Reichman and Greenberg, 2009) and operation-related risk factors including prolonged hospitalization before surgery, operating environment, duration of the operation, tissue trauma, poor haemostasis, and presence of foreign material in the wound-this greatly increases the risk of serious infection despite a relatively small number of bacteria (Prakash, 2010). In effect, location of found, exposure of patients and hospital hygiene to a large extent contribute to wound infections.

Nosocomial infection is a great threat to surgical wound infection and management moreso, since most microbes involved are resistant to conventional antibiotics in surgical wound management. As the resistant level to the use of most antibiotics is increasing it becomes a big threat to the recovery of patients with surgical wound infection. Therefore, the study was done to determine the effect of metronidazole and amoxicillin on isolated *Bacteroides* species from surgical wounds.

Materials and Methods

Collection of Specimen

A total of 100 clinical specimens (wound swabs) were collected into transport media (pre-reduced thioglycollate broth) from both male and female patients attending Christiana Specialist Hospital Owerri and Braithwaite Memorial Specialist Hospital Port Harcourt. This study was carried out within the period of May to August 2016.

Isolation and Identification

Specimens were streaked on freshly prepared fastidious anaerobe agar LAB M supplemented with 5% defibrinated blood and incubated at 37^0C for 7 days to allow for pigmentation to in an anaerobic jar containing 90% N_2 and 10% CO_2 generated by sachets of gas generating kit (oxoid). The growth on the plate was examined macroscopically, Gram stained and subculture onto fastidious anaerobic agar (Lab M). Biochemical tests were performed using API 20A (bio Mérieux SA, Mercy-l'Etoile, France) according to the manufacturer's instructions. The identified isolates were stored in 10% skimmed milk at -80^0C.

Bile Salt Sensitivity Test

Disks of 6 mm in diameter were punched off from Whatman's No 1 filter paper. All disks were collected into a Petri dish and sterilized in hot air oven at 160^oC for 1 hour. The bile salt was prepared by dissolving the bile salt; the oxgall-water suspension was placed in a 56 °C incubator for 20 minutes. The bile salt solution was then autoclaved at 121 °C for 15 minutes (Leslie *et al*., 1983). The disks were then impregnated into bile salt solution and then sterilize at $60^{o}C$ until it was properly dried (Talaro and Talaro, 2002; Selvamohan and Sandhya, 2012; Obi, 2014).The test organisms were inoculated on the surface of fastidious anaerobe agar; aseptically with a sterile forceps, the impregnated discs was properly placed on the surface of each agar plate and incubated at $37^{o}C$for 24hours. After 24hours incubation, the plates were examined and the zones of inhibition were recorded as sensitive or resistant.

Antibiotic Susceptibility Testing

Preparation of 0.5 Macfarland turbidity standard; It was prepared by preparing a 1% v/v solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99ml of water, it was mixed well. 1% v/v solution of barium chloride was prepared by dissolving 0.5g of dehydrate barium chloride in 50ml of distilled water. 0.6ml of the barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed.

Antibiotics: The potency powder of the following antibiotics will be used for the minimum inhibitory concentration (MIC), metronidazole, and amoxicillin. The agar dilution procedure recommended by Clinical Laboratory Standard Institute will be used (CLSI, 2015). Double dilution of each antibiotic agent (ranging from 0.5-8µg/ml) was incorporated into the sterile molten fastidious anaerobic agar supplemented with hemin. The agar plates were inoculated and incubated anaerobically for 2days at $37^{0}C$. Control plates without metronidazole, and amoxicillin was inoculated and incubated in the same way. The MICs was recorded as the lowest concentration of the antibiotic in the medium that inhibited bacterial growth, gave a faint haze of growth or with no more than one discrete bacterial colony. The MICs was interpreted as resistant or sensitive by applying the breakpoints that were proposed by CLSI (CLSI, 2015).

Statistical Analysis

The results obtained from this study were subjected to Analysis of variance (ANOVA) to determine the significance at 95% interval.

Results

A total of seventy-one (71) isolates were obtained from the one hundred (100) wound specimens collected from both male and female patients attending Christiana Specialist Hospital Owerri and Braithwaite Memorial Specialist Hospital Port Harcourt. Bacterial species isolated were *Bacteroides fragilis* (57.8%) and *Bacteroides ureolyticus* (42.3%). Table 1 showed the frequency occurrence of *Bacteroides* species isolated from surgical wounds at different locations. *B. fragilis* had the highest occurrence with 23(32.4%) while the least *B. ureolyticus* with 18(25.4%) from Braithwaite Memorial Specialist Hospital Port Harcourt. Also, *B. fragilis* with 18(25.4%) was the highest while the least *B. ureolyticus* with 12(16.9%) from Christiana Specialists Hospital Owerri. The percentage of *B. fragilis* observed was higher than *B. ureolyticus* in both locations; ($p<0.05$) showing there is a significant difference in the frequency occurrence of *Bacteroides* species obtained from both locations.

Table 1: Frequency occurrence of *Bacteroides* species isolated from surgical wounds at different locations

	Bacteroides fragilis			*Bacteroides ureolyticus*		
Location	Male	Female	Total	Male	Female	Total
BMSHP	10(14.1%)	13(18.3%)	23(32.4%)	8(11.3%)	10(14.1%)	18(25.4%)
CSHO	6(8.5%)	12(16.9%)	18(25.4%)	4(5.6%)	8(11.3%)	12(16.9%)

Key: BMSHP – Braithwaite Memorial Specialist Hospital Port Harcourt, CSHO – Christiana Specialists Hospital Owerri

Table 2 shows the susceptibility pattern of *Bacteroides fragilis* from different locations. *Bacteroides fragilis* with 16(69.6%) was sensitive to metronidazole and 13(56.5%) to amoxicillin obtained from Braithwaite Memorial Specialist Hospital Port Harcourt. For Christiana Specialists Hospital Owerri; 12(66.7%) of *Bacteroides fragilis* was sensitive to metronidazole and 10(56.6%) to amoxicillin. Results obtained from both locations shows similarity in the susceptibility pattern based on the number of isolates susceptible to the anibitoics; ($p<0.05$) showing there is a significant difference in the susceptibility pattern of *Bacteroides fragilis* from the different locations.

Table 2: Susceptibility pattern of *Bacteroides fragilis* from different locations

	BMSHP		CSHO	
Antibiotics	Sensitive	Resistance	Sensitive	Resistance
Metronidazole	16(69.6%)	7(30.4%)	12(66.7%)	6(33.3%)
Amoxicillin	13(56.5%)	10(43.5%)	10(56.6%)	8(44.4%)

Table 3 showed the susceptibility pattern of *Bacteroides ureolyticus* from different locations. *Bacteroides ureolyticus* with 12(66.7%) was sensitive to metronidazole and 9(50%) to amoxicillin obtained from Braithwaite Memorial Specialist Hospital Port Harcourt. Also from Christiana Specialists Hospital Owerri; *Bacteroides ureolyticus* 8(66.7%) was sensitive to metronidazole and 5(41.7%) to amoxicillin from Christiana Specialists Hospital Owerri. This shows that there is a similarity in the susceptibility pattern from both locations based on the number of isolates susceptible to both antibiotics ($p<0.05$) indicating there is a significant difference in the susceptibility pattern of *Bacteroides ureolyticus* from both locations.

Table 3: Susceptibility pattern of *Bacteroides ureolyticus* from different locations

	BMSHP		CSHO	
Antibiotics	Sensitive	Resistance	Sensitive	Resistance
Metronidazole	12(66.7%)	6(33.3%)	8(66.7%)	4(33.3%)
Amoxicillin	9(50%)	9(50%)	5(41.7%)	7(58.3%)

Table 4 shows the minimum inhibitory concentration of the antibiotics on the *Bacteroides* species. metronidazole was shown to be more effective on the isolates than amoxicillin using the Minimum inhibitory concentration obtained.

Table 4: Minimum inhibitory concentration of antibiotics on isolates from patients attending BMSHP and CSHO

	Amoxicillin		Metronidazole	
Isolates	Range tested (ug/ml) Breaking point (ug/ml)	MIC obtained	Range tested (ug/ml) Breaking point (ug/ml)	MIC obtained
1	≤ 0.5-8	0.25	≤ 0.5-8	2
2	≤ 0.5-8	1	≤ 0.5-8	0.5
3	≤ 0.5-8	0.5	≤ 0.5-8	0.5
4	≤ 0.5-8	0.25	≤ 0.5-8	2
5	≤ 0.5-8	0.5	≤ 0.5-8	0.25
6	≤ 0.5-8	0.25	≤ 0.5-8	0.125
7	≤ 0.5-8	0.125	≤ 0.5-8	0.5
8	≤ 0.5-8	0.25	≤ 0.5-8	1
9	≤ 0.5-8	0.5	≤ 0.5-8	0.25
10	≤ 0.5-8	0.5	≤ 0.5-8	0.5

Discussion

Bacteroides is the most frequently isolated pathogen from clinical specimens, including blood. Several studies have documented an alarming gradual increase of resistance rates of *Bacteroides*, *Prevotella* and *Porphyromonas* species worldwide. Antimicrobial agents active against 99% of clinical isolates of *Bacteroides* are metronidazole, chloramphenicol, amoxicillin, tetracyclines and carbapenems (Mathew and Elias, 2000).

This study investigated the susceptibility patterns of metronidazole and amoxicillin on *Bacteroides* species isolated from two different locations. The species identified were *Bacteroides fragilis* and *Bacteroides ureolyticus*. *B. fragilis* was the highest occurring species as this agrees with work done by Lassmann *et al*., 2007 which emphasized that *B. fragilis* is the commonest anaerobe, accounting for 45% to 65% of clinically important bacteremia, both nosocomial and community acquired infections.

Metronidazole and Amoxicillin used in this study showed effectiveness on the *B. fragilis* and *B. ureolyticus*. Both were susceptible to the metronidazole. This is in agreement with the work done by Sjolin *et al*., 2003 where cefotaxime and metronidazole was used to treat *Bacteroides* isolated from brain abscess. Also, studies done to investigate and compare the susceptibility profiles of pre-operative and post-operative intestinal *B. fragilis* and *B. ureolyticus* against antimicrobials to evaluate their resistant development pattern due to prophylactic antibiotic administration, showed that both *Bacteroides* species isolated from all specimens before and after the surgery were uniformly susceptible to amoxicillin-clavulanate, metronidazole, and chloramphenicol. Although, metronidazole, imipenem, and amoxicillin-clavulanate seem highly effective against both *Bacteroides* species (Toprak *et al*., 2005).

It has been seriously demonstrated that antimicrobial agent are capable of different microbial groups although few studies hard controversial results. A synergistic in vitro effect between gentamicin and penicillin, clindamycin, rifampin, nalidixic acid, ticarcillin, erythromycin and spectinomycin showed synergy with metronidazole against less than 50% of *B. fragilis* strains (Brook *et al*., 2010). The increase of the resistance rates of *Bacteroides* and order anaerobic bacteria to antimicrobials noted by several investigators during the last few years will inevitably lead to a more intensive investigation of the possible role of combinations of different classes of antibiotics against the *B. fragilis* group (Ralph and Amatnieks, 2008).

Conclusion

Metronidazole and amoxicillin still showed effectiveness in the treatment of some of the *Bacteroides* species identified. The similarities in susceptibility pattern observed from these two locations emphasized the need to continue monitoring the emergence of resistance against the most frequently used anti anaerobic drugs. The rate of susceptibility and resistance may vary among members of the *Bacteroides* group; however, there is need for further studies on comparison between other antibiotics other than the one used in this research to demonstrate the susceptibility of *Bacteroides* strains from different specimen.

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