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MULTIDRUG RESISTANT ACINETOBACTER INFECTION AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A NIGERIAN TERTIARY HOSPITAL ICU.

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Abstract

Background: *Acinetobacter baumannii*, a non-glucose fermenting Gram negative bacillus, has emerged in the last three decades as a major etiological agent of hospital-associated infections giving rise to significant morbidity and mortality particularly in immunocompromised patients. Multidrug resistant *A. baumannii* (MDR-AB) is fast becoming a global threat, having developed resistance to major classes of antibiotics and carbapenem-resistant isolates have increasingly been reported worldwide as a cause of nosocomial outbreaks. Despite intensive efforts, nosocomial acquisition of MDR-AB is still a problem due to the organism's great ability to colonize human and environmental reservoirs.
Objectives: This study was aimed to determine the prevalence of (MDR) AB and their antibiotic susceptibility pattern.
Methodology: A total of 400 specimens which include tracheal aspirates, catheter specimens of urine, wound biopsies and blood culture collected from 100 patients admitted at the Intensive Care Unit of our hospital over a period of nine months were processed following standard microbiologic procedure.
Results: A total of 155 non-lactose fermenters were isolated out of which 14 (9.0%) were Acinetobacter spp. Eleven (79.0%) out of the 14 Acinetobacter spp were *A. Baumanii*, while 2 (14.0%) were *A.lwoffi* and 1(7.0%) *A.calcoaceticus.* All the isolates were resistant to Amoxicillin-clavulanate, Ceftriaxone, Ciprofloxacin, Ofloxacin, gentamicin and Ampicillin-sulbactam; while susceptibility to Meropenem, Amikacin and Levofloxacin were 64.3%, 50.0% and 35.7% respectively.
Conclusion: The high rate of antibiotic resistance shown by Acintobacter isolates in this study demonstrates the need for antibiotic stewardship protocols to be set up in health facilities to prevent outbreaks of multi-resistant bacterial infections.

Key words: Acinotebacter infection, Multidrug resistant, Intensive care unit.

Introduction

Acinetobacter baumannii, a non-glucose fermenting Gram negative bacillus, has emerged in the last three decades as a major etiological agent of hospital-associated infections giving rise to significant morbidity and mortality particularly in immunocompromised patients. Multidrug resistant *A. baumannii* has become a global threat to the seriously infected patients who critically rely on antibiotic therapy. It has developed resistance to major classes of antibiotics and carbapenem-resistant isolates have increasingly been reported worldwide as a cause of nosocomial outbreaks (Bergogne-Bérézin, 2001; Prashanth and Badrinath, 2006).

Therapeutic options have become very limited raising infection control concern worldwide. Meanwhile, the ability of these bacteria to develop resistance rapidly has raised the suggestion that unless newer therapeutic options are developed we may be closer to the end of the antibiotic era with *A. baumannii* compared to methicillin-resistant *Staphylococcus aureus* (Giamarellou *et al*, 2008). The capacity of *Acinetobacter* species for extensive antimicrobial resistance may be due in part to the organism's relatively impermeable outer membrane and its environmental exposure to a large reservoir of resistance genes (Bonomo and Szabo, 2006; Maragakis and Perl, 2008). Although the definitions of multidrug-resistant Acinetobacter species vary, referring to a wide array of genotypes and phenotypes, the two most common definitions of multidrug resistance are carbapenem resistance or resistance to □3 classes of antimicrobials (Falagas et al, 2006; Maragakis and Perl, 2008).
Intensive care units (ICUs) of hospitals harbour critically ill patients who are extremely vulnerable to infections. These units, and their patients, provide a niche for the spread of opportunistic microorganisms that are generally harmless to healthy individuals but are often highly resistant to commonly used antibiotics. Infections by such organisms are difficult to treat and can lead to an increase in morbidity and mortality. Furthermore, their eradication from the hospital environment can require targeted measures, such as the isolation of patients and temporary closure or even reconstruction of wards. The presence of these organisms, therefore, poses both a medical and an organizational burden to health-care facilities (Dijkshoorn et al, 2007).

Acinetobacter baumannii (AB) has emerged as an important pathogen, especially in intensive care units (ICUs).The increasing development of multiple antimicrobial resistances in this pathogen has severely restricted the therapeutic options available for infected patients, and increased the length of stay in ICUs and mortality. Available data regarding therapeutic options for multidrug-resistant *Acinetobacter* infection are from in vitro, animal, and observational studies as there are no well-designed clinical trials to compare treatment regimens (Maragakis and Perl, 2008).

Carbapenems remain the treatment of choice if isolates retain susceptibility to this antimicrobial class (Maragakis and Perl, 2008). Unfortunately, carbapenem-resistant *Acinetobacter* isolates are increasingly reported worldwide. Sulbactam, a β lactamase inhibitor, has been used to successfully treat 14 patients with multi-drug Acinetobacter ventilator associated pneumonia (Wood et al, 2002); while tigecycline, a relatively new glycylcycline agent has been reported to have antimicrobial activity against multi-drug resistant Acinetobacter species (Pachon-Ibanez et al, 2004; Seifert et al 2006). Other therapeutic options include aminoglycoside agents like tobramycin and amikacin if susuceptibility is retained. These agents are usually used in conjunction with another active antimicrobial agent (Maragakis and Perl, 2008).
Despite intensive infection control efforts, nosocomial acquisition of multi-drug resistant (MDR) AB is still a problem due to the great ability of AB to disseminate from and colonize human and environmental reservoirs (Jung et al, 2010).

The aim of this study was to determine the prevalence of multidrug resistant Acinetobacter and their antibiotic susceptibility pattern.

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Methodology

This cross sectional study was carried out in University College Hospital, a tertiary hospital located in southwest region of Nigeria. The hospital has 850 beds capacity, with a 12 bedded ICU that take care of a monthly turnover of 25 patients. The study population consists of 100 patients who were admitted and have spent at least 48 hours in the Intensive Care within a period of nine months (January to September, 2011). This population comprises of various categories of patients requiring critical care, including post-surgical patients, majority of who were on ventilators and had a prior history antibiotic use. Ethical approval was obtained from the institution's ethical committee.

Verbal and/or written informed consents were sought and obtained from caregivers of subjects; thereafter relevant medical history, socio-demographic data and other information obtained from the caregivers and case files were entered into a semi-structured close-ended questionnaire.

Laboratory Methods

The tests were done in the Bacteriology laboratory of the institution. Tracheal aspirate, blood, catheter urine and wound biopsy (in patients with wounds) were collected from all recruited patients for microscopy, culture and sensitivity. Blood specimens were collected aseptically into Bactec blood culture bottles after cleaning proposed venepuncture sites with 70% alcohol, then povidone iodine and finally 70% alcohol to remove the iodine at the end of venepuncture. Five milliliters of blood was collected from each patient, injected into the bottle and transported to the microbiology laboratory for incubation in the Bactec blood culture system (B.D). Gram stain and subcultures using MacConkey and blood agar plates were done for culture bottles were growths were indicated.

Other specimens were inoculated on MacConkey agar and blood agar and incubated at 35-37^{o}C for 18-24hrs. *Acinetobacter* species grew on MacConkey agar appearing as a non lactose fermenter. All Gram-negative coccobacilli isolates were tested for catalase and motility. All catalase positive, non-motile Gram negative coccobacilli were subjected to an oxidase test. All oxidase negative organisms were inoculated into peptone broth for about 30mins. Subsequently 1ml of the broth was inoculated into the various cups of Microbact Identification kit and incubated for 18-24hrs. After the stated period, Gram negative coccobacilli were identified as *Acinetobacter* spp based on the reactions on the identification panel which was read with the help of the identification software that accompanies the kit.

Antibiotic Susceptibility Testing

This was done using the disc diffusion method (Modified Kirby-Bauer test). The inoculum was prepared from a suspension of the organism made by picking 2 or 3 colonies of the organism and making an emulsion of it in peptone water. This suspension was then compared against a turbidity standard (0.5 McFarland standard). Using a sterile swab stick, Mueller-Hinton agar plates were inoculated with the broth cultures after about 3mins. Antibiotic impregnated discs (ceftriaxone 30ug, Ampicillin-sulbactam 5ug, amoxicillin-clavulanate 10ug, amikacin 5ug, meropenem 10ug, ciprofloxacin 5ug, Ofloxacin 5ug, gentamicin 10ug, levofloxacin 5ug) were placed on the surface of the Agar and incubated at 35-37^{0}C for 24hrs. The diameter of the zones of inhibition was measured with a calibrated meter rule and interpreted with standard interpretative CLSI charts. *Escherichia coli* ATCC *25922* was used as control.

Results

A total of 100 patients were recruited into the study, comprising of 52 (52.0%) males and 48 (48.0%) females, giving a male to female ratio of 1.1: 1. Majority of the patients studied (82.0%) were admitted into the ICU through the accident and emergency (A/E) unit while the remaining 18.0% were from the different wards. The ages of the patients range from 2 - 95 years, with the mean age of 38.2 ± 12.4 years. Majority of the patients (40%) were in the 31-40year age group while the 10-20 year age group constituted the least age group (4%), as shown in Table 1.

Out of the 100 patients studied 14 were found to be having Acinetobacter infection, giving an prevalence rate of 14.0%. These 14 isolates comprises of 9% of all the isolated organisms. Six (11.5%) out of 52 male patients and 8 (16.7%) out of 48 female patients studied were infected, but this female preponderance observed was not statistically significant ($p > 0.05$) (Table 2). Age group >60 years had the highest rate of infection (40.0%), followed by 51-60 years age bracket. Those ≤20 years recorded no acinetobacter infection (Table 3). Majority of the isolates (86.0%) were from tracheal aspirates, while 7.0% each were from urine and blood culture (Table 4). Eleven (79.0%) of the Acinotobacter species were *Acinetobacter baumannii,* 2(14.0%) *Acinetobacter lwoffi* and 1(7.0%) *Acinetobacter calcoacetius*(Table 4). Out of the 82 patients admitted through the accident and emergency unit 11 (13.4%) had acinetobacter infection while 3 (16.7%) out of 18 admitted through the wards were susceptible (Table 5).

All the isolates were resistant to Amoxicillin-clavulanate, Ceftriaxone, Ciprofloxacin, Ofloxacin, gentamicin and Ampicillin-sulbactam. Nine (64.3%) of the isolates were susceptible to Meropenem while five (35.7%) were resistant. Seven (50%) of the isolates were susceptible to amikacin and the other 50% were resistant. Five (35.7%) of the isolates were susceptible to levofloxacin while nine (64.3%) of the isolates were resistant to the antibiotic Figure1.

Discussion

A cross-sectional study was carried out to determine the prevalence of acinetobacter in the ICU of the University College Hospital Ibadan. In this study, prevalence rate of acinetobacter was found to be 14.0%. This figure is higher than 9.0% reported in France and 4.6% reported in Lagos, Nigeria. Also acinetobacter consisted of 9% of all isolates in the study, this finding is low compared to 14.5% obtained by Kessaris *et al.*(2006) and 13.9% by Lul *et al*. (2009) but higher than 8.4% reported by Oberoi *et al.*(2009) and 3% reported by Iregbu *et al*.(2002). The observed differences may have to do with variation in the study design and methodology. For example, the study in Lagos by Iregbu *et al.* (2002) was a hospital wide project as compared to this study which was limited to the ICU.

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Multidrug resistant *Acinetobacter* isolates as found in this study have been reported to be associated with almost all types of nosocomial infections like urinary tract infections, respiratory tract infections and septicemia (Patwardhan et al, 2008). In recent years, *A. baumannii* has become an important pathogen especially in intensive care units. Persistence of endemic *A. baumannii* isolates in ICU seems to be related to their ability for long-term survival on inanimate surfaces in patient's immediate environment and their widespread resistance to the major antimicrobial agents (Oberoi et al, 2009).

Table 1: Age, Sex and Source of Admission Distribution of Patients (n=100)

Variable	Number	Percent
Age group(yrs)		
≤10	13	13.0
11-20	4	4.0
21-30	13	13.0
31-40	30	30.0
41-50	20	20.0
51-60	10	10.0
>60	10	10.0
Sex		
Male	52	52.0
Female	48	48.0
Source of Admission		
A/E	82	82.0
Wards	18	18.0

A/E: Accident and Emergency.

Table 2: Distribution of Acinetobacter Infection by Gender

Sex	Number Studied	Number with Infection	Percentage with Infection
Male	52	6	11.5
Female	48	8	16.7
Total	100	14	14.0

Table 3: Distribution of Acinetobacter Infection by Age group

Age group	Number Studied	Number with infection	Percentage with infection
≤10	13	-	-
11-20	4	-	-
21-30	13	1	7.7
31-40	30	4	13.3
41-50	20	2	10.0
51-60	10	3	30.0
>60	10	4	40.0
Total	100	14	14.0

Table 4: Source and Species of Isolates Distribution (n=14)

Source of Isolate	Number of Isolate	Percent
Tracheal Aspirate	12	86.0
Urine	1	7.0
Blood	1	7.0
Wound Biopsy	-	-
Species of Isolate		
Acinetobacter baumannii,	11	79.0
Acinetobacter lwoffi	2	14.0
Acinetobacter calcoacetius.	1	7.0

Table 5: Distribution of Acinetobacter Infection by Source of Admission

Source of Admission	Number Studied	Number with Infection	Percentage with infection
A/E	82	11	13.4
Wards	18	3	16.7
Total	100	14	14.0

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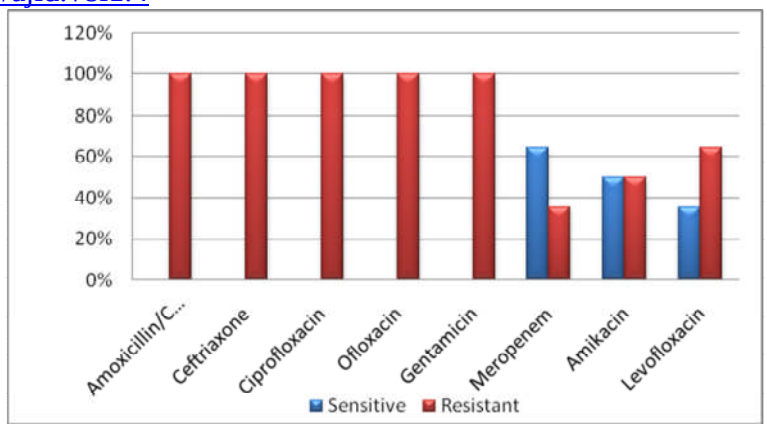

Figure 1: Antibiotic susceptibility profile.

Members of the genus *Acinetobacter* have been showing increasing resistance to β-lactams, aminoglycoside antibiotics and also thought to be a reservoir of antibiotic resistant genes in hospital environment (Boon et al, 2011). This was confirmed in this study where all of the isolates were found to be resistant to ceftriaxone, amoxicillin-clavulanate, ampicillin-sulbactam, gentamicin, ciprofloxacin, ofloxacin. *Acinetobacter* isolates have a propensity to readily develop resistance to third generation cephalosporins and fluoroquinolones thus giving rise to therapeutic problems. As newer generation antibiotics are being developed to overcome problem of resistance against available antibiotics, bacteria are also developing mechanisms to resist newer antimicrobials (Shete et al, 2010).

Partial susceptibility was observed to levofloxacin (35%) and amikacin (50%). The carbapenems (Meropenem) showed the highest susceptibility (64.5%) in this study. Similar finding has been reported by Boon Hong Kong *et al*. (2011), where the susceptibility to Meropenem was 98.2%, while 100% resistance were observed for both amoxicillin-clavulanic acid and ceftriaxone (Boon et al, 2011).

The susceptibility profile showed that the antibiotic combination such as ampicillin/sulbactam and amoxicillin/clavulinic acid did not show promising activities against these isolates despite it being a recommended antibiotic treatment for *Acinetobacter* infections (Dhabaan et al, 2011).

The high resistance rates found in this study may be associated with the high frequency at which these antimicrobial drugs were used for both empirical and therapeutic treatments of hospitalized patients. This practice may have exerted selective pressure leading to the emergence of multidrug resistant strains which in turn may have stimulated the acquisition of genes encoding resistance mechanisms (Federico et al, 2007; Souli et al, 2008).

The role of exposure to certain antibiotics provides a selective advantage to a small number of resistant organisms in patients already colonized, thereby enabling them to become pathogens at the earliest opportunity (Oberoi et al, 2009).

Susceptibilities to *Acinetobacter* spp. against antimicrobials is considerably different among countries and even among the wards of different hospitals. For many years in the ICU of the University College Hospital, the cephalosporins and the quinolones were the drug of choice in empiric treatment and they have been used without any restrictions not only in ICUs but also on other hospital wards and Emergency Units. This could explain the high resistance rates of *Acinetobacter baumannii* to antimicrobials. Multidrug resistance of *Acinetobacter* isolates is a growing problem and has been widely reported (Aharon et al, 2005).

The wide array of intrinsic and acquired resistance determinants that have emerged in *A.baumanni* have justifiably brought it great scientific attention. As determined by the Infectious Diseases Society of America (Ecker et al, 2006), *A. baumannii* is one of the "red alert" pathogens that greatly threaten our current antibacterial armoury. Prior to the 1970s, it was possible to treat *Acinetobacter* infections with a range of antibiotics, including aminoglycosides, β-lactams, and tetracyclines. However, resistance to all known antibiotics has now emerged in *A. baumannii*, thus leaving the majority of today's clinicians in unfamiliar territory.

Conclusion

The high rate of antibiotic resistance demonstrated by Acinetobacter isolates observed in this study demonstrates that antibiotic stewardship protocols should be set up in health facilities to prevent an explosion of resistant bacteria in our health care facilities.

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Conflicts of Interest: The authors declare no conflict of interest.

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