MOLECULAR DETECTION OF CEFOTAXIMASE MUNICH (CTX-M) RESISTANT *ACINETOBATER BAUMANNII* IN A TERTIARY CARE HOSPITAL IN SOUTHEAST NIGERIA

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menace associated with this organism.

Key Words: *Multidrug resistance, Acinetobacter baumannii, hospital infections*

Abstract

Background: *Acinetobacter baumannii* (*A. baumannii*) is one of the numerous organisms responsible for multidrug resistant, hospital acquired infections in many tertiary health care institutions worldwide.

Aim: To detect the presence of cefotaximase (CTX) resistant *A. baumannii* in NAUTH Nnewi, Southeast, Nigeria.

Materials and Methods: Clinical isolates of Gram-negative bacilli (n=210) were obtained from several clinical specimens. All isolates were phenotypically identified in the laboratory using standard cultural methods. Antimicrobial susceptibility tests were performed on all *A. baumannii* species detected using the Kirby Bauer disc diffusion technique. Isolates resistant to any of the 3rd generation Cephalosporins were screened for ESBL activity using the Double disc synergy test. Polymerase Chain Reaction was then used to detect the presence of CTX gene from the ESBL positive isolates.

Results: Out of the 210 Gram-negative bacilli, 20 (9.5%) were Acinetobacter species. The organisms were most resistant to amoxicillin-clavulanic acid and the 3rd generation cephalosporins. Cefotaximase (CTX) gene was observed in 3/20 (5%) of the *Acinetobacter species.*

Conclusion: Multidrug resistant *Acinetobacter baumannii* was prevalent in the study area, although the rate of CTX production was relatively low. Consequently strict application of good infection prevention and control measures in the hospital setting is necessary to curb the menace associated with this organism.

Key Words: *Multidrug resistance, Acinetobacter baumannii, hospital infections*

Introduction

Acinetobacter baumannii (*A. baumannii*) accounts for about 80% of reported *Acinetobacter* human infections. The organism is a ubiquitous, intrinsically multidrug-resistant and rapidly emerging pathogen. It causes a range of healthcare associated infections including sepsis, pneumonia, meningitis, urinary tract infection, and wound infection. The organism has the ability to survive under a wide range of environmental conditions. Its persistence for extended period of time on surfaces makes it a frequent cause of outbreaks in healthcare settings [1,2]. *Acinetobacter baumannii* preferentially colonizes aquatic environments, and in healthcare settings, these include fomites like irrigating solutions and intravenous solutions[3]. These organisms can be cultured from hospitalized patients' sputum or respiratory secretions, wounds, and urine [4].

In recent years, multidrug-resistant *A. baumannii* isolates have been increasingly reported worldwide and has been seen to be emerging as a cause of numerous global outbreaks [5,6]. Multidrug-resistant *A. baumannii* strains are associated with an enhanced risk of morbidity, mortality and prolonged duration of hospitalization[7]. Although several studies on multidrug resistant (MDR) *A. baumannii* (MDR-AB), have been done worldwide, there is insufficient information about the pathogen in the South eastern parts of Nigeria hence the design of the present study.

Materials And Methods

Study area:
The study was conducted at the Medical Microbiology and Parasitology Laboratory of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Southeastern Nigeria. The hospital is a tertiary institution and a major referral Centre serving individuals from most parts of South-east, Nigeria.

Study Design:
This was a prospective hospital based cross-sectional study conducted from March to October 2019.

Study Population
Twenty *Acinetobacter species* were obtained from 210 Gram negative bacilli isolated from several clinical specimens of patients attending the Nnamdi Azikiwe University Teaching Hospital, Nnewi, a major referral centre serving individuals from most parts of South-East, Nigeria. The bacteria were collected within from March to May 2019.

Ethical approval
Ethical approval was obtained from the Research and Ethics Committee of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi.

Isolation and characterization of *Acinetobacter baumannii*
Duplicate swabs (HVS and wound swabs) were collected by rolling moistened sterile swab sticks over the infected sites for about 5 seconds [8]. Urine and sputum specimens were collected in their appropriate containers. Blood specimens were collected in appropriate BactAlert blood collection bottles. The blood culture bottles were incubated in a BactAlert 3D 60 automated blood culture machine until the bottles were flagged positive or when no growth was observed after 5 days of incubation [8]. Positive blood culture specimens were sub-cultured on chocolate agar and Mac Conkey agar (Oxoid, UK) and the culture plates were incubated at 35-37°C for 24 hours. All other specimens were sent to the laboratory immediately after collection and cultured on chocolate agar and Mac Conkey agar (Oxoid, UK) and incubated at 35-37°C for 24 hours [8].

The isolates were Gram-stained, and the Gram-negative rods were subjected to confirmatory identification of *Acinetobacter* species using the Microbact™ Gram-negative bacteria identification kit (Oxoid, UK) [8] motility and oxidase tests [8].

Antimicrobial Susceptibility testing
The Modified Kirby-Bauer antimicrobial susceptibility testing technique was performed on all isolates confirmed as *A. baumannii* [8]. A lawn of each bacterial inoculum equivalent to 1.5 X 10^8 CFU/ml, was made on the surface of a Mueller-Hinton agar (Oxoid, UK) plate using a sterile swab stick and left to dry for 3-5 minutes. Antibiotics were then placed on the lawn, and the plates were incubated aerobically at 35-37°C for 16-18 hours. The zones of growth inhibition around each antibiotic disc were then measured and reported based on the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [8]. The antibiotics tested against the isolates include: Ceftazidime (30 µg), Cefuroxime (30 µg), Ciprofloxacin (30 µg), Ceftriaxone (30 µg), Piperacillin Tazobactam (10 µg), Amikacin (30 µg), Imipenem (10 µg), Etarpenem (10 µg), Meropenem (10 µg), Cefixime (5 µg), Colistin and Gentamicin (10 µg) (Oxoid UK). The test was controlled using *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 as the control strain [8].

Screening for suspected Extended Spectrum Beta Lactamase (ESBL) production
The isolates were screened for resistance to 3rd generation cephalosporins according to the 2020 CLSI guidelines [9]. In this method, 3rd generation cephalosporins (cefotaxime (30 µg) and ceftazidime (30 µg)) discs (Oxoid, UK) were placed on the surface of Mueller Hinton Agar (Oxoid, UK) plates inoculated with each isolate and then incubated for 16-18 hours at 35-37°C after which zones of inhibition were read off. Isolates showing zones of inhibition ≤ 27 mm in

plates inoculated with each isolate and then incubated for 16-18 hours at 35-37^{0}C after which zones of inhibition were read off. Isolates showing zones of inhibition ≤ 27 mm in diameter for cefotaxime or ≤ 22 mm for ceftazidime were considered as suspected ESBL producers. These isolates were then tested using the Combined Disc Method for phenotypic confirmation of ESBL production. The screening test was quality controlled using a strain of *Escherichia coli* ATCC 25922. [9]

Phenotypic confirmation of Extended Spectrum Beta Lactamase (ESBL) production (Combination Disc Test)

The *A. baumannii* isolates which were suspected of producing ESBLs were then subjected to confirmatory ESBL testing using the combination disc technique[10]. Ceftazidime (30 µg) and cefotaxime (30 µg) were used alongside their combinations with clavulanic acid. Standard disc diffusion procedure was followed as described earlier and plates were incubated at 35-37^{0}C for 16-18 hours. Following incubation, a 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate versus the zone diameter of the agent when tested alone was confirmatory for ESBL production[11].

Molecular Detection of CTX gene

Bacteria DNA from the confirmed *A. baumannii* isolates were extracted using the boiling method for DNA extraction previously described by De Medici *et al*. [14]. The Gene encoding CTX group beta-lactamases were detected using conventional PCR reactions that were based on the protocols published previously by Iroha *et al.* (2012) [24]. The primers used were designed and supplied by (Inqaba, SA) and are listed in Table 1. The PCR conditions were as follows: Initial denaturation at 94^{0}C for 5 minutes; followed by 32 cycles of denaturation at 95^{0}C for 30 seconds; annealing at 54^{0}C for 1 minute; extension at 72^{0}C for 2 minutes, then final extension at 72^{0}C for 5 minutes.

Table 1: Primer sequence

Primer	Sequence (5'? 3')[a]	Location	Product Size
CTX-M (F)	CGCTTTGCGATGTGCAG	bla$_{CTX-M}$	517bp
CTX-M (R)	ACCGCGATATCGTTGGT	bla$_{CTX-M}$, reverse primer	

Key: CTX-M = Cefotaximase Munich

The products were resolved on a 2.0% agarose gel at 100V for 30 minutes and visualized on a blue light trans- illuminator at 280 nm.

Results

Out of the 210 Gram negative bacilli isolated in the course of the study, the prevalence of *A. baumannii* was 10 (4.8%) (Table 2). Out of the 10 *A. baumannii* isolated in the study, 4 (40%) were obtained from urine, and 3 (33%) were from swab samples. (Table 3). The *A. baumannii* isolates were most resistant to amoxicillin-clavulanic acid (80%) and cefuroxime (80%). They were least resistant to ciprofloxacin (33.3%) and Piperacillin-tazobactam (33.3%) (see Figure 1). The CTX gene was detected in 3/10 (30%) of the *A. baumannii* isolates. (Figure 2).

Table 2: PREVALENCE OF *ACINETOBACTER BAUMANNII* AT NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL NNEWI, ANAMBRA STATE SOUTHEASTERN NIGERIA

Total No of Isolates cultured	No of A. baumannii	Prevalence (%)
210	10	4.8

Table 3: DISTRIBUTION OF *ACINETOBACTER BAUMANNII* ACCORDING TO SAMPLE USED

Total No of *A. baumannii*	No in Urine (%)	No in HVS (%)	No in Wound Swab (%)
10	4 (40%)	2 (20%)	4 (40%)

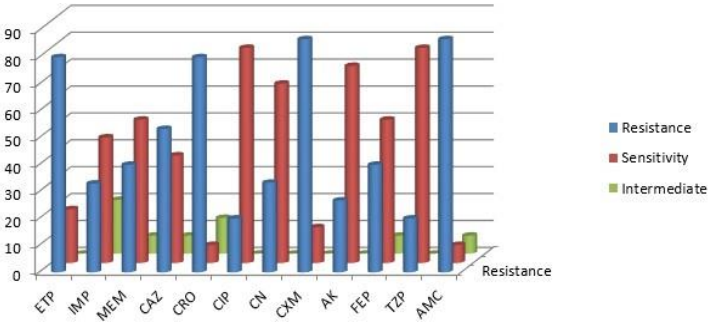

Figure 1: Antibiogram of the *A. baumannii* isolates. ETP=Etarpenem; IMP=imipenem; MEM=Meropenem; CAZ = Ceftazidime; CRO = Ceftriaxone; CIP=Ciprofloxacin; CN=Gentamycin; CXM=Cefexime; AK=Amikacin; FEP=Cefepime; TZP=Tazobactam piperacillin; AMC=Amoxicillin clavulanic acid.

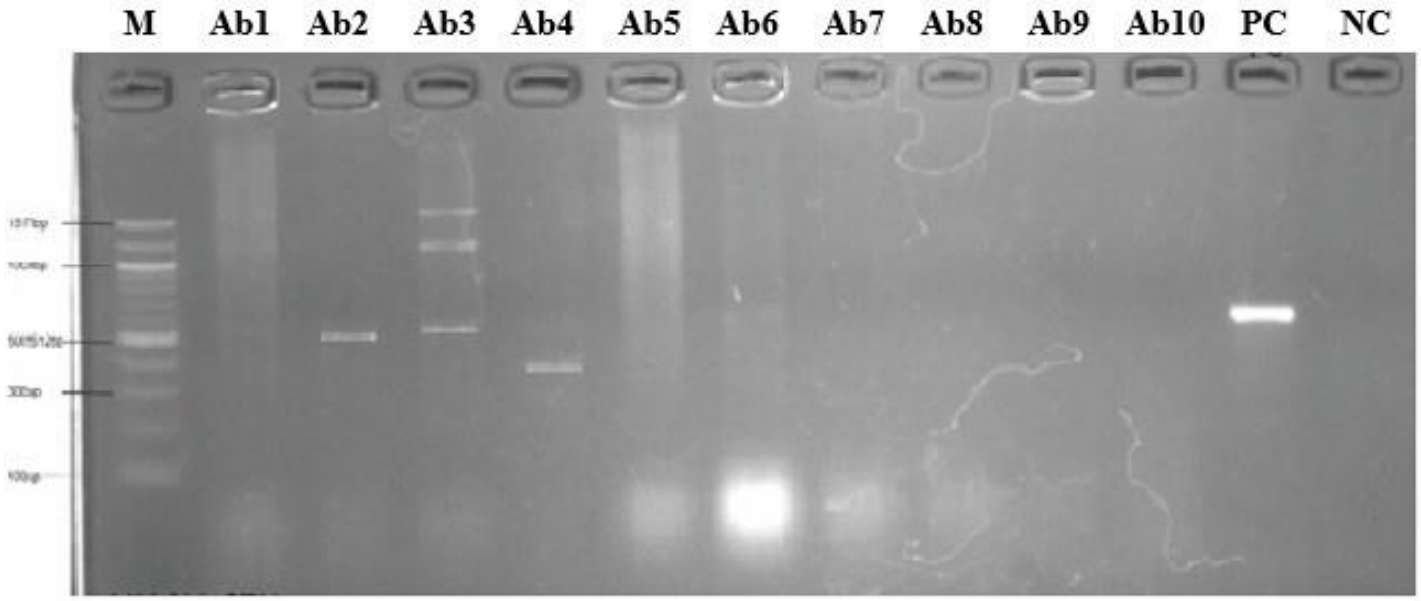

Figure 2: Agarose gel electrophoresis showing the amplified CTX gene from *A. baumannii* isolates AB1-AB10. M = Marker, Ab = *Acinetobacter baumannii*, PC = Positive control, NC = Negative control.

Discussion

Acinetobacter baumannii as one of the leading causes of health care associated infections can be easily contacted because it is found everywhere and spread from person to person. Antibiotic resistant *A. baumannii* has emerged as one of the most problematic hospital acquired pathogens around the world [13]. The emergence of this multidrug strain has gained attention due to its role in mortality and its potential threat to society, particularly in healthcare institutions. While several studies have been conducted on the organism, relatively few have been conducted in Nigeria, particularly in the southeastern region. It has been identified as a cause of nosocomial infection.

This study found that *A. baumannii* occurred at a rate of 4.8%. This is lower than 79% prevalence found by Nwadike *et al.* [7] in their study, which only examined blood samples. In contrast, this study used samples from various sources (e.g. blood, urine, swab, aspirate). Neetu *et al.*[10] found a prevalence of 72% in their study, which is also higher than 4.8% found in this research. It is possible that higher prevalence in these other studies may be due to the use of more accurate identification techniques. This study also used a reliable identification method.

Furthermore, a prevalence of 25.4% and 8.4% were obtained by Parisa *et al.* and Hilina *et al.* [13,15] for *A. baumannii*, which is way higher than that of this study, probably because the study was carried out within a longer period of time and their samples were collected from two different hospitals. That is, the more time given to research, the more chances of obtaining organisms. Also, different environmental practices (e.g. hygiene, proper health care services etc.) play a role in the presence and survival of micro-organisms. Zahra *et al.*[16] also discovered a very high prevalence (30%) of *A. baumannii*, although, it was from air, water and inanimate surface samples. *A. baumannii* is know to be ubiquitous in nature (e.g. CDC 2010 etc) and is also known to be resistant to several disinfectants hence the possibility of isolating a large number from the environment.

According to the site of infection, swab samples showed the highest prevalence (40%) of *A. baumannii* followed by urine (30%) but this is contrary to previous studies [3,11], and Alkali *et al.*[1] who reported their highest prevalence to be in urine and suction respectively [17]. Methodology used by each study varies and this could be the reason for the differences in result.

About 80% of the isolates were resistant to cefexime, and amoxicillin/clavulanic acid; ceftriaxone, (66.7%); ceftazidime (40 %), cefepime 33 . 3 %; while ciprofloxacine, gentamycin, amikacin, tazobactam-piperacillin had few organisms that are resistant. This resistance to cephalosporins is similar to a study done by Velma *et al.* [21] in which about 80% of the organism were highly resistant to some cephalosporins. This study showed a high sensitivity pattern to ciprofloxacin but however, is different from the result obtained by Odewale *et* al. [8]. The report by Odewale *et* al. [8] showed 78.3% resistance to ciprofloxacin although their study population was specifically patients with nosocomial infections unlike the present study where all patients were recruited.

The CTX-M family members hydrolyze cefotaxime and ceftriaxone better than ceftazidime, and they are inhibited more by tazobactam than by clavulanic acid, although point mutations leading to increased activity against ceftazidime can occur. CTX-M enzymes have disseminated rapidly worldwide and are now among the most prevalent ESBLs in Europe and South America [22]. CTX-M gene was detected in three of the

A. baumannii (AB2, AB3, and AB4) isolates at 500bp, 1000bp, 1517bp respectively. (Figure 5) These isolates happen to be the ones obtained from urine and swab samples. This result is similar to findings made by Nwadike *et al*. [18] and Alkali *et al*. [7,1] because they were isolated from moist environment and it proves that *A. baumannii* is a known cause of urinary tract infection (UTI) and wound infections. CTX-M β-lactamases produced by *A. baumannii* strains is plasmid-mediated hence the wide spread and long-time survival in hospitals. The CTX-M gene activity conferring resistance to cefotaxime and ceftazidime (Essam *et al*. 2015) [9].

Conclusion

Multidrug resistant *A. baumannii* was prevalent in the study area, although the rate of CTX production was relatively low. To effectively address the problems caused by this organism, it is essential to strictly follow good infection prevention and control measures in the hospital setting.

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