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The Antibiotics Susceptibility Profile of *Acinetobacter spp* Isolated from Clinical Specimens in University Teaching Hospital

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ABSTRACT

The genus Acinetobacter currently contains 34 species, the vast majority of which are not regularly implicated in causing infection. However, Carbapenems have long been thought of as the agents of choice for serious *Acinetobacter baumannii* infections. The objective of this study is to determine the Antibiotics susceptibility profile of *Acinetobacter spp* Isolated from clinical specimen University of Ilorin Teaching Hospital, Ilorin. The descriptive cross-sectional study is conducted in UITH located in the North Central Nigeria. 10 strains of *Acinetobacter spp*. were isolated from clinical samples between February to July 2018. The isolated strains were identified using standard microbiological methods, API20NE. Antimicrobial susceptibility was performed using Modified Kirby Bauer method with the organism tested against Amoxicillin, Amoxicillin-Clavulanate, Ceftriaxone, Cefixime, Cefuroxime, Streptomycin, Perfloxacin, Gentamicin, Imipenem, Nitrofurantoin. Most of the antibiotics used in this study are mostly multi-drug resistant. Amoxicillin (100%), Cefixime, Amoxicilln Clavulanate (100%), Cefuroxime (100%), Gentamicin (100%), Nitrofurantoin (100%), Ceftriaxone (100%) but 80% are susceptible to Imipenem. Carbapenems are the best antibiotic treatment option for infections arising from these organisms although a coordinated rational usage is desired along with a functional antibiotic prescription policy to avoid treatment failures. Continuous monitoring of resistance patterns is necessary to strengthen infection control policies.

Keywords: *Acinetobacter species*, Intensive care unit, Cerebrospinal fluid (CSF), Blood, and Macconkey agar.

INTRODUCTION:

Acinetobacter spp are Gram negative, coccobacillus and strict aerobes Munoz, Robert and Weinsten, (2007). They play significant role in the colonization and infection of patients admitted to the hospital Schreckenberger, Daneshvar and Weyant, (2007). It is an opportunistic pathogen found to be associated with a wide spectrum of infection including nosocomial pneumonia, meningitis, endocarditis, skin and soft tissue infections. Others are Urinary tract infection, conjunctivitis, burns, wound infections and bacteraemia Gerner, Tjernberg and Ursing, (1991). Carbapenems have long been thought of as the agents of choice for serious *A. baumannii* infections Davis *et al.* (2004). However, the clinical utility of carbapenems is increasingly jeopardized by the production of carbapenemases. Outbreaks of infection caused by strains of *A. baumannii* resistant to multiple antibiotics classes including carbapenems,

are a serious concern in many specialized hospitals units, including intensive care unit (ICUS). The foremost implication of infection with carbapenem-resistant *A. baumannii* is the need to use "last-line" antibiotics such as colistin, polymyxin B, or Tigecycline David *et al.* (2012). Selection of empirical anti-biotic therapy when *A. baumannii* is suspected, it is challenging & must rely on knowledge of local epidemiology (Sarker *et al.,* 2021; Shahen *et al.,* 2019). The interval from onset of infection to initiation of effective empirical therapy clearly influences outcome. Given the diversity of resistance mechanisms in *A. baumannii*, definitive therapy should be based on the results of antimicrobial susceptibility testing.

METHOLODOGY:

This study was carried out collaborately at the Microbiology laboratory department of the University of Ilorin Teaching Hospital (UITH) and Bangladesh; UITH is located in Ilorin which provides quality health care services to the neighbouring state such as Oyo, Kogi, Niger, Osun and Ekiti states. UITH has bed space of about four hundred and fifty (450) and admission rate of about twelve thousand (12,000) patients per annum. Ilorin is the capital of Kwara state. The study population includes patients admitted into medicals, surgical wards and intensive care unit (ICU) OF University of Ilorin Teaching Hospital, Ilorin, Nigeria during February to July, 2018 whose clinical samples were sent to the Medical microbiology laboratory for routine investigation.

Study Design

This is a descriptive cross sectional study.

Sampling Method

Purposive sampling, a non probability method was employed for this study. All *Acinetobacter spp* isolates from clinical samples collected in a repetitive manner were used for this study. All patients' data to each isolate available in the laboratory register were used for this study. The age, sex and unit/wards were noted in the proforma.

Ethical Clearance

Ethical approval was obtained from the Ethical Review committee of the University of Ilorin Teaching Hospital, Ilorin.

Sample Size

Sample size was determined using the sample size formula for estimating single proportion Lislie and Wiley, (2007).

$$N = \frac{z^2pq}{d^2}$$

Where,

N=Minimum sample size

Z=Standard normal deviation, usually set at 1.96 which corresponds to 95% confidence level

P=The best estimated prevalence of target population; 8% from a study, Odewale *et al*. (2016) = 0.08%

Q=I-p; and 1-0.08=0.92

d=minimum statistically significant difference to be measured at 0.05 precision.

$$\text{Hence, } N = \frac{1.96X0.08X0.92}{0.05X0.05} = 113$$

The minimum sample size is 113. Therefore, 113 clinical samples were collected for this study. However, they were some multiple samples collected from more than one patient which made it 150 clinical samples.

Specimen collection

Various clinical samples were collected through aseptic technique by medical practitioners to prevent contamination. For optimal results, specimen was collected in clean sterile, wide bore containers. The clinical samples include urine, pus, blood, fluid (pleural, pericardial, synovial and peritoneal), wound swab, cerebrospinal fluid (CSF), sputum, central venous pressure (CVP), catheter tip , stool, throat swab, ear swab, nasal swab, from the tip of Foley catheter, tissue (wound and soft tissues), and bronchial washing samples. They were transported immediately to the Medical Microbiology Laboratory of the University of Ilorin Teaching Hospital for microbiological analysis.

Characterization of bacterial isolates

Colonial morphology of suspected colonies from both blood agar and Macconkey agar plate was characterized based on their appearances. Gram staining was done on the isolates and biochemical testing such as oxidase, catalase and triple sugar iron was furtherly done on the gram negative, coccobacillus isolates to characterize it. Moreso, the identified *Acinetobacter-spp* was furtherly confirmed by API20NE multi test

system. These tests were used according to manufacturer's protocol for Enterobacteriaceae and non-enteric bacteria.

Susceptibility Testing

Antimicrobial Susceptibility test was determined using disc diffusion method. The disc diffusion method is a modification of the Kirby Bauer technique that has been carefully standardized by CLSI as described by Lalitha M. K in Manual of Antimicrobial Susceptibility Testing. This is meant to designate isolates as either Carbapenem - resistant *Acinetobacter*. *Baumannii* (CRAB) (with Imipenem MIC> 8ug/ml). The tested agents included Amoxicillin, Cefixime, Streptomycin, Amoxicillin-clavulanate, Perfloxacin, Gentamycin, Imipenem, Nitrofurantoin and Ceftriaxone. Quality Control for the MIC analysis was performed with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Statistical analysis

Data obtained was fed into a computer and analyses were done using statistical package for the social sciences (SPSS) version 20.0. Data was presented in frequency tables, charts and prose. Cross tabulation of important table was done.

RESULTS:

Acinetobacter Speciation

Acinetobacter baumannii constituted 7(70%) of the species isolated followed by *Acinetobacter iwoffi* 2(20%) with least from *A. haemolyticus* 1 (10%) using API20NE which are majorly male prepondence as shown in **Table 1**.

Antibiotics Susceptibility Testing

The Antimicrobial susceptibility pattern of *Acinetobacter spp* isolated from this study as shown in **Table 2**. Overall, 11 different antibiotics were tested and the isolates were resistant to at least two or more antibiotics. All the isolates were resistant to amoxicillin, amoxicillin clavulanic acid, gentamicin, nitro-furantoin, cefixime, ceftriaxone and cefuroxime. Significant resistance is observed with Streptomycin 90%, perfloxacin 90% and least resistance is observed with imipenem 20%.

Table 1: Species Distribution of Acinetobacter.

Species	Number of Isolates (%)	Males N (%)	Females N (%)
Acinetobacterbaumannii	7(70)	5(71.4)	2(28.6)
Acinetobacteriwoffi	2(20)	2(100)	0(0)
Acinetobacterhaemolyticus	1(10)	0(0)	1(10)
Total	10(!00)	7(70)	3(30)

Table 2: Antibiotics susceptibility pattern of *Acinetobacter spp.*

Antibiotics	Number	Sensitive	Intermediate	Resistant
Amoxicillin	10	0(0.0)	0(0.0)	10(100.0)
Cefixime	10	0(0.0)	0(0.0)	10(100.0)
Amoxicillin Clavulanate	10	0(0.0)	0(0.0)	10(100.0)
Cefuroxime	10	0(0.0)	0(0.0)	10(100.0)
Streptomycin	10	0(0.0)	0(0.0)	9(90.0)
Perfloxacin	10	0(0.0)	0(0.0)	9(90.0)
Gentamicin	10	0(0.0)	0(0.0)	10(100.0)
Imipenem	10	7(70.0)	1(10.0)	2(20.0)
Nitrofurantoin	10	0(0.0)	0(0.0)	10(100.0)
Ceftriaxone	10	0(0.0)	0(0.0)	10(100.0)

Table 3: Resistance pattern of Multi-Drug Resistance (MDR) *Acinetobacter spp*.

Antibiotics	Number	Sensitive	Intermediate	Resistance
Amoxicillin	10	0(0.0)	0(0.0)	10(100.0)
Cefixime	10	0(0.0)	0(0.0)	10(100.0)
Amoxicillin clavulanate	10	0(0.0)	0(0.0)	10(100.0)
Cefuroxime	10	0(0.0)	0(0.0)	10(100.0)
Gentamicin	10	0(0.0)	0(0.0)	10(100.0)
Nitrofurantoin	10	0(0.0)	0(0.0)	10(100.0)
Ceftriaxone	10	0(0.0)	0(0.0)	10(100.0)

Table 3 below shows the resistance pattern of Multi-Drug Resistance (MDR) *Acinetobacter spp* which includes seven antibiotics such as Amoxicillin, Cefixime and others.

DISCUSSION:

Antimicrobial resistance among *Acinetobacter species* has increased significantly in the past decades Maragakis and Perl, (2008). The ability of *Acinetobacter species* to extensively resist antimicrobial agents may be explained in part by the organisms' relatively impermeable outer membrane, selective pressure & environmental exposure to a large reservoir of resistance genes (Bonomo & Szabo, 2006; Sarker *et al.,* 2021). Definition of MDR *Acinetobacter spp* varies, referring to a wide array of genotypes and phenotypes Falagas *et al.* (2006).

Two of the most common definitions of multidrug resistance are carbapenem resistance or resistance to >3 classes of antimicrobial agents investigated in this study. In this study all the strains exhibited resistance to >6 antimicrobial agents tested. All (100%) strains were resistant to amoxicillin, amoxicillin clavulanate, cefixime. Cefuroxime, ceftriaxome, nitrofurantoin and gentamicin used. In 1970s, Acinetobacter infections were treated with ampicillin second generation cephalospirins, minocycline, colistin, carbenicilin and gentamicin Iregbu *et al.* (2002). Today, most strains are resistant to ampicillin, cefotaxime and chloramphenicol with reports of 84% resistant to gentamicin in some institutions Iregbu *et al.* (2002). This is consistent with the observation made in this study. Among the classes of penicillins used in this study, all the strains (100%) were resistance to amoxicillin-clavulanate and amoxicillin. The beta-lactamase inhibitors used, amoxicillin-clavulanate did not improve the antimicrobial activity with all the isolates showing resistance. This is in agreement with previous observation made by Higgins *et al.* (2004). For the antibiotic class cephalosporins, all the isolates were resistance to cefuroxime the only second generation subclass of cephalosporin investigated. Similarly zero susceptibility rates were also recorded against the third generation subclass of cephalosporin, cefixime and ceftriaxone. These observations were comparable to report made by Iregbu *et al.* (2002) against ceftriaxone and cefuroxime. Generally, Cephalosporin's are beta-lactam antibiotics with reportedly high antimicrobial activity and low toxicity. Increased resistance observed from this institution could partly be explained by high level production of extended-spectrum antimicrobial therapy and frequent prescription of the drugs. Carbapenems remain the antibiotic of choice to treat *A. baumannii* and other Gram-negative infections due to both a wider spectrum of antibacterial activity and less frequent side effects. The report of high susceptibility pattern to carbapenem in this study is similar to Farahani *et al.*

However, there was high rate of resistance to carbapenem reported by Wang *et al.* (2013) in South Africa reported 63% resistance and Ramoul *et al.* (2013) reported 91.3% in Algeria health care centers. It is important to note that this wide differences associated with carbapenem sensitivity and Acinetobacter infection in this study may be due to the difference in the time of the study, the kind of antibiotics sensitivity disc used and can also be due to inappropriate use of this antibiotic in the hospital setting where this study was carriedout. Aminioglycosides are usually used in combination with another active anti-microbial agent. In this study, the only amino-glycoside used in gentamicin having 100% resistance rate, similar finding was reported by Nemecand Maixnerova, (2004) with gentamicin having similar trend of 87% resistance.

CONCLUSION:

The prevalence rate of *Acinetobacter spp* in this study is high and they are generally Multi-Drug Resistant (MDR). However, Carbapenem, Streptomycin and Perfloxacin remain useful therapy for the infections caused by these organisms. As such, rational usage of Carbapenems, Streptomycin, Perfloxacin and functional antibiotic prescription policy are desired for the management of Acinetobacter infection to avoid treatment failures. Continuous surveillance through well-equipped laboratories for prompt accurate detection of *Acinetobacter spp.*

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CONFLICTS OF INTEREST:

There are no conflicts of interest.

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