**Antibiotic Susceptibilities of Two Multidrug Resistant *Acinetobacter* *species* Clinical Strains Showed Significant Variation to Amoxicillin Resistance and Susceptibilities to Quinolones**

**Abstract**

*Acinetobacter* species is now considered among the three most important nosocomial bacterial pathogens worldwide. *Acinetobacter* species possess an inherent capacity to withstand environmental stress and the bacterium pathobiology is complicated with limited therapeutic options available for its treatment due to widespread resistance to antimicrobials of different classes. A total of 75 swabbed surfaces at the nurses’ station paediatric surgical ward ATBUTH were examined for Acinetobacter species and the susceptibility to antibiotics of the isolates investigated. A low prevalence 2.67% (2/75) was obtained. The isolates were completely resistant to three of the 10 antibiotics tested namely: chloramphenicol, augmentin (ampicillin + sulbactam) and amoxicillin and wholly susceptible to five of the 10 antibiotics tested: septrin (sulfamethaxozole + trimethoprim), sparfloxacin, perfloxacin, ofloxacin and streptomycin. The zone of inhibition of the isolates differ significantly with four of the antibiotics tested; ofloxacin (p<0.01), streptomycin (p<0.04), gentamicin (p<0.002) and amoxicillin (p<0.005). The study showed strains of *Acinetobacter* species even though β-Lactam resistant could vary in the degree of resistance to β-Lactam antibiotics evident with significant difference in zone of inhibitions and high susceptibilities to quinolones antibiotics of the strains. Further work will determine whether strains are clonal and whether the determinant and expression of β-Lactam resistance are the same in those strains background, as well as if the different susceptibilities to β-Lactam as shown by zone of diffusion will translate to different MIC values, and if whether such resistance determinants do not encode quinolone resistance.

**Keywords**: *Acinetobacter*, β-Lactam, Carbapenems, Quinolones, Multidrug Resistance, Nosocomial pathogens

**INTRODUCTION**

*Acinetobacter* species is a genus of gram-negative bacteria belonging to the γ-proteobacteria family. *Acinetobacter* species are coccobacillus, non-motile, non-fermentative, oxidase negative occurring in in pairs on microscopy. Several species of the genus are found in the environment but the clinical relevant species globally is *Acinetobacter baumanii*.  *A. baumanii* is considered as the second most important nosocomial pathogen worldwide after *Pseudomonas aeruginosa* among aerobic gram- negative pathogens, the bacterium is responsible for outbreaks of hospital infections. *A baumanii* is a key source of infections in debilitated patients in the hospital and the etiological agents of respiratory and urinary tract infections, meningitis, endocarditis, burn infections and wound sepsis especially in intensive care units (ICUs).[[1]](https://paperpile.com/c/rvX9ea/qKRc) Presently *Acinetobacter baummanii*is recognized as the cause of the largest nosocomial outbreak  in history and some cases of community–acquired infection caused by *Acinetobacter* species have been reported[[2]](https://paperpile.com/c/rvX9ea/P5ow).

Antibiotics are the drug of choice in combating bacterial infections. The treatment of *A. baumanii* infections in many centres worldwide is problematic due to widespread resistance to many antibiotics and disinfectants as a result of innate and acquired resistance mechanisms[[3]](https://paperpile.com/c/rvX9ea/7Odk). The widespread use of β-Lactam antibiotics class of antibiotics attributed to  its selective toxicity have been rendered useless by the acquisition of β-Lactam resistance mediated by β-Lactamases express by many gram-negative pathogens[[4]](https://paperpile.com/c/rvX9ea/YzXD). Multidrug resistance in Gram negative bacteria including Acinetobacter species especially to β-Lactams antibiotics is commonly associated with the expression of β-Lactamases such as extended spectrum β-Lactamases (ESBLs) and metallo β-Lactamase (MBL). The ESBLs have evolved into the classical TEM- , SHV- and CTX-M- β-Lactamases with different affinities to cefotaxime , ceftazidine and other broad spectrum cephalosporins and monbactams.[[1]](https://paperpile.com/c/rvX9ea/qKRc)

 The limited therapeutic options available for the treatment of ESBLs-producing Enterobacteriaceace and other Gram negative bacteria  necessitated the introduction of carbapenems (such as imipenem and meropenem)  presently considered the drug of choice for the treatment of multidrug resistant gram negative pathogens, but increased resistance to carbapenems by  the emergence of carbepenems-hydrolyzing β-Lactamase of Amber class D β-Lactamases particularly the OXA-23 and OXA-58 enzymes, OXA-40 enzyme and carbapenemase of molecular class B are now widespread[[5]](https://paperpile.com/c/rvX9ea/t9BZ). The study was carried out to investigate the prevalence of *Acinetobacter* species that may be incriminated in outbreaks and to suggest possible source and mode of transmission of the pathogen. We also sought to know the degree of susceptibilities in a quantitative manner to different classes of antibiotics. *Acinetobacter* strains obtained showed significant difference to susceptibility to four antibiotics of the β-Lactam class, quinolone and aminoglycoside a further evidence of the use of any of these classes in combination therapy for the treatment of *Acinetobacter* infections.

**MATERIALS AND METHODS**

**Specimens Collection**

A total of 75 swabbed sample of surfaces that includes sink, examination bedrails, examination bedside, table, vital signs monitor equipment such as thermometers, blood pressure touch pads, ventilator adjustments knob, floor on both side of examination bed, tables, drawers handles at the nurses’ station of the Pediatric surgical ward Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), a 1000-bed tertiary hospital in Bauchi Bauchi State Nigeria. Samples were collected within a month (December, 2015). The areas sampled were cleaned regularly according to hospital directives and different disinfectants containing chlorhexidine- and quaternary ammonium compounds- containing agents were used at different times within the month of sampling. The efficacy of cleaning was not measured during the study. The study was approved by the ATBUTH Research and Ethics Committee (ATBUTH- REC) and was assigned ATBUTH-REC number 03/11/2015. After sampling, each swab was placed in 5ml cooked meat broth (Oxoid, UK), mixed by gentle shaking for approximately a minute and incubated overnight at 37oC aerobically for 24h.

**Isolation, Identification and Preservation of *Acinetobacter* Isolates**

The overnight cooked meat broth culture was rocked and 100µl of the broth inoculated on MacConkey agar (Fluka, Germany) and blood agar (5% v/v human blood in nutrient agar (Fluka,Germany) using the spread plating technique. Inoculated plates were incubated aerobically, at 37oC for 24h. Isolates were identified as *Acinetobacter* species on the basis of Gram stain reaction, morphology of culture on microscopy after Gram stain, colonial morphology on human blood agar plates (5% v/v), biochemical tests such as catalase test, lactose fermentation, oxidase test and test for motility. Strains that were Gram negative, coccobacilli on microscopy, catalase positive, non-lactose fermenters, oxidase negative and non-motile were identified as *Acinetobacter* species. Identified strains were streaked to purity unto blood agar (5% human blood in nutrient agar) plates, discrete colony for each strain picked and sub-cultured separately unto sterile nutrient agar slants, strains were labeled MM1\_15 and MM2\_15 accordingly and preserved in 4oC refrigerator for further used.

**Determination of Antibiotic Susceptibilities**

Antibiotic susceptibilities of the isolates were determined with a disc diffusion method. Discrete colonies from an overnight (O/N) culture plate were picked emulsified in 3ml normal saline (saline at physiological concentration- 0.85% w/v), and the inoculum density standardized with Densi-Chek™ (Biomeriux-SA France) to be equivalent to 0.5 McFarland standard. A sterile swab stick wasdipped into the inoculum suspension, drained of excess moisture by pressing against the wall of the test tube and used to inoculate the surface of Mueller-Hinton agar (Fluka, Germany) plates poured to uniform depth, to obtain a confluent growth. Commercially prepared antibiotic impregnated discs (Optudisc™, Optun Laboratory, Nigeria) were placed on the lawn culture, allowed to stand for five minutes on the bench. The plates were then incubated aerobically at 37oC for 24h. Zone of inhibitions (ZoI) were measured with a metre rule, recorded and interpreted according to modified EUCAST 2013 interpretative criteria(Committee et al. 2013). The experiments were carried out with two repeats in two independent experiments, results averaged and plotted with standard deviations. The potency of the antibiotic impregnated discs was as follows: septrin (30µg), chloramphenicol (30µg), sparfloxacin (10µg), amoxicillin (30µg), augmentin (30µg), gentamicin (10µg), perfloxacin (10 µg), ciprofloxacin (10µg), streptomycin (30µg), and ofloxacin (10µg).

**BIOSTATISTICS**

Experiments were performed with two repeats in two independent experiments, mean and standard deviation values calculated and plotted in figures. Test for statistical significance was assessed by carrying out an independent two sample t-test. Values of p<0.05 were considered statistically significant.

**RESULTS/DISCUSSION**

A low prevalence of 2.67% (2/75) of positive isolation of *Acinetobacter* species was obtained. According to the antibiogram results presented in Table 1, the strains are completely sensitive to septrin (a trimethoprim + sulphonamide), streptomycin (aminiglycoside) sparfloxacin, perfloxacin and ofloxacin (quinolones). Similarly the zone of inhibition (ZoI) of 50% of the strains (ZoI50) and ZoI90 were equal to or greater than five units to the breakpoints in five antibiotics: septrin (ZoI50 =20mm and ZoI90=21mm) , streptomycin (ZoI50=22mm and ZoI90=29mm) ,sparfloxacin (ZoI50 =23mm and ZoI90 =25mm) perfloxacin (ZoI50 =21mm and ZoI90 =29mm) and ofloxacin (ZoI50 =22mm and ZoI90 =25mm), which signifies a high degree of susceptibility to these antibiotics by the strains. The susceptibilities of *Acinetobacter* strains to fluoroquinolones( such as sparfloxacin, perfloxacin ofloxacin) and aminoglycosides( such as streptomycin)  antibiotics  have led to recommendation  that these antibiotics could be used with β-Lactams for the treatment of infections due to *Acinetobacter* species.[[6]](https://paperpile.com/c/rvX9ea/PghP) All the strains are out rightly resistant to chloramphenicol, amoxicillin and augmentin (ampicillin + sulbactam).Transferable plasmids encoding resistance to β-Lactams do encode resistance determinants such as those for resistance to aminoglycosides, tetracyclines, chloramphenicol ,trimethoprim and sulfonamides.[[7]](https://paperpile.com/c/rvX9ea/jo93) The strains were resistant to amoxicillin- a semisynthetic penicillin with low affinity to β-Lactamase –and augmentin- ampicillin conjugated with the β-Lactamase inhibitor sulbactam. Combining β-Lactams or carbapenems have boosted the efficacy of those drugs but report of increased resistance to sulbactam by *Acinetobacter baumanii* strains are on the increased.[[8]](https://paperpile.com/c/rvX9ea/QnxD)

The zone of inhibitions to different antibiotics tested are as shown in Figure 1, the zone of inhibitions of the strains showed no significant difference to six of the ten antibiotics tested namely: septrin (p<0.522), chloramphenicol (p<0.852) , sparfloxacin (p<0.345), augmentin (p<0.456) , perfloxacin (p<0.245) and ciprofloxacin(p<0.545). The ZoI of the strains were significantly different to four antibiotics: amoxicillin (p<0.005), gentamicin (p<0.002), streptomycin (p<0.04) and ofloxacin (p<0.01).*Acinetobacter* strain MM2\_15 more susceptible to ofloxacin than strain MM1\_15, while MM1\_15 showed more susceptibility to amoxicillin, gentamicin and streptomycin. Many resistance mechanisms are said to operate in the species such as β-Lactamases, multidrug efflux pumps , aminoglycoside modifying enzymes, permeability defects  and aleteration to target sites these mechanisms may act against a class of antibiotic or many classes.[[8]](https://paperpile.com/c/rvX9ea/QnxD) It has been established that *Acinetobacter* though possessing intrinsic  chromosomal β-Lactamase genes the expression of resistance to β-Lactams such as amoxicillin used in these study  may vary in  *Acinetobacter* strains as a result of the presence or absence of a strong promoter provided by *IS*Aba1 upstream of the gene encoding resistance , furthermore the different ESBLs  (classical TEM- , SHV, and CTX-M-β-Lactamases) are known to confer different levels of resistance to β-Lactams such as cefotaxime, ceftazidine and other broad spectrum cephalosporins and monobactams.[[9]](https://paperpile.com/c/rvX9ea/OAf9) The β-Lactamases express by our local strains may have different affinities and enzyme kinetics to the β-Lactam substrate.

**Table 1 Antibiotic Susceptibilities of Two β-Lactam Resistant *Acinetobacter* species Isolated in Abubakar Tafawa Balewa University Teaching Hospital Bauchi Nigeria**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotics Tested****(Disc Potency in µg)** | **Total No.** **Screened** | **EUCAST 2015****Breakpoints (mm)****R/S** | **ZoI50:ZoI90****(mm)** | **Proportion Susceptible±** |
| Septrin | 2 | ≤15> | 20:21 | 100% |
| Chloramphenicol | 2 | ≤17> | 16:17 | 0% |
| Sparfloxacin | 2 | ≤15> | 23:25 | 100% |
| Amoxicillin | 2 | ≤16> | 12:16 | 0% |
| Augmentin | 2 | ≤16> | 14:15 | 0% |
| Gentamicin | 2 | ≤12> | 11:22 | 50% |
| Perfloxacin | 2 | ≤15> | 21:24 | 100% |
| Ciprofloxacin | 2 | ≤15> | 15:19 | 50% |
| Streptomycin | 2 | ≤11> | 22:29 | 100% |
| Ofloxacin | 2 | ≤14> | 22:25 | 100% |

ZoI= Zone of Inhibition of 50% of Strains (ZoI50) and 90% of strains (ZoI90) , ± Susceptibilities defined by modified Clinical European Committee for Antimicrobial Susceptibility Testing (EUCAST) 2013 breakpoints

Fig 1: Zone of Inhibitions in millimetres of *Acinetobacter* strains (MM1\_15 and MM2\_15) to ten (10) antibiotics tested. Discrete colonies of each strain from an O/N plate were emulsified in normal saline separately to match 0.5 Mc Farland standards using a Densi-Chek ™ (Biomerieux, SA France) approximately 108 cells/ml. A sterile cotton swab was dipped into the suspension drained of excess moisture used to inoculate the surface of MHA (Fluka, Germany), kept on bench for 5 minutes before the placement of antibiotic discs. The plates were then incubated aerobically at 37oC for 24h. Two repeats in two independent experiments were carried out, results averaged and plotted with standard deviations. Susceptibilities of the strains differ significantly with AMX (Amoxicillin) (p<0.005), GEN (Gentamicin) (p<0.002), STR (Streptomycin)(p<0.04) and OFL (Ofloxacin) (p<0.01). NB: SEP-Septrin, CHL-Chloramphenicol , SPAR- Sparfloxacin , AMX- Amoxicillin, AUG- Augmentin, GEN- Gentamicin, PERF- Perfloxacin, CIP-Ciprofloxacin, STR- Streptomycin , OFL- Ofloxacin.

**CONCLUSION**

We report *Acinetobacter* strains isolated from the same clinical environment displaying varied resistance to amoxicillin- a β-Lactam antibiotic and ofloxacin -a quinolone, gentamicin and streptomycin. We observed no significant variation in the degree of resistance to augmentin (a β -Lactam conjugated with anti-β-Lactamase sulbactam) and three other quinolones. Further work will seek to determine the ability of these strains to withstand environmental stress as obtained in a hospital setting, to establish the capacity of the strains to persist and be transmitted, we also seek investigate systematic relationship between the strains, the location of the resistance determinants that may explain variation to the expression of resistance.

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