

**DETERMINATION OF THE ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF  
ENTEROBACTERIACEAE IN A TERTIARY HEALTH CENTER  
OF NORTHEASTERN NIGERIA.**

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## ABSTRACT

**Background:** Gram negative resistance particularly amongst species of *Enterobacteriaceae* has emerged as a worldwide threat affecting the efficacy of our commonly used antimicrobial agents. These threats affect the quality of care and results in prolonged hospitalization and increase in health care cost. We set out to evaluate the occurrence and profile of antimicrobial resistance from clinical isolates of *Enterobacteriaceae* in a tertiary health center of north-eastern, Nigeria.

**Method:** Confirmed species of *Enterobacteriaceae* isolated from 225 patients that were admitted in various units of University of Maiduguri Teaching Hospital (UMTH) Maiduguri were tested for susceptibility to 6 antimicrobial agents; ampicillin (10µg), aztreonam (30µg), gentamicin (10µg), ceftriaxone (30µg), ciprofloxacin (5µg) and amoxicillin/clavulanic acid (20/10µg) using the clinical and laboratory standards institute (CLSI) breakpoints.

**Results:** A total of 225 clinical variants of *Enterobacteriaceae* were isolated during the study period. The specie with the highest proportion was *Klebsiella pneumoniae* with 73(32.4%) and this was followed closely by *Escherichia coli* with 61(27.1%). The result of the susceptibility testing on this species of *Enterobacteriaceae* showed ampicillin (10µg) with the highest resistance of 118(52.4%) while aztreonam(30µg) has the lowest resistance of 58(25.8%).

**Conclusion:** There is the existence of widespread resistance to mostly the beta lactam agents among species of *Enterobacteriaceae* with the exception of Aztreonam. Irrational use of antibiotics must be discouraged so as to reduce this emerging threat. Stringent infection control and antibiotic stewardship program should be established and sustained in our hospitals nationwide.

**Keywords:** Resistance, *Enterobacteriaceae*, Maiduguri, north-east.

## INTRODUCTION

Antimicrobial resistance has now emerged as a global - local problem that threatens health security of all nations. Gram negative bacteria particularly members of *Enterobacteriaceae* harbor's the majority

of this resistance threat.<sup>1</sup> *Enterobacteriaceae* are inhabitants of the intestinal flora of more than seventy (70) different bacteria and are among the most common human pathogens, causing infections such as cystitis and pyelonephritis with

fever, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections.<sup>2</sup> The species of *Klebsiella pneumoniae* and *Escherichia coli* predominates as the one with highest burden of resistance.<sup>3</sup>

Antibiotic use in Nigeria is unregulated and there are no regional or national surveillance data on bacterial resistance to antibiotics - only a few localized institutional studies have reported the prevalence or level of antibiotic resistance, particularly to beta lactams.<sup>4</sup> These resistant bacteria have the potential to spread rapidly within the hospital environment and also across continents causing great morbidity, mortality, prolonged hospitalization and indirect healthcare cost.<sup>5</sup>

This study set out to determine the antimicrobial resistant profile of *Enterobacteriaceae* in a tertiary health centre of northeastern Nigeria. These resistant profiles of *Enterobacteriaceae* would enable the understanding of the magnitude of the problem in our locality with a view to institute appropriate measures. It would also enable us to make an appropriate rational choice of antimicrobial agents for *Enterobacteriaceae* in our setting.

## MATERIALS AND METHOD

**Study area:** University of Maiduguri Teaching Hospital (UMTH), Borno State. It is a 500 bed capacity tertiary health center which serves as a principal reference center for the whole of northeastern Nigeria.

**Study design:** Descriptive and cross-sectional.

**Study population:** All hospitalized patients that consented for the study and from whom *Enterobacteriaceae* were isolated.

**Sample size:** A total of 225 species of *Enterobacteriaceae* were isolated between July 2014 January, 2015 during the study period.

**Sampling method:** Convenient sampling.

**Specimen collection:** The isolates of *Enterobacteriaceae* were obtained from hospitalized patients in various units and wards of UMTH. All specimen were collected and transported according to standard methods.<sup>6</sup>

**Bacterial identification:** The specimens were inoculated on MacConkey agar. After 24-48 hours of aerobic incubation at 36-37°C, colonial appearance and characteristics of isolates on

MacConkey agar was noted and they were then subjected to Gram staining reaction and also motility testing according to standard methods.<sup>6</sup> All suspected isolates of *Enterobacteriaceae* were confirmed by the Microbact Gram negative identification system™ (Oxoid) 24E according to the manufactures instructions.

**Antimicrobial susceptibility testing using disc diffusion (modified Kirby Bauer) method:** All identified clinical strains of *Enterobacteriaceae* were subjected to *in-vitro* susceptibility testing using Kirby Bauer disk diffusion method as described in the clinical and laboratory standard institute (CLSI)<sup>7</sup> guidelines and were subsequently interpreted accordingly. The commercially available Oxoid England single disc comprising of Ampicillin (10 µg), Ceftazidime (30 µg), Aztreonam (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Amoxicillin plus Clavulanic acid (AMC 30µg) discs were used. *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 35218 were used as control strains for the susceptibility studies.<sup>7</sup>

**Data analysis:** Data collected was recorded into a computer and analysis was be done using statistical package for social sciences version 16.0 (SPSS Chicago Ill. USA). Results were presented when necessary as tables and figures.

**Ethical consideration:** The study protocol was reviewed and approved by the ethical review committee of UMTH.

## RESULTS

We isolated 225 isolates of *Enterobacteriaceae* from 225 patients over a period of six months. The distribution of the various species of the 225 *Enterobacteriaceae* isolated were as shown in Table 1 with 73(32.4%) being *Klebsiella pneumoniae* as majority while *Citrobacter freundii* has the least proportion of 2(0.9%). A total of 115(51.1%) clinical isolates of *Enterobacteriaceae* were obtained from urine, 45(20.0%) from blood specimen, 31(13.8) from swabs, 21(9.3%) from sputum and 13(5.8%) from cerebrospinal fluid.

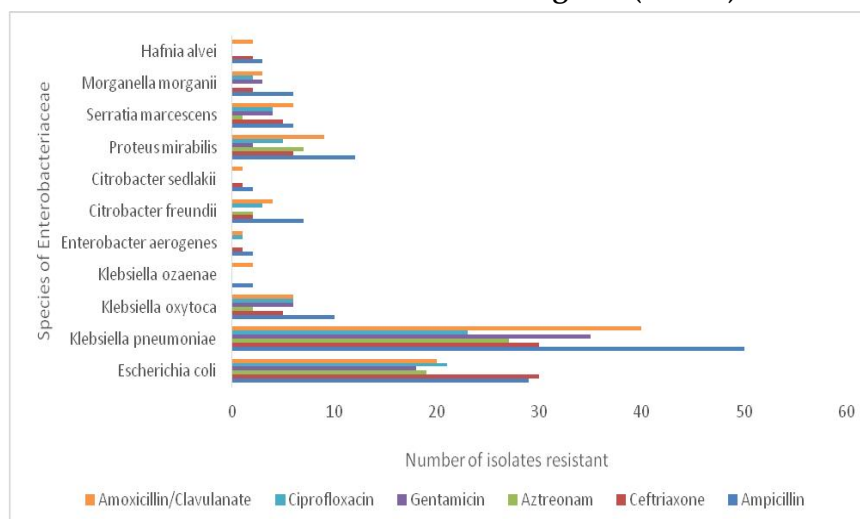
Figure 1, 2 and 3 showed the general pattern of resistance, intermediate sensitivity and sensitivity respectively for the isolates of *Enterobacteriaceae* to the tested antimicrobial agents. There was

demonstrable resistance of *Escherichia coli* to ceftriaxone and ampicillin in 30(49.2%) and 29 (47.5%) of the tested isolates respectively. The highest resistance for *Klebsiella pneumoniae* and *Klebsiella oxytoca* was with Ampicillin with 50(68.5%) and 10(55.6%) respectively. The highest resistance for *Klebsiella ozaenae* of 2(75.0%) and 2(66.1%) was detected in amoxicillin/clavulanic acid and ampicillin. *Enterobacter aerogenes* has the highest resistance of 2(28.6%) for ampicillin and the lowest of 0(0%) for both aztreonam and gentamicin. The highest resistance for *Citrobacter freundii* and *Citrobacter sedlakii* were both in ampicillin of 7(58.3%) and 2(100%) respectively. The highest resistance for *Proteus mirabilis* were in ampicillin with 12(57.1%) and the lowest resistance of 2(9.5%) was recorded in gentamicin. There was demonstrable resistance to all the tested antimicrobial agents for *Serratia marcescens* with aztreonam having the lowest resistance of 1(8.3%) in the isolates. All the strains of *Morganella morganii* tested were resistant to ampicillin with a value of 6(46.2%). The highest resistance of 3(100%) for *Hafnia alvei* was recorded in ampicillin while aztreonam, gentamicin and ciprofloxacin has the lowest resistance of 0(0%) in the tested isolates.

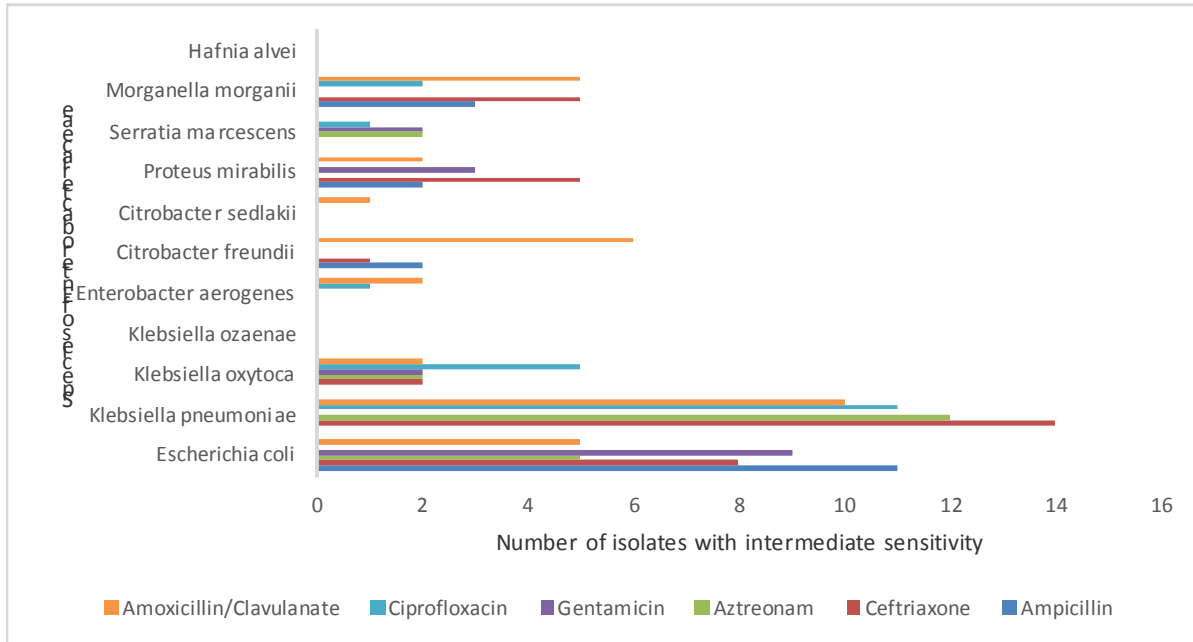
**Table 1: Distribution of species of Enterobacteriaceae isolated**

Species of Enterobacteriaceae	Frequency (Percentage)
<i>Klebsiella pneumoniae</i>	73(32.4%)
<i>Escherichia coli</i>	61(27.1%)
<i>Proteus mirabilis</i>	21(9.3%)
<i>Klebsiella oxytoca</i>	13(5.8%)
<i>Morganella morganii</i>	12(5.3%)
<i>Citrobacter freundii</i> ,	12(5.3%)
<i>Enterobacter aerogenes</i>	7(3.1%)
<i>Klebsiella ozaenae</i>	3(1.4%)
<i>Hafnia alvei</i>	3(1.4%)
<i>Citrobacter sedlakii</i>	2(0.9%)

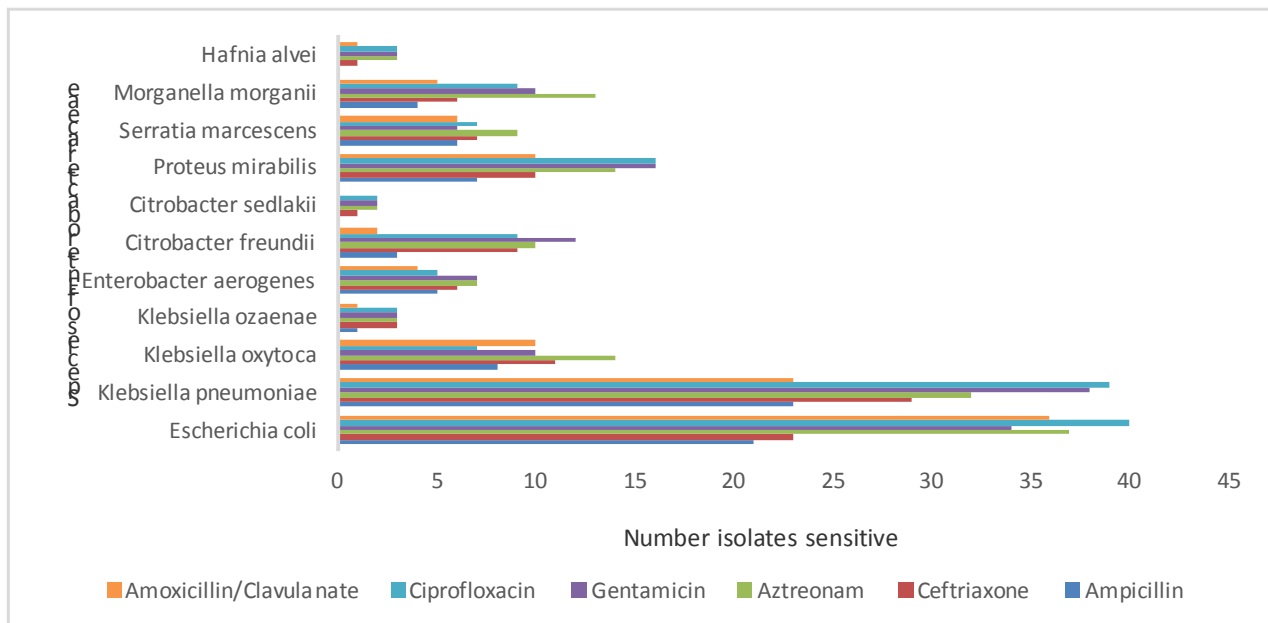
**Figure 1: General pattern of the Enterobacteriaceae isolates that were resistant to the tested antimicrobial agents (N=225)**



**Figure 2: General pattern of the *Enterobacteriaceae* isolates with intermediate susceptibility to the tested antimicrobial agents (N=225)**



**Figure 3: General pattern of the *Enterobacteriaceae* isolates that were sensitive to the tested antimicrobial agents (N=225)**



## DISCUSSION

In this study, the general susceptibility pattern of *Enterobacteriaceae* in the north-eastern part of Nigeria was established with the finding of highest resistance of *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Klebsiella ozaenae* observed to ampicillin, amoxicillin/clavulanic acid and ceftriaxone. This finding is in conformity with the finding of Iroha in Enugu state, Nigeria.<sup>8</sup>

The high resistance to ampicillin may be due to the fact that these antibiotic classes of drug are often available over the counter and are affordable which leads to their widespread use hence creating selective pressure for antimicrobial resistance. This study also recorded a high resistance of *Escherichia coli* to ampicillin and ceftriaxone. The finding of high resistance to ampicillin and ceftriaxone of the *Escherichia coli* strains observed from this study is in conformity with the work of Wariso and Ibe from Port Harcourt, Nigeria and also Kibret and Abera in Ethiopia.<sup>9-10</sup>

The clinical implication of this is that ampicillin and ceftriaxone may be ineffective as an initial therapy for *Enterobacteriaceae* isolates in our patients. Even though, other studies<sup>11</sup> have shown documented resistance of *Enterobacter aerogenes* to cephalosporins, this study finds a low resistance to that class of drug for *Enterobacter aerogenes*. This study records the finding of high resistant of ampicillin and cephalosporins to *Citrobacter freundii* and *Citrobacter sedlakii*. Sofie and colleagues have shown that *Citrobacter freundii* are generally resistant to ampicillin but sensitive to amoxicillin and cephalosporins while *Citrobacter sedlakii* are generally resistant to ampicillin, amoxicillin and cephalosporins.<sup>12</sup>

*Serratia marcescens* has been widely reported as multi drug resistant from numerous studies, similarly in this study *Serratia marcescens* was resistant to all the tested agents except for aztreonam with the lowest resistance in the tested isolates.<sup>13</sup> The strains of *Morganella morganii* tested from this study were resistant to ampicillin but sensitive to aztreonam and gentamicin. Similarly, Caroline and colleagues have observed *Morganella morganii* to have a low resistance to most of the beta lactam agents they tested in addition to ciprofloxacin and gentamicin.<sup>13</sup>

The low resistance of *Hafnia alvei* to aztreonam, gentamicin and ciprofloxacin in this study is similar to a study done by Sharon and colleagues where a high sensitivity of *Hafnia alvei* and *Hafnia paralvei* was recorded to quinolones, aminoglycosides, carbapenems and monobactams.<sup>14</sup>

## CONCLUSION

This study demonstrated a pan resistant of *Enterobacteriaceae* family to mostly the beta lactam agents like ampicillin and ceftriaxone, except for aztreonam which has the lowest resistance. Gentamicin and Ciprofloxacin can be used with caution.

A large multi-center study is recommended to validate these findings. Summarily, all stakeholders must come together to address this rising spade of antimicrobial resistance in our community by aggressive healthcare provider's education, antimicrobial stewardship and infection control practices.

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