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Molecular Detection of AMPCs Producing Enterobacterales from Patients Attending Some Hospitals in Bauchi State, Northern, Nigeria

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Abstract

The burden of antimicrobial resistance (AMR) is rapidly growing across antibiotic classes, with an increase in detection of isolates resistant to cephalosporins. Data regarding the incidence of AmpC-producing enterobacterales (CRE) is still lacking. It is therefore pertinent to encourage surveillance studies along this line with epidemiological purposes, thus prompting the current work. The study aimed at establishing the prevalence of AmpC-producing Enterobacterales among patients attending some hospitals in Bauchi state, Nigeria. A prospective cross-sectional study was carried out in which 382 samples were collected from consented and assented patients attending six different hospitals in Bauchi state, Nigeria. The samples include Urine, Sputum, Catheter tips and Wound swabs. The isolates were isolated and confirmed using standard techniques based on their cultural morphology, Gram staining and were confirmed biochemically using API20E strips. Susceptibility profiles of the isolates were determined by the disc diffusion technique according to Clinical Laboratory Standards Institute (CLSI). Isolates resistant to one or more cephalosporin antibiotics were selected for the detection of AmpC Enzymes by the Disc approximation assay and were confirmed using molecular techniques. Out of 382 samples, the study isolated 205(53.6%) Enterobacterobacterales. The most frequently detected pathogens include *Escherichia coli* (47.8%), *Klebsiella pneumoniae* (36.0 %), *Citrobacter freudii* (5.3%), *Proteus mirabilis* (2.9%), *Serratia marcescens* (2.9%), *Morganella morganii (*1.9%), *Hafnia alvei* (0.9%), and *Enterobacter aerogenes* (1.9%). Highest resistance was recorded for Ampicillin (68.3%), followed by Ciprofloxacin (39.4%) and Amoxicillin-clavulanate (47.3%), while Imipenem exhibited the lowest resistance (9.2%). Among the 205 Enterobacterales isolates, 51.7% were multidrug-resistant isolates. Seventy (70) species were found to be AmpC producers, with only 12 isolates confirmed by polymerase chain reaction (PCR). These results emphasize the importance of continuous screening and surveillance programs for the detection of Betalactamase resistance genes in enteric bacteria of public health importance.

Keywords: *Beta-lactamases; AmpC; Enterobacterales,· Antibiotic resistance*

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1. Introduction

Increasing resistance of Gram-negative bacteria to β-lactam antibiotics currently represents one of the main concerns worldwide (Mahmud et al., 2024). The primary mechanism of resistance is the production of β lactamase enzymes, which have the ability to hydrolyze β-lactams antibiotics. Over the past 30 years, bacteria from the otder Enterobacterales have increasingly developed enzymes that can break down expanded-spectrum cephalosporins, posing a serious threat to human health. These resistant strains have now become endemics in many countries. AmpC-type β-lactamases represent the two groups of β-lactamases that

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play a key role in expanded-spectrum cephalosporins resistance, but display several peculiarities between each other (Meini *et al*., 2019). AmpCs are naturally occurring enzymes encoded by chromosomal genes in certain Enterobacterales species, but they can also be acquired through plasmids, making them more easily transferable; These two scenarios are distinct from both a microbiological and clinical perspective. Plasmid-mediated AmpCs (pAmpCs) have spread widely among Enterobacterales, but their overall prevalence remains significantly lower than that of extended-spectrum β-lactamases (ESBLs) (Manandhar et al., 2020).

Enterobacterales are common causes of both community- and hospital-acquired infections, including lower respiratory tract infections, ventilator-associated pneumonia, urinary tract infections with catheter-related bloodstream infections, and intensive care unit-acquired sepsis, including surgical site infections (Somda et al., 2024). Infections caused by gram-negative bacteria resist the most commonly prescribed antibiotics by acquiring genes encoding multiple antibiotic resistance mechanisms; examples include extended-spectrum β-lactamases (ESBLs), AMPCs, and carbapenemases (Rana et al., 2024). Currently, Enterobacterales resistance is on the rise, resulting in infections that are difficult to manage and whose effects cannot be simply estimated (Kawa et al., 2023). A systematic review conducted in Nigeria revealed significant resistance to several commonly prescribed drugs used for treating urinary tract infections, gastrointestinal infections, and systemic infections in the country (Mohammed et al., 2015). However, high rates of resistance to cephalosporins (74%) and fluoroquinolones were reported in some part of the country (Okesanya et al., 2024). Most organisms exhibited complete (100%) resistance to ampicillin and cotrimoxazole, both of which have traditionally been used as first-line treatments for urinary tract infections (Zhang et al., 2024). Betalactam antibiotics are one of the most commonly prescribed antibiotics for the treatmen of community and hospitals acquire bacterial infections cause by both gram positive and gram negative bacteria, an example includes Penicillin, Cephalosporins, Carbapenem and Monobactam (Uwanibe et al., 2024). Many Bacterial pathogens are resistant to penicillin and Cephalosporins antibiotics due to the acquisition of resistance genes such as AmpC and have been detected among the order Enterobacterales not limited to the family Enterobacteriaceae. AmpC β-lactamases belong to class C enzymes according to the Ambler classification system and possess serine residues at their active site, which are essential for catalysis (Richter et al., 2024). The resistance mechanisms of AmpC β-lactamases are categorized into three types: (1) inducible resistance mediated by chromosomally encoded *ampC* genes found in bacteria such as *Enterobacter cloacae, Serratia marcescens, Citrobacter freundii*, and *Pseudomonas aeruginosa*; (2) non-inducible chromosomal resistance arising from promoter or attenuator mutations, commonly observed in *Escherichia coli, Shigella species*, and *Acinetobacter baumannii*; and (3) plasmid-mediated resistance, which occurs in pathogens like *Klebsiella pneumoniae, Escherichia coli,* and *Salmonella* species (Olalekan et al., 2020). Exposure to β-lactam antibiotics can activate a series of molecular processes that lead to elevated AmpC enzyme production, conferring β-lactam resistance even in bacterial strains that were initially susceptible. In almost every nation of the world, including Nigeria, reports of the spread of ampicillin and carbenicillin (AmpC) resistance among Enterobacterales are rising, which is not encouraging for infection prevention (Al-Abdely et al., 2021). Numerous studies have examined the incidence and prevalence of AmpC resistance among Enterobacteria in African nations. Study from Uganda reported a prevalence of 23.3% (53/270) among pathogenic *Klebsiella pneumoniae* isolates (Medugu et al., 2022). In Bauchi State, the incidence and prevalence of AmpC and their distribution among those Enterobacterales family from six different Hospitals studied were unknown. Infection caused by these resistant bacteria fails to respond to standard treatment, resulting in prolonged hospital stays, disability, and a greater risk of death due to the delay in the administration of effective treatment. The treatment of these patients should be timely, aggressive, and rapidly efficacious. However, therapeutic options are obviously limited (Dubey et al., 2024). This study assists the public health sector in prevention and control of AmpC producing Enterobacterales infections. Knowing which antibiotic has the highest resistance rate can inform the ideal choice of antibiotic for treatment. This study provides a summary of AmpC producing Enterobacterales that established from clinical samples in Bauchi state, Nigeria and which antibiotics they were susceptible to and how this developed over time. It can also assist the State government in reducing the purchases of antibiotics that are not sensitive, thereby saving millions of naira, which will help the state's economy.

2. Methodology

2.1 Study design

This study uses two approaches which includes qualitative and quantitative approach, in the quantitative approach cross sectional survey was carried out as described by (Abad-Fau et al., 2024**).**

2.2 Study area

The study was carried out in Bauchi State. The state is located at altitude 10º30'N 10º00'E. The state has total land area of 49,596km^2 with a projected population of 8,308,800 in 2022. The state comprises of three senatorial zone; Bauchi north, Bauchi south and Bauchi central.

2.3 Study population

The study population includes hospitalized and non-hospitalized patients of all age groups.

2.3.1 Inclusion criterion

Patients sent to the Microbiology laboratory for suspected cases of Urinary Tract Infection, Respiratory Tract Infection or wound infection and who was consented.

2.3.2 Exclusion criterion

Those participants that did not agree to participate in the study was excluded.

2.4 Sampling techniques
A stratified random sampling technique were employed in which the population is divided according to variation of the geographical locations Bauchi central (General Hospital Ningi& General Hospital Darazo), Bauchi North (General Hospital Azare& General Hospital Shira) Bauchi south (General Hospital Dass& Abubakar Tafawa Balewa University Teaching Hospitals Bauchi) and each sample is selected randomly in proportion to the size of the population.

2.5 Ethical considerations
The protocol for this study was submitted to the Health Research Ethics Committee of Bauchi State Nigeria, for review and approval before the commencement of data collection.

2.6 Sample collection
Three hundred and eighty-two clinical samples were collected aseptically as described by Jack *et al.*, (2018), from both inpatients and out-patients of the General Hospital across the state, namely: Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), General hospital Ningi (GHN), General Hospital Azare (GHA), General Hospital Dass (GHD) General hospital Shira (GHS) and General hospital Darazo (GHDR). The sample includes; urine, wound swab, Catheter tips Urethral swab.

2.7 Cultural Identification
Sputum was considered acceptable for the culture if it contained <25 epithelial cells per lowpower field and >25 polymorphonuclear leukocytes. Sputum and wound samples were cultured on chocolate agar, blood agar and macconkey agar, and then incubated in a 5% CO2 incubator at 37°C for 24 hours. In dwelling medical devices and urine samples were cultured on MacConkey agar and blood agar then incubated at 37°C in an incubator for 24 hours (Mohammed *et al.*, 2017). Sputum was considered acceptable for the culture if it contained <25 epithelial cells per low-power field and >25 polymorphonuclear leukocytes. Sputum and wound samples were cultured on chocolate agar, blood agar, and Macconkey agar and then incubated in a 5% CO2 incubator at 37°C for 24 hours. In dwelling medical devices, urine samples were cultured on MacConkey agar and blood agar, and then incubated at 37°C in an incubator for 24 hours (Mohammed *et al.*, 2015).

2.8 Gram reaction
Positive growth after culture were gram stained using gram staining technique and all Gram-positive bacteria are stained violet or purple and Gram-negative bacteria don't retain the primary stain (crystal violet) due to their less complex morphology of their cell wall, which make them appear pink or red (the colour of safranin, the counter stain).

2.9 Biochemical Characterization of the family Enterobactarales
Biochemical characterization of the isolates was performed using commercially biochemical kit, Analytical profile index which contain the substrates in micro wells to identify the family of enterobacteriaceae (Mohammed *et al.*, 2015).

2.9.1. Oxidise Test
The oxidase test is a technique for detecting the presence of the terminal enzyme system in aerobic respiration called cytochrome C oxidase, or cytochrome A. Typically, bacteria in the *Enterobacterales* family yield a negative result, while species like *Pseudomonas spp.*, *Aeromonas spp.*, *Vibrio spp.*, and *Neisseria spp.* produce a positive result. The oxidase test is employed to detect bacteria that produce cytochrome c oxidase, an enzyme involved in the bacterial electron transport chain. When present, this enzyme oxidizes the reagent (tetramethyl-p-phenylenediamine dihydrochloride), resulting in the formation of indophenols, a purple or dark blue end product. In the absence of the enzyme, the reagent remains colorless, indicating a negative result.

2.10 Antibiotic Susceptibility testing of Enterobacterales
Antibiotic Susceptibility testing of enterobacterales isolates were carried out using the disc diffusion method as described by CLSI, 2020. The antibiotics used (Oxoid, Ltd. Basingstoke, UK)) were as follows: Ceftazidime (30 µg), Cefotaxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ampicillin (10 µg), Augmentin (30 µg), Imipenem (10 µg) and Meropenem (10 µg) (Le Page *et al.*, 2016; CLSI, 2020).
Standardized inoculums equivalent to 0.5 McFarland standards (1.5×10^8 cfu/mL) was achieved and a sterile swab is submerged in the suspension and inoculated the entire surface of the Müeller Hinton agar plate three times by rotating the plate 60 degrees between each inoculation. The inoculum is allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs were placed on the nine aforementioned antibiotics (Oxoid, UK) were carefully and firmly placed on the surface of the agar using sterile forceps. Afterwards, the plates were incubated at 37 °C for 18-24 hours. After incubation, the zones of inhibition around the antibiotics were measured Base on CLSI criteria (CLSI, 2020). *P. aeruginosa* ATCC 27853 and *Escharicia coli* ATCC 25922 were used as a routing quality control for aminoglycoside, carbapenem and Betalactam combination agent respectively (CLSI, 2020). Resistance patterns of bacterial isolates were determined. Isolates were suspected as AmpC producer when resistance to one or more cephalosporins antibiotics (Ajigbewu et al., 2024).

2.10.1 AmpC screening and test
All isolates were tested for AmpC production by assessing their susceptibility to a 30µg cefoxitin disc. Isolates showing an inhibition zone diameter of ≤14mm were considered AmpC positive. The detection of AmpC producers was carried out using the AmpC disc test. On a MHA plate *E.coli* ATCC 25922 was inoculated with a lawn culture method and allowed it to dry and a moistened blank disc with sterile normal saline was inoculated with few colonies of test strain and then placed next to a cefoxitin disc (30 µg) already placed on MHA plate. An indentation in the cefoxitin inhibition zone near the disc containing the test strain was regarded as an indication of AmpC production.

2.11 Genotypic confirmation of AmpC genes

2.11.1 Primer Design

Primers was designed using the NCBI BLAST (Basic Local Alignment Search Tool) program to detect AmpC *β*-lactamase genes (blaCMY-2). The sequences of the prima were CCAGAACTGACAGGCAAACA for CMY-2-F and CCTGCCGTATAGGTGGCTAA for CMY-2-R.

2.11.2 DNA extraction

The DNA template was prepared (Accu prep Genomic DNA extraction kit from Bioneer) by taking 20 µl of Proteinase K and added to a clean 1.5 ml tube. A 200 µl of bacterial cells was added to the tube containing proteinase K then 200 µl of Binding buffer (GC) was also added to the sample and mix immediately by vortex mixer.

2.11.3 Preparation of the DNA template

The preparation of the DNA template from bacterial suspension using Accu power Hotstart PCR premix, Bioneer.

3. Results and Discussion

Out of 382 samples the study isolated 205(53.6%) Enterobacterobacterales. The five most frequently occurring pathogens were *Klebsiella pneumoniae* (36.0 %), *Morganella morganii (*1.9%), *Escherichia coli* (47.8%), *Proteus mirabilis* (2.9%), *C. freudii* (5.3%), *H. alvei* (0.9%), *S. marcescens* (2.9%) and *Enterobacter aerogenes* (1.9%) as shown in **table 1**.

The resistance profile of Enterobacterales among 205 species of *Enterobacterales* isolates, Ampicillin was (68.3%), followed by Ciprofloxacin (39.4%), Amoxyclav, (47.3%), Ceftazidime (27.9%), Meropenem (12.6%), Gentamycin (42.4%), Cefotaxime (40.5%, Imipenem (9.2%), Cefoxitin (28.2%), ciprofloxacin (25.8**%) table 2.**

The multidrug resistance profile shows that one hundred and six Enterobacterales isolates (51.7%) were resistant to two or more classes of antibiotics. The multidrug resistance isolates include 58 *E. coli*, followed by 32 *Klebsiella pneumoniae* followed by 2 *shigella spp*, 6, *E. aerogenes*, 2 *P. mirabilis*, 2 *S. marcescens* and 4 *Morganella morganii* **table 3.**

AmpC -producing strains were confirmed phenotypically from 382 samples collected catheter tips have the highest frequency of occurrence with 34.3% and the least was observed from wound swabs (13.4%), the species identified to be AmpC were *S. marcescens* 40%, *Escherichia coli (*34.7%), *Klebsiella pneumoniae* (24.0%), and *Enterobacter aerogenes* (25%).

The prevalence of AmpC from the six different hospitals studied was shown in **table 4**. Abubakar Tafawa Balewa University Teaching Hospital Bauchi, which is a tertiary health care facility harbored the highest percentage of AmpC with 37(24.%) followed General Hospital Dass which is a secondary health care facility with 14 (18.7%), General Hospital Ningi 7 (13.3%) General Hospital Shira 3(12.9%) General Hospital Azare 2(6.2%), and the least prevalence rate was observed at General Hospital Darazo 2(5.7%).

Table 1. Prevalence of *Enterobacterales* from various clinical samples collected in Bauchi State, Nigeria (2021)

Enterobacterales	Urine	Wound swab	Catheter tips	Urethral swab	Total	Percentage
E. coli	70	0	21	7	98	47.8
K. pneumoniae	60	0	11	4	75	36.0
E. aerogenes	3	1	0		4	1.9
C. freundii	10	0	1	0	11	5.3
P. mirabilis	3	0	1	2	6	2.9
S. marcescens	5	0	0	0	5	2.9
M. morganii	4	0	0	0	4	1.9
H. alvei	2	0	0	0	2	0.9
Total	157	1	34	13	205	

Table 2: Antibiotics Susceptibility Profile of Enterobacterales from the study area.

				Number in parenthesis indicate percentage out of total number of *Enterobacterales* isolates		
S/N	**Antibiotics**	**Code**	**Concentration**	Sensitive	Intermediate	Resistance
1	Ampicillin	AMP	10 µg	61 (29.7)	4 (1.9)	140 (68.3)
2	Amoxy-clav	AMC	20 µg	107 (52.1)	1 (0.4)	97 (47.3)
3	Cefoxitin	FOX	10µg	144(70.2)	3 (1.4)	58 (28.2)
4	Cefotaxime	CTX	30 µg	122 (59.5)	1 (0.4)	82 (40.5)
5	Ceftazidime	CAZ	30 µg	131 (63.9)	4 (1.3)	88 (27.9)
6	Gentamicin	GEN	10 µg	113 (55.1)	1(0.4)	87 (42.4)
7	Ciprofloxacin	CIP	5 µg	151 (73.6)	1(0.4)	53 (25.8)
8	Imipenem	IPM	10 µg	185 (90.2)	1(0.3)	19(9.2)
9	Meropenem	MEM	10 µg	162 (88.7)	0(0.0)	26 (12.6)

Table 3: Sample-wise distribution of AmpC producing Enterobacterales

Samples	Frequency	AmpC Positive	Percentage (%)
Urine	198	28	14.1
Sputum	82	11	13.4
Wound	52	16	30.7
Catheter tips	35	12	34.3
Urethral swabs	15	3	20.0
Total	382	70	18.3

Table 4: Species wise distribution of AmpC producing Enterobacterales

Isolates	Frequency	AmpC Positive	AmpC gene (%)
E. coli	98	34(34.7)	4(11.7)
K. pneumonia	75	18(24)	6(33.3)
E. aerogenes	4	1(25)	0(0.0)
C. freundii	11	6(54.5)	2(0.0)
P. mirabilis	6	0(0.0)	0(0.0)
S. marcescens	5	2(40)	0(0.0)
M. morganii	4	0(0.0)	0(0.0)
H. alvei	2	0(0.0)	0(0.0)
Total	205	61(29.7)	12(19.6)

Table 5: Hospital-wise distribution of AmpC

Isolates	Frequency	AmpC positive	Percentage
ATBUTH	154	37	24.0
GHD	75	14	18.7
GHN	15	2	13.3
GHDR	52	3	5.7
GHS	54	2	12.9
GHA	32	2	6.2
Total	382	70	18.3

This study shows that out of the total Enterobacterales isolated ATBUTH has the highest prevalence of *Enterobacterales* (30.5%) with general Hospital Shira having the lowest (5.7%) These could be because the former is the tertiary health care facility with high number of patients visiting the Hospital while the latter is the secondary heath care facility with few numbers of patients. The emergence and spread of AmpC producing *Enterobacterales* (AmpC) have become an increasing concern for healthcare services worldwide, especially when mediated by transferable genes (Mohammed *et al.*, 2017). Infections caused by these bacteria have been associated with significant morbidity and mortality and treatment options have been limited (Nkengkana et al., 2023).

The following resistance profile was observed from this study 68.3% 47.3%, 42.4 25.8%, Ampicillin, Amoxy-clav, Gentamicin and Ciprofloxacin respectively but susceptible to the imipenem (90.2%) and Meropenem (88.7%). This is due to the fact that the hydrolysis rate for fourth-generation cephalosporins (e.g., cefotaxime and ceftaxidime) and carbapenems (e.g., imipenem and meropenem) is very low, so that susceptibility to these drugs is usually maintained and highly stable against most β-lactamases, and retains *in vitro* activity against AmpC-producing *Enterobacterales(Chelaru et al., 2024;*Sivarajan et al., 2025). The present study demonstrated that only 28.2% of the AmpC -producing strains carries the resistance gene that mediate to cefoxitin resistance; this disagrees with the study of (Ibaideya et al., 2024) in Tanzania who reported that 100% of Enterobacterales that produce AmpC were positive for the ampcillinase resistance gene (bla CMY). The remaining 71.8% of isolates were negative for the genes in spite of resistance to cefoxitin antibiotics, this is due to the fact that these bacteria may contain some resistance genes other than AmpC genes, or the bacteria may have other mechanisms of resistance such as drug efflux and porin loss.

4. Conclusion

This study highlights the significant prevalence of AmpC-producing Enterobacterales among patients attending selected hospitals in Bauchi State, Northern Nigeria. Among the 205 isolated Enterobacterales, 70 species were identified as AmpC producers, with only 12 isolates confirmed by polymerase chain reaction (PCR). The high resistance to commonly used antibiotics, particularly ampicillin (68.3%), ciprofloxacin (39.4%), and amoxicillin-clavulanate (47.3%), raises concerns about the effectiveness of conventional treatment regimens. The detection of multidrug-resistant strains (51.7%) underscores the urgent need for continuous

surveillance, antimicrobial stewardship programs, and stringent infection control measures. These findings contribute to the growing body of evidence on antimicrobial resistance in Nigeria and emphasize the necessity for molecular screening in routine diagnostic practices to mitigate the spread of resistant bacterial strains.

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Conflicts of Interest
Authors declare that there is no conflict of interest

Ethical approval
Ethical clearance was obtained from the Ethics Committee of Bauchi State Ministry of Health. Written informed consent was obtained from all participants prior to sample collection, ensuring confidentiality and compliance with ethical research standards.

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