Emerging Multidrug Resistant Ampc Beta-Lactamase and Carbapenamase Enteric Isolates in Abeokuta, Nigeria

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Abstract: Rapid emergence of multidrug co-resistant AmpC β-Lactamase and Carbapenamase Enteric Isolates in Abeokuta, Nigeria was investigated. A total of 426 faecal samples was obtained and tested for **AmpC** ß-lactamases using Inhibitor based test method and carbapenamase production. Their antibiotics susceptibility to various commonly used antibiotics was evaluated accordingly. Only 5(45.5%) express AmpC beta-lactamase among hospital patients while 17/59(63.0%) from community residents. Carbapenamase producing ESBL isolates among the hospital patients shows 0.76% rate compare to 3.3% recorded among the community residents. High resistant of 100% was recorded in some enteric isolates such as *Klebsiella spp*, *Pseudomonas spp* and *Proteus spp* to ampicillin while 81.0%, 67.5% and 63.0% were resistant to ceftazidime, azithromycin and cefuroxime (MIC≤8). Proliferation of multi-drug resistance AmpC beta-lactamase and carbapenamse strains may become predominant in our health care facilities and in the community as a result of the indiscriminate use of antimicrobial agents and fecal carriage among the people who serve as risk factor, and thereby making formulation of public health guidelines unachievable.

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INTRODUCTION

AmpC ß-lactamase had been known to be cephalosporinases and are resistant to beta-lactamase inhibitors but not to the 4th Generation Cephalosporins (4GC) (eg. cefepime). They (AmpC ß-lactamases) are clinically important because they could confer resistance to a wide variety of ß-lactam drugs, including α-methoxy-ß-lactams, such as cefoxitin, narrow-, expanded-, and broad-spectrum Cephalosporins, ß-lactam-ß-lactamase-inhibitor combinations, and aztreonam while Group 1 AmpC ß-lactamases are poorly inhibited by clavulanic acid. However, they are inhibited by cloxacillin (1). AmpC is normally produced in low levels by many organisms but it can be produced at high levels and cause resistance to all beta-lactams, except carbapenems and 4th Generation Cephalosporins (2). This AmpC ß-lactamase gene is commonly found on the chromosome of *Enterobacter spp*, *Hafnia alvei*, *Morganella morganii*, *Citrobacter freundii*, *Serratia marcescens*, *Providencia spp*, *Aeromonas spp*, and *Pseudomonas aeruginosa* (3). It was observed that chromosomal AmpC ß-lactamases can be induced when exposed to agents, such as cephamycins (i.e cefoxitin), while ampicillin and carbapenems are constitutively (3). However, plasmid-mediated AmpC ß-lactamases had been found in organisms that do not carry the chromosomal AmpC such as *E coli*, *Klebsiella spp*, *Proteus spp* and *Salmonella spp* (4). Many nosocomial enteric isolates which were plasmid-mediated AmpC ß-lactamase producers have been reported to be involved in several worldwide outbreaks of infections (5) and even confer resistance to the carbapenems (6,7). Patients colonized with Carbapenem-resistant Enterobacteriaceae (CRE) are thought to be a source of transmission in the healthcare setting, especially the asymptomatic colonization with carbapenem-resistant *Klebsiella pneumoniae* among intensive care unit patients (8) but identifying such patients who are colonized with CRE and placing these patients in isolation, may be an important step in preventing its transmission. Continue emergence of CRE among Gram negative bacilli usually occurs when an isolate acquires a carbapenemase resistance gene or its produces an extended-spectrum cephalosporinase, such as an AmpC-type β-lactamase, in combination with porin loss and of such common isolate is the *Klebsiella pneumoniae* carbapenemase (KPC) (9). In view of this, AmpC ß-lactamase and ESBL-producing isolates are becoming prevalent in our society despite various epidemiological measures implemented to curtail and control this spread, while the rate of in-patients, out-patients and healthy volunteers colonised with CRE and AmpC ß-lactamase producers has not been addressed in some national studies.

Recently, studies have shown that faecal carriage and colonisation with ESBL-producing bacteria is a risk factor for acquisition of severe enteric infections caused by these pathogens and a potential source of transmission among households (10,11). Acquisition of resistant strains, are generally found in post treatment with broad-spectrum cephalosporins or through acquisition of a resistant strain via nosocomial transmission. Therefore, self medication with the use of antibiotics can considerably accelerate the selection pressure for diversification and dissemination of mutant extended-spectrum beta-lactamses (12, 13). **Therefore,** the risk factor for the continue emergence and spread of AmpC ß-lactamase and CRE enteric isolates are investigated in this locality.

MATERIALS & METHODS

Study population: This study surveyed the 479 faecal samples of patients attending the major hospitals serving as major health facilities in the Abeokuta metropolis and randomly selected community residents who have not been on antimicrobial agents in the preceding two weeks either as therapy for gastro-intestinal complication or prophylaxis.

Isolation: All the faecal samples collected were cultured within 2 hours on Chocolate agar containing 1mg/L Ampicillin (Palmer et al, 2000), Salmonella-Shigella agar and MacConkey agar without salt (Oxoid CM 516, UK); and were incubated at 37^{O}C for 18-24hours. **The isolates obtained were** identified according to the modified method of Stokes protocol (1998) and **further characterisation was done according to WHO manual for laboratory investigation of acute enteric infections (1983) (14, 15).**

ß-Lactamase test: All isolates were tested for ß-lactamase production by a starch-iodide paper acidometric method as described by Odugbemi et al (16).

Carbapenemase Detection: Carbapenemase activity of the isolates was detected using Modified Hodge Test (MHT) method described by Landman et al, (2005) (17) as it was recommended by CLSI (18) using 0.5 McFarland suspension of each isolates spread on Muller-Hinton Agar with 10-µg Imipenem disc placed at the centre of the plate touching each streaked isolates and incubated overnight at incubated at 37^{O}C for 18-24hours.

AmpC ß-lactamase detection (*Inhibitor based test)*: All isolates were tested for AmpC ß-lactamase production with discs containing cefoxitin (30µg) and another containing Boronic acid/cefoxitin (400µg/30µg) which was placed at 30mm distance on the agar (19). Similarly, discs of ceftazidime (30µg) and ceftazidime- clauvulanic acid (30/10 µg) were also placed on the medium at a distance of 30 mm. Inoculated plates were incubated overnight at 37°C. An organism demonstrating a zone diameter around the disk containing cefoxitin and Boronic acid >5mm, the zone diameter around the disk containing cefoxitin alone were considered an Amp C producer.

Antibiotic susceptibility testing: Susceptibility testing was performed on Mueller-Hinton (MH) agar plates by the disk diffusion method (Diagnostics Pasteur, Paris, France). Antibiotics tested were Ampicillin (10µg), Amoxicillin/clavulanic acid (20µg/10µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Cefuroxime (30ug), Gentamicin (10ug), Ciprofloxacin (5µg), Pefloxacin (10ug), Tetracycline (30ug) and Azithromycin (5ug) using 0.5 McFarland pure culture suspension. The inhibition zones were interpreted according to CLSI guidelines using Kirby-Baure method (18). Standard broth micro-dilution method was used to determine the **Minimum Inhibitory Concentration (MIC)** of each identified isolates to the following antibiotics dilution ranges; Ampicillin (0.5–64 ug/mL), Augumentin (0.5-32 ug/mL), Gentamycin (0.5–64 ug/mL), Ciprofloxacin (0.5–64 ug/mL), Cefuroxime (1–64 ug/mL), Ceftazidime (0.25–128 ug/mL), Ceftazidime/Clavulanate (0.25/4–128/4ug/mL), Tetracycline (0.25–64 ug/mL), Cefotaxime/Clavulanate (0.25/4–64/4 ug/mL) and Imipenem (0.25–16 ug/mL).

Statistical Analysis: Significance of AmpC ß-lactamase producing isolates and carbapenamse producers were determined using T-test, at p value <0.05 at confidence interval of 95%. Comparison of resistant rates of different antibiotic proportions was performed using the X^2 method.

Result: Assessment of the risk factor for the continue emergence of the multi-resistance **AmpC** ß-lactamases and CRE enteric isolates in Abeokuta show that, out of 80(30.3%) beta-lactamase enteric isolates obtained, only 5(45.5%) express **AmpC** ß-lactamases among hospital patients while 17(63.0%) out of 59(27.4%) from community residents respectively. Carbapenamase producing ESBL isolates among the hospital patients shows 0.76% rate compare to 3.3% recorded among the community residents (Table 1). Antibiotic susceptibility pattern shoiwn in Table 2; reveal a very high resistant of 100% among some enteric isolates such as *Klebsiella spp*, *Pseudomonas spp* and *Proteus spp* to ampicillin while 81.0%, 67.5% and 63.0% were resistant to ceftazidime, azithromycin and cefuroxime (MIC≤8).The MIC and ESBL results were interpreted in accordance with NCCLS criteria (22).

Table 1. Distribution of ß-lactamase and ESBLs producing enteric isolates obtained from the Hospital patients.

Isolates	HOSPITAL Hospital patients		COMMUNITY	
	AmpC-producer n (%)	Carbapenamase producers n (%)	AmpC-producer n (%)	Carbapenamase producers n (%)
Eschericial coli	5(6.7)	1(1.3)	2(3.2)	3(4.8)
Klebsiella oxytoca	7(13.5)	0(0.0)	1(2.7)	2(5.4)
Salmonella spp	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Citrobacter freundii	2(33.3)	0(0.0)	0(0.0)	0(0.0)
Enterobacter cloacae	0(0.0)	0(0.0)	1(100)	0(0.0)
Pseudomonas aeruginosa	1(1.7)	1(1.7)	1(2.4)	1(2.4)
Proteus mirabilis	1(3.0)	0(0.0)	1(3.2)	0(0.0)
Total	17(6.4)	2(0.76)	5(2.3)	7(3.3)

Table 2. Pattern of resistance of AmpC beta-lactamase and CRE isolates to various antibiotics

Isolate	Antimicrobial agents (MIC >16ug/ml)[a]							
	AMP	AMC	CFX	AZT	CPX	CTX	CFZ	IMP[b]
Eschericial coli	7	1	4	3	2	5	4	2
Klebsiella oxytoca	8	2	7	4	3	6	4	3
Citrobacter freundii	2	0	1	0	0	2	1	0
Enterobacter cloacae	1	0	0	0	0	1	1	0
Pseudomonas	2	1	2	1	1	2	2	0
Proteus mirabilis	2	0	1	1	0	2	2	1
Resistance rate (%)	22(100.0)	4(18.2)	15(67.5)	9(40.5)	6(27.0)	18(81.0)	14(63.0)	6(66.7)

Key: a:break point limit for resistance**; b:**resistance carbapenamase producer**; AMP-***Ampicillin***, AMC** Amoxicilli/Clavulanate**, CFX-**Cefuroxime**, AZT-**Azithromycin**, CPX-**Ciprofloxacin**, CTX-**Cefotaxime**, CFZ-**Ceftazidime,IMP-imipenem

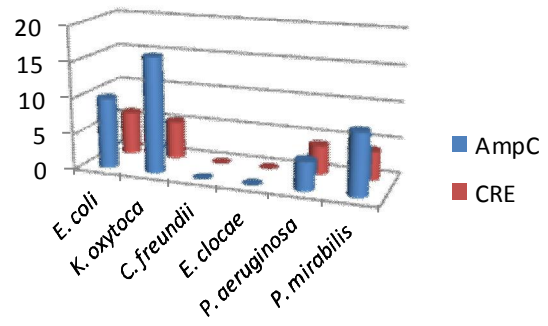

Figure 1. The pattern of distribution of AmpC and CRE enteric isolates obtained

DISCUSION

In the last decade, there has been an increasing rate of co-existence of AmpC ß-lactamase and carbapenamase resistance enterobacteriae (CRE) among pathogenic enteric isolates both in hospitals and the community, which is now becoming a global threat due to various risk factors that promote its prevalence. AmpC ß-lactamase, which is normally produced in low levels by many organisms, was detected in 6.4% with 0.76% CRE isolates obtained from the hospital patients showing an increasing emergence of hospital-acquired AmpC ß-lactamase strains which could be elicited by self-medication, use of unprescribed drug or uncontrolled use of antibiotics. From the survey of the faecal of community residents, 2.3% rate of AmpC ß-lactamase was recorded while only few 3.3% CRE was obtained. This rate reveals stemming rate of antibiotic misuse and unhygienic ways of eating habits of most residents in poorly located food joints, restaurants and street food vendors. Similar incidence was recorded in 18% multi-drug resistant ESBL strains found obtained in sachet-water supplied for drinking in most cafeteria and restaurants in Abakaliki town (20). This poses a grave danger to the community and also suggesting faecal colonisation

with enteric isolates co-existed as AmpC ß-lactamases and CRE producing isolates among food vendors who were also a risk factor for acquisition and a potential source of transmission among households and the community residents patronising their restaurants. Acquisition of resistant strains, are generally common in post treatment with broad-spectrum cephalosporins or via nosocomial transmission as a result of considerable acceleration of the selection pressure due to diversification and dissemination of mutant ESBL (21,22).

Outbreaks of AmpC ß-lactamase enterobacteriaceae caused by these multidrug-resistant strains have been reported in the United States and in many countries in Europe, Asia and South America (23). The outcome of this carbapenamase resistant incidence usually increase mortality in systemic infections while morbidity in terms of lengthy stay in the hospital with severe complications and attributable financial loss. The risk of such adverse outcomes has been found to be higher in patients with infections caused by an antibiotic-resistant organism compared with infections caused by susceptible strains of the same pathogen, even after adjustment to underlying infections.

There was moderate resistance pattern of the AmpC ß-lactamase isolates to various antibiotics (MIC >16ug/ml). Of the 22 AmpC-producing isolates obtained, 9(40.5%) showed resistance to Azithromycin, likewise 4(18.2%) to Augmentin and 6(27.0%) Ciprofloxacin while Cephalosporins 15(67.5%) to cefuroxime, 18(81.0%) Cefotaxime and 14(63.0%) ceftazidime. With the exception of *K. oxytoca* which shows complete susceptibility to imipenem, other isolates shows a multi-resistance activity to more than two antibiotics. However, highly diverse antibiotics resistance rates against all cephalosporin, such as ceftazidime, cefuroxime and cefotaxime show MIC >8µg/mL; representing a very critical situation as compared to investigations from other regions of the world reporting resistance towards first, second and third generations of cephalosporin (24). The spread of antimicrobial resistance among bacterial pathogens to most antibiotics in this locality is fast becoming an important fatal challenge for the community and health institutions due to unguided use of over-the-counter drugs mostly antibiotics. It was observed that the excessive and over-use of cephalosporin antibiotics to various undiagnosed infections could lead to emergence of resistance isolates majorly in the community. The cause for this upsurge in resistant of community-acquired AmpC-producing organisms is not yet clear, but associations with foodstuffs, animal consumption of antibiotics, and frequent patient contact with relatives during illness may be a major risk factor to be considered.

The spread of AmpC ß-lactamase strains of ESBL may continue to proliferate and become predominant in our health care facilities and the community as a result of the indiscriminate use of antimicrobial use and fecal carriage among the people who serve as a risk factor. However, dissemination of antibiotic resistance genes from food-borne pathogens to human emerging infections especially in areas where resources are limited couple with poor antimicrobial surveillance could make formulation of public health guidelines unachievable.

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