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ORIGINAL ARTICLE

Mediate ampc β −lactamases among escherichia coli, klebsiella spp, and proteus mirabilis isolated from urinary tract infection of subjects in delta state university health centre.

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Abstract

The study was carried out to determine mediate AmpC beta-lactamases among Escherichia coli, Klebsiella spp, and Proteus mirabilis isolated from urinary tract infection from symptomatic subjects in the University Health Center Abraka. Midstream urine was collected from 100 patients having urinary tract infection. The urine sample was cultured using MacConkey agar to isolate gram negative organisms. All isolated organisms were identified by standard cultural and biochemical test. The antibiotics susceptibility test was done by using Kirby- Bauer disk diffusion method. Screening test for AmpC beta-lactamase was done using disk diffusion method with cefoxitin disc (30µg). Confirmatory test was done based on phenotypic inhibition test for AmpC beta-lactamase using phenylboronic acid. The occurrence of the uropathogens are E. coli (85%), klebsiella spp (12%) and proteus mirabilis (3%), this shows that E.coli is the most common uropathogen. Highest susceptibility of the isolates was to levofloxacin, ofloxacin, gentamicin, cefepime, nalidixic acid while they show high resistance to cefotaxime, imipenem and augmentin. There was moderate susceptibility to ampiclox, this indicates the prescence of AmpC beta-lactamase as cloxacillin is an inhibitor of AmpC beta-lactamase. The isolates were resistant to penicillins and the cephalosporins except cefepime, this also indicates the presence of the AmpC beta-lactamase enzyme. Zone of inhibition of ≤18mm is considered resistant and organisms that had the diameter of their zone of inhibition ≤18mm were selected for confirmatory test. Organisms with zone of inhibition of the cefoxitin disc (30µg) with phenylboronic acid (300µg) ≥5mm are positive for AmpC beta-lactamase. 80% of Klebsiellaspp, 75% of E.coli and 50% of Proteus mirabilis were positive for AmpC beta-lactamase enzyme. The detection of AmpC beta-lactamase should be practiced as it will contain the spread of organisms possessing this resistance mechanism, help in choosing the right drugs for the treatment and learn about the clinical implications of AmpC beta-lactamase.

Keywords: AmpC, resistant, UTI

1 | BACKGROUND OF STUDY

Urinary tract contaminations are one of the foremost common diseases which are display within the clinic setting and the community. Thirty-five percent of nosocomial contaminations are made up of urinary tract diseases. It has been shown that almost twelve percent of males and forty percent of females have at least one symptomatic infection during their life [1]. Within the structural classification of beta-lactamases mediate AmpC enzymes belong to the C-Class, whereas within the functional classification they relegated to Group one [2]. Genes for AmpC β-lactamases may be plasmid or chromosomal mediated in the members of Enterobacteriaceae. The overproduction of AmpC in conjunction with porin changes of the external layer can lead to a decrease in susceptibility to carbapenems, particularly in plasmid mediated AmpC producers [2]. Although there have been several studies on Mediate AmpC beta-lactamases among Escherichia coli, klebsiella spp and Proteus mirabilis isolated from urinary tract infections there is few literatures on Mediate AmpC beta-lactamases among Escherichia coli, klebsiella spp and Proteus mirabilis isolated from urinary tract infections from both symptomatic and asymptomatic subjects in Delta state.

Mediated AmpC β-lactamases are unused hazard since they present resistance to cephamycins and are not impacted by β-lactamase inhibitors. This resistance instrument has been found around the world, can cause nosocomial episodes, and shows up to be expanding in prevalence [3]. The disturbing increment within the rate at which these bacteria obtain anti-microbial resistance qualities has restricted therapeutic options particularly for UTIs for which broad utilizing of anti-microbials has been seen in both community and healing center settings [4,5]. This emergence of resistant bacteria responsible for urinary tract infections has led to the difficulty in reatment of infections caused by these bacteria leading to increased morbidity and mortality rates. [6]. Consequently, this study is carried out to address to ascertain the predominance of mediate AmpC beta lactamase among Escherichia coli, klebsiella spp and Proteusmirabilis segregated from urinary tract infections in symptomatic and asymptomatic subjects.

The findings of this study is to show the incidence of Mediate AmpC beta- lactamase among Escherichia coli, klebsiella spp and Proteus mirabilis isolate in symptomatic and asymptomatic subjects, and also to contribute to already existing literatures on the prevalence of mediate AmpC beta lactamase among Escherichia coli, klebsiella spp and Proteus mirabilis isolate from urinary tract infections in symptomatic and asymptomatic subjects. The general objective of this study was to determine Mediate AmpC beta-lactamases among Escherichia coli, Klebsiella spp and Proteus mirabilis isolated from urinary tract infections from symptomatic subjects in Delta State University Health Center, Abraka.

2 | MATERIALS AND METHODS

Apparatus and equipment

Autoclave, light microscope, incubator, digital weighing balance. MacConkey agar, CLED agar, nutrient agar, nutrient broth, Mueller Hinton agar, Simmon citrate agar, peptone water, phenylboronic acid, Antibiotics susceptibility disc (CELTECH DIAGNOSTIC MUILTIPURPOSE PACK Belgium Inc. Code no BDR003).

Supplementary information: The online version of this article(https://doi.org/10.15520/arjmcs.v8i09.4 61) Contains supplementary material, which is available to authorized users.

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Method

Collection of sample

A total of one hundred (100) samples were obtained from the midstream urine of human subjects both asymptomatic and symptomatic patients having urinary tract infections in Delta State University Health Centre, Abraka from 1st of November, 2019 to 31st of January, 2020.

Isolation of Bacteria in the Urine

Midstream urine samples were collected from 100 patients both symptomatic and asymptomatic using a sterile universal container. With the aid of a wireloop they were aseptically inoculated on MacConkey and CLED agar plates and labelled. The culture plates were inverted and incubated at 37° for 24hrs and observed for growth through the formation of colonies. The colonies obtained were further inoculated in a nutrient agar slant to obtain purer strain of the test organisms. These pure strains that were stored in slants were taken for identification test.

Preservation of Bacterial Colony

In order to preserve the bacterial colonies gotten from the MacConkey and CLED cultures, slants were made using nutrient agar following the manufacturer's instructions on the label of the agar. The prepared agar together with washed bijou bottles wrapped in aluminum foil was sterilized with the autoclave for 121°C for 15 minutes. After allowing the agar to cool to a temperature of 50 - 55°C, then poured into the sterilized bijou bottles and then kept in a slant position and left to solidify. After which a sterilized wire loop was used to inoculate each bacterial inoculum on the nutrient agar slant and then incubated for 24 hours at 37°C.

Identification Test

Identification test was done using standard microbiological method including gram staining, colony morphology on media, biochemical test as described by Monica [7]

Antibiotics Susceptibility Test

Antimicrobial susceptibility test was performed using disc diffusion method using the following antibiotics: amoxicillin clavulanate (30µg), cefotaxime (25µg), imipenem/cilastatin (10µ), ofloxacin (5µg), gentamicin (10µg), nalidixic acid (30µg), nitrofurantoin (30µg), cefuroxime (30µg), ceftriaxone (45µg), ampiclox (10µg), cefepime (5µg), levofloxacin (5µg).

Mueller Hinton agar was prepared following the manufacturer's directions and decanted on petri dishes. The antimicrobial disc were placed on the surface of the Mueller Hinton agar using a sterilize forcep and incubated at 37°c for 24 hours. Thereafter, the diameter of the zone of inhibition was measured [8]

Screening and Confirmation Test for AmpC Beta -Lactamase

Screening test was done using the disc diffusion method. Mueller Hinton agar was prepared according to direction on the manufacturer's label. Then sterilized by autoclaving at 121°C for 15 mins and when cooled poured into sterile petri dish and allowed to solidify. Sterile wireloop was dipped into an overnight broth containing the test organisms and used to streak the agar to obtain uniformity in all directions and left for 5 minutes for the surface to dry before adding the cefoxitin (30µg) disc. The cefoxitin (30µg) disc is placed on the surface using a forcep after being allowed to drip. The impregnated plates were incubated at 37°c for 24 hours after which the results were recorded.

Isolates that had zone of inhibition <18mm in the screening test were selected for the confirmation test. The confirmation test was based on phenotypic inhibitor-based method for detection of AmpC beta -lactamase using phenyl boronic acid method following CLSI [9]. The surface of the prepared Mueller Hinton agar plate was inoculated by making a streak on the surface in all directions from an overnight broth. A 30µg cefoxitin disc was placed on the surface of the Mueller Hinton agar at one end while a 30µg cefoxitin disc impregnated with 300µg phenyl boronic acid was placed at one end. The culture plates were inverted and incubated at 37°c for 24 hours. The zone of inhibition was measured for the cefoxitin disc alone and the cefoxitin disc impregnated with phenyl boronic

acid. The organisms that had zone of inhibition ≥5mm in the cefoxitin disc with phenyl boronic acid are AmpC producers.

Table 1: *Frequency of E.coli, Klebsiella spp and Proteus mirabilis isolated from urinary tract infections of patients Delta.*

Bacteria	Frequency	Percentage (%)
Escherichia coli	52	85.2
Klebsiella spp	7	11.6
Proteus mirabilis	2	3.2
Total	61	100

Table 2: *Susceptibility pattern of clinical isolates*

S/N	Sample	Organism	AUG	CTX	IMP	CFX	CN	NA	NF	CXM	CRO	ACX	CFP	LBC
1	41	*Proteus mirabilis*	R	R	R	S	S	I	S	R	I	R	S	S
2	85	*Proteus mirabilis*	R	R	I	S	S	S	S	I	S	R	S	S
3	56	*Klebsiella spp*	R	R	R	R	S	S	S	I	I	R	S	S
4	65	*Klebsiella spp*	S	R	R	S	S	S	S	S	S	S	S	S
5	71	*Klebsiella spp*	R	R	R	S	R	R	R	R	R	R	I	S
6	84	*Klebsiella spp*	R	R	R	S	R	R	R	R	R	R	R	S
7	37	*Klebsiella's spp*	R	R	R	S	I	R	I	R	R	R	I	S
8	13	*E. coli*	R	R	R	S	R	S	R	R	S	R	S	S
9	19	*E. coli*	R	R	R	S	S	S	I	R	I	R	S	S
10	20	*E. coli*	R	R	R	S	S	S	S	I	R	S	S	S
11	54	*E. coli*	R	R	R	S	S	I	S	R	I	R	I	S
12	62	*E. coli*	R	R	R	S	S	S	S	R	S	S	S	S
13	63	*E. coli*	R	R	R	I	R	R	R	R	R	R	R	S
14	70	*E. coli*	S	R	R	S	S	S	S	S	S	S	S	S
15	79	*E. coli*	S	S	R	S	S	S	S	S	S	S	S	S
16	80	*E.coli*	R	R	R	S	S	S	S	I	I	R	S	S
17	81	*E. coli*	S	R	S	S	S	S	R	S	I	S	S	S
18	18	*E. coli*	R	R	R	R	S	S	R	R	R	S	S	S

Keywords

S - Susceptible

I - Intermediate

R – Resistant

Table 3: *Screening Test for AmpC beta-lactamases*

S/N	Sample Code	Inhibition Zone Diameter (mm) Cefoxitin
1	41	16
2	85	4
3	56	2
4	65	10
5	21	-
6	84	-
7	37	-
8	13	5
9	19	9
10	70	3
11	54	-
12	62	2
13	63	-
14	70	21
15	79	2
16	80	-
17	81	20
18	82	-

3 | DISCUSSION

This study evaluates the incidence of mediate AmpC beta- lactamases among *Escherichia col*i, *Klebsiella spp*, and *Proteus mirabilis* isolated from patient's urinary tract infections in Delta State University, Health Centre, Abraka.

From the study *Escherichia coli* isolates showed a higher occurrence (85.2%) followed by *Klebsiella spp* (11.6%) *and Proteus mirabilis* (3.2%). The high occurrence rate of *E. coli* in this study could be attributed to its presence in the gut and is shed in the faeces where the bacteria can spread to the opening of the urinary tract and end up in the bladder where it can cause problem. This is comparable to the result obtained by Department of chemical pathology, kidney care centre, Ondo [10] with *E. coli* (37.9%) been the most predominant isolate causing UTI while *Klebsiella spp* (34.2%) ranked second. High rate of resistance to Cefotaxime (94.4%), Imipenem (84.9%), and Augmentin (77.7%) was common among the

uropathogens. The widespread resistance was mostly on some of the cephalosporins. The high resistant to these antibiotics is due to the acquisition of resistance conferring DNA through highly mobile genetic elements such as plasmids [11]. Augmentin is readily misused and commonly prescribed antibiotics. Appreciable susceptibility by all uropathogens was found in Levofloxacin (100%), Ofloxacin (83%), Gentamicin (72.2%), Cefepime (72%), Nalidixic acid (66.7%). from the result stated above, Levofloxacin, ofloxacin, gentamicin, cefepime, nalidixic acid showed highest susceptibility to all isolates and should be included as first line drugs in antibiotics therapy involving urinary tract infections.

The human health consequence of resistant pathogens include more serious infections and increased frequency of treatment failures. Patients may experience prolonged duration of illness, increased frequency of blood stream infections, greater likelihood of hospitalization and increased mortality [12].

High level AmpC production is associated with resistance to all beta-lactam except for cefepime [13] that is bacterial organisms that produce AmpC beta-lactamases are resistant to cephamycins but susceptible to the fourth-generation cephalosporin, cefepime. In addition, treatment failure with broad spectrum cephalosporins have been documented. These enzymes are not affected by available beta - lactamases inhibitors and in association with the loss of outer membrane porins can produce resistance to carbapenems [14]. From the result obtained from this study, 88.9% of the isolate was resistant to imipenem while 72% was highly susceptible and 3% was moderately susceptible to cefepime. This indicates the presence of AmpC beta- lactamase enzyme. There was moderate susceptibility to ampiclox, this is an indication of the presence of AmpC beta-lactamase as Cloxacillin present in ampiclox is a known inhibitor of AmpC beta-lactamase.

From the results obtained using cefoxitin disc (30µg), zone of inhibition <18mm was taken as cefoxitin resistant in with the work done by Coudron *e t al*., [15]. From this study 87% of the isolates had zone of inhibition <18mm. Both *Proteus mirabilis* and *Klebsiella spp* were all resistant to cefoxitin while *E. coli* (82%) was also resistant to cefoxitin.

The phenotypic inhibitor-based test for confirmation using cefoxitin disc (30µg) and cefoxitin disc with phenylboronic acid (30µg) was used because it was simple, convenient and an accurate method for determining plasmid mediated AmpC beta-lactamases in organisms that lack a chromosomally mediated AmpC beta-lactamase [16]. The isolates that had zone of inhibition <18mm in the screening test was used for the detection test of AmpC beta - lactamases. Organisms with zone of inhibition of the cefoxitin disc (30µg) with phenylboronic acid (300µg) ≥5mm are positive for AmpC beta-lactamase. From the result of the study *E.coli* (75%), *Klebsiella spp* (80%), *Proteus mirabilis* (50%) are positive for AmpC beta-lactamases enzyme. This shows AmpC producing *E.coli, Klebsiella* spp and *Proteus mirabilis* isolated in this study are resistant to most penicillins and cephalosporins. This is significant and contributes to the growing frequency of antibiotics resistance.

Detection of AmpC beta-lactamase isolates is clinically importance because of their resistance to cephalosporins and further resistance to carbapenems. The detection of AmpC beta-lactamase enzyme should be practiced as it will contain the spread of the organisms possessing this resistance mechanism, reduce multidrug resistance, helps in choosing the right drug for the treatment, learn about the clinical relevance of AmpC beta-lactamases.

4 | CONCLUSION

Based on the results of this research work, it can be concluded that isolates of *Escherichia coli*, *Klebsiella spp and Proteus mirabilis* present the occurrence of mediate AmpC beta - lactamase enzyme. *E. coli* is the most common uropathogen. Antibiotics which are not readily used by patients tend to have high susceptibility to most of the

uropathogens. Given that there is high prevalence of antimicrobial resistance and this resistance has clinical implications, there is need for mitigation effort. Hence AmpC beta-lactamase detection will help to determine the most appropriate antibiotic and reduce the multidrug resistance.

Recommendation

Identification of the type of AmpC beta-lactamase enzyme may help to prescribe the most appropriate antibiotics, this can help to reduce antibiotics resistance. Since there is no major method for detection of AmpC beta-lactamase, comparison of different methods should be done to determine the best method.

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