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Detection of AMPC and ESBL Producers among Enterobacteriaceae in a Tertiary Health Care in, Kano- Nigeria

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ABSTRACT

Resistance to broad spectrum β lactams, mediated by extended spectrum beta lactamase (ESβL) and AmpC βL enzymes is fast spreading in Nigerian hospitals. Production of these enzymes has resulted in treatment failure especially if one of the second or third generation cephalosporins is used. A study was undertaken to determine the prevalence of each enzyme and coproduction of the two enzymes among members of Enterobacteriaceae at the Aminu Kano Teaching Hospital (AKTH), Kano. A total of 550 clinical isolates of *Escherichia coli, Klebsiella pneumoniae, K. aerogenes, Proteus mirabilis, P. vulgaris, Serratia spp., Morganella spp., Enterobacter spp.* and *Enteropathogenic E. coli* from different clinical specimens were screened for both AmpC and ESBLs using AmpC Disc Test and Double Disk Synergy Test, respectively, over a period of 12 months. The overall prevalence of ESβL- and AmpC βL-producing isolates in study was 15.8% (87/550) and 11.3% (62/550), respectively. Co-production of ESβL and AmpC βL was detected in 6.04% (9/149) of the isolates. Co-production was highest among *Enterobacter spp* (12.5%), followed by *K. aerogenes* (11.1%). This indicates that some members of Enterobacteriaceae in Kano possess the capacity to elaborate both AmpC and ESβLs concurrently, which may render them resistant to a multiple antibiotics.

Keywords: *AmpC β-lactamases, Extended Spectrum Betalactamases, Co-production, Prevalence, Enterobacteriaceae*

I. INTRODUCTION

The production of Beta-lactamases is an important mechanism of resistance to Beta-lactam antibiotics among gram negative bacteria [1]. Extended-Spectrum-Beta-Lactamases (ESBLs) are plasmid-mediated beta-lactamases of predominantly Bush class A, so far described only in gram negative bacilli [2]. ESBLs are mutants of TEM-1, TEM-2 and SHV-1, that are capable of efficiently hydrolyzing penicillin, narrow spectrum cephalosporins (e.g. cefotaxime, ceftazidine) and monobactams (e.g aztreonam), but they do not hydrolyze cephamycin or carbapenems (imipenem, meropenem) [2]. Beta-Lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam generally inhibit ESBL producing strains [3]. ESBL producing isolates are most commonly found in *Klebsiella spp*, (predominantly *Klebsiella pneumoniae)* and *Escherichia coli* [4].

Class C Betalactamase enzymes are primarily chromosomal and plasmid-mediated. The most prevalent enzyme in this group, which is found among the *Enterobacteriaceae* and *P. aeruginosa*, is AmpC. Class C Beta-lactamases are resistant to Beta-lactamase inhibitors and mutations in the regulatory gene, which occurs at a high frequency among *Enterobacter cloacae*, can result in high-level constitutive production resulting in resistance to all Beta-lactams except carbapenems [5].

Gram negative *Enterobacteriaceae* expressing AmpC and Extended-Spectrum-Beta-Lactamases are among the most multidrug-resistance pathogens in hospitals and they are spreading worldwide [6]. Infection caused by these organisms have resulted in poor chemotherapeutic outcomes, reduced rate of clinical and microbiological responses, longer hospital stay and greater hospital expenses [7, 8]. Multiple outbreaks rate of illness and death, especially in neonatal intensive care units (ICUs) have been reported [9, 10]. Physical contact is the most likely mode of transmission [11]. The gastrointestinal tract of colonized or infected patients is the most frequent reservoir [8]. Studies have indicated that transient carriage of bacteria on the hands of healthcare workers may lead to transmission to patients [11].

Detection of ESBL or AmpC beta lactamase remains a challenge to most of our hospitals in Nigeria. Increasing resistance of members of Enterobacteriaceae to antibiobic in our hospitals and community is highly worrisome. Ability to produce these enzymes by members of Enterobacteriaceae may confer on these organisms multiple antibiotic resistances resulting in treatment failure, increased morbidity and mortality. Thus it is necessary to know the prevalence of each of the enzymes and their co production among hospital isolates so as to formulate a policy that will aid empirical therapy. This study was therefore aimed at detecting each of ESBL and AmpC beta lactamase and their co-production among members of Enterobacteriaceae in Aminu Kano Teaching Hospital Kano (AKTH), a tertiary health center in Kano, Nigeria.

II. MATERIALS AND METHODS

Collection of Clinical Isolates

A total of five hundred and fifty (550) consecutive non repetitive clinical bacterial isolates belonging to Enterobacteriacea cultured from samples of urine, catheter tip, stool, semen, urogenitals, and abscesses were collected from pathology Department of Aminu Kano Teaching Hospital (AKTH) over a period of 12 months. The isolates included *Escherichia coli* (268), *Klebsiella pneumoniae* (104), *Klebsiella aerogenes* (24), *Proteus mirabilis* (89), *Proteus vulgaris* (32), *Salmonella typhi* (4), *Morganella spp* (2), *Serratia spp* (1), Enteropathogenic *E. coli* (10) and *Enterobacter spp* (16). *E. coli* ATCC 25922 was also obtained from Aminu Kano Teaching Hospital, Kano and used as a control strain.

Screening of isolates for β-lactamase

All the isolates were concurrently screened for both AmpC and ESBL lactamases.

Screening for ESBLs

A suspension of the organism to be tested was prepared with turbidity equivalent to 0.5 Mc Farland standard and inoculated on Mueller-Hinton Agar (MHA) (Oxoid, England) plates for disc diffusion testing, in accordance with Clinical Laboratory Standard Institute, CLSI [12] guidelines. Two discs, Cefpodoxime (10µg, Oxoid, England) and Ceftazidime (30µg, Oxoid, England), were placed on the inoculated MHA and the plates were incubated at 37^0C for 24 hours. Zones of inhibition of the two discs were measured by means of a ruler [12]. Organisms showing a zone of inhibition of ≤ 21mm to cefpodoxime or ≤ 20mm to ceftazidime were subjected to confirmatory test (Double Disc synergyTest). **Confirmatory Test (Double Disc Synergy Test)** Amoxicillin-clavulanic acid (augmentin) disc (30µg, Oxoid, England) was placed toward the center of MHA plate, a ceftazidime disc (30 µg) (Oxoid, England) was placed 15 mm out from the edge of augmentin disc at 90° angle, so that its inner edge is 15 mm from it. The same was performed with cefpodoxime (10 µg) disc so that they were spaced 90° apart and 15 mm from augmentin disc. Plates were incubated at 37°C; aerobically for 24 hours. Zone diameters and zone-enhancement toward Augmentin disc were recorded for the all cephalosporins, as per CLSI guidelines [12].

AmpC Screening Test

The susceptibility of the isolates to cefoxitin disc (30µg) (Oxoid, England) was determined by the standard Disc Diffusion method [12]. Isolates that yielded a cefoxitin zone diameter less than 18 mm were tested for AmpC enzyme production by AmpC disk test.

Confirmatory Test (AmpC Disk Test)

The method of Black *et al*. [13] was adopted. A 0.5 McFarland suspension of ATCC *E. coli* 25922 was inoculated on the surface of a MHA plate. A 30 µg cefoxitin disc (Oxoid, England) was placed on the inoculated surface of the agar. A sterile plain disc inoculated with several colonies of the test organism was placed beside the cefoxitin disc almost touching it, with the inoculated disk face in contact with the agar surface. The plates were incubated at 37^0C for 24hours.

After incubation, the plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

III. RESULTS AND DISCUSSION

The overall prevalence of ESBL producers among the clinical bacterial isolates was 15.8% (Table 1). The prevalence of ESBL producing isolates is high when compared with the findings of Yushau *et al*. [14] who reported 9.3% prevalence in Kano Nigeria, Spanu *et al*. [15] who found 6.3% in Italy and Gangoue-Pieboji [16] who reported a prevalence rate of 12% in Younde Cameroun. In Nigeria and elsewhere, some studies had reported higher prevalence of ESBL producers. For instance 20% in Lagos-Nigeria [17] and 15.8% in Riyadh, Saudi Arabia [18]. From previous studies in Kano, reports of higher rates of ESBL among clinical isolates have been made even among the immune-compromised patients such as tuberculosis patients [19]. The highest prevalence of ESBL producing organisms was found in Morganella spp (50.0%), followed by *Enterobacter spp* (31.3%), *Salmonella typhi* (25.0%) *K. aerogenes* (20.8%) and *E. coli* (17.2%) No ESBL production was detected in *Serratia spp*. (Table 1).

Table 1: Prevalence of ESBL among gram negative bacterial clinical isolates

Bacterial species	No Screened	No Positive	% positive
E. coli	268	46	17.2
Proteus mirabilis	89	12	13.5
Proteus vulgaris	32	2	6.3
Enterobacter spp	16	5	31.3
Salmonella typhi	4	1	25.0
Morganella spp	2	1	50.0
Serratia spp	1	0	0
K. aerogenes	24	5	20.8
K. pneumoniae	104	14	13.5
EPEC	10	1	10.0
Total	**550**	**87**	**15.8**

EPEC-Enteropathogenic *E. coli*

On contrary, earlier study in Kano, Nigeria revealed highest prevalence rates among *E. coli* (42.6%) and *K. pneumoniae* isolates (25.5%) [20]. The high occurrence of ESBLs among these pathogens as observed in this and previous studies is of great concern since infections caused by these bacteria are very common in the area and are major cause of hospital-acquired infections and nosocomial outbreaks in Kano-Nigeria.

Increasing ß- lactam resistance among our isolates is thought initially to be caused by pathogens elaborating ESBLs alone following their first report in 2007 [14], but due to the fact that some isolates that are screened negative for ESBL still showed decreased or no susceptibility to cefpodoxime, ceftriaxone, ceftazidime, cefoxitin and/or carbapenem invitro [19].

The overall AmpC production by our isolates was 11.3 %. *Morganella spp* had the highest AmpC prevalence (50.0%), followed by *Enterobacter spp* (18.8%), *K. aerogene* (16.7%), K. *pneumoniae* (16.4%) *P. mirabilis* (15.7%) and E. coli (8.6%). However, Amp C β-lactamases were not detected in *S. typhi, Serratia spp,* and *Enteropathogenic Escherichia coli* (EPEC*)* (Table 2).

Table 2: Prevalence of Amp C beta lactamase among clinical bacterials isolates

Bacterial species	No Screened	No Susceptible to cefoxitin (zone of inhibition ≥18mm) (%)	No Resistant to cefoxitin (Zone of inhibition <18mm) (%)	Positive AmP C	% positive
E. coli	268	174(65)	94 (35)	23	8.6
Proteus mirabilis	89	56 (63)	33 (37)	14	15.7
Proteus vulgaris	32	15 (47)	17 (53)	2	6.3
Enterobacter spp	16	5 (31.2)	11 (67.8)	3	18.8
Salmonella typhi	4	4 (100)	0 (0)	0	0
Morganella spp	2	1 (50)	1 (50)	1	50.0
Serratia spp	1	1 (100)	0 (0)	0	0
K. aerogenes	24	10 (42)	14 (58)	4	16.7
EPEC	10	9 (90)	1 (10)	0	0
K. pneumoniae	104	50 (48)	54 (52)	15	16.4
Total	**550**	**325(59)**	**225(41)**	**62**	**11.3**

EPEC-Enteropathogenic *E. coli*

This prevalence rate is higher than that reported from other parts of the world. For instance, Singhal *et al*. [21] reported 8% in India, Gazouli *et al*. [22] reported 2.6% in Greece. Eyitayo *et al.* [23] however reported a higher prevalence (23%) in Lagos-Nigeria among cancer patients. It should be noted that cancer patients are more likely to contract antibiotic resistant infections than other members of the community, due to their weakened immunity. In Delhi India, however, Manchanda and Singh [10] observed higher rates of AmpC production among *K. pneumoniae* (33.3%) and *P. mirabilis* (33.3%) isolates, but a lower rate among *E. coli* isolates (14.3%). In the study, 73.9% (215/291) of isolates that are resistant to cefoxitin are non-AmpC-producers. Cefoxitin resistance in this type of AmpC negative isolates could be due to a decreased permeability of porins [24, 25].

The study also demonstrated co-existence of both ESBL and AmpC enzymes in nine isolates (6.04%), with the highest prevalence in Enterobacter spp (12.5%), followed by *K. aerogenes* (11.1%), *P. mirabilis* (7.7%) and *E. coli* (5.8%) (Table 3).

Table 3: Co existence of Amp C and ESBL among bacterial clinical isolates

Bacterial species	Amp C	ESBL	AmpC +Esbl (%)
E. coli	23	46	4 (5.8)
Proteus mirabilis	14	12	2 (7.7)
Proteus vulgaris	2	2	0 (0.0)
Enterobacter spp	3	5	1 (12.5)
Salmonella typhi	0	1	0 (0.0)
Morganella spp	1	1	0 (0.0)
Serratia spp	0	0	0 (0.0)
K. aerogenes	4	5	1 (11.1)
EPEC	0	1	0 (0.0)
K. pneumoniae	15	14	1 (3.4)
Total	**62**	**87**	**9 (6.04)**

EPEC-Enteropathogenic *E. coli*

Similar co-existence has been reported previously. For instance, Sinha *et al*. [26] reported 8% co existence. A prevalence of 1.25% (1/80) was demonstrated by Mathur *et al*. [27] among *E. coli*. The co-production of both AmpC and ESBL by our isolates as observed in this study suggests the existence of multi-drug resistant strains of Gram negative bacterial pathogens which may lead to treatment failure and/or outbreaks of infections caused by resistant organisms. In addition, resistance to amino glycosides and trimethoprin sulfamethotazole, as well as high frequency of flouroquinolones resistance are likely to occur among the isolates because plasmids conferring resistance to these antibiotics also carry ESBL and AmpC genes. The findings therefore highlight the need to adopt strict control measures in order to prevent the spread of multi-resistant pathogens in the hospitals.

IV. CONCLUSION

It could be concluded that some gram negative bacterial pathogens in Kano possess the capacity to elaborate both AmpC and ESβLs concurrently, which would renders them resistant to a multitude of antibiotics.

REFERENCES

[1]. Coudron, P.E., Moland, S.E. and Sanders, C.E. Occurrence and detection of Extended spectrum beat lactamases in members of the family Enterobacteriaceae at a veterans medical confers *J. Clin. Microbial* 35: 2593-2597 (1997).

[2]. Emery, C.L. and Weymouth, L.A. Detection and clinical significance of Extended spectrum beta lactamases in a tertiary care medical center. 35(4). 61067 (1997).

[3]. Naumorskil, L. and Palzkill, T. Outbreak of ceftazidime resistance due to moral extended spectrum beta-lactamases in isolates from cancer patients. *Antimicrob agents another 36(4)*: 91-95 (1999).

[4]. Winokur, P.L. and Brueggemann-Desalvo, D.L. Animal and human multidrug-resistant cephalosporin-resistant Salmonella isolates expressing a plasmid-mediated CMY-2, AMP-C beta-lactamases. *Antimicrob. Agents Chemother*., 44: 2777-2783 (2005).

[5]. Livermore, D.M. and Woodford, N. Laboratory detection and reporting of bacteria with extended spectrum betalactamases. Antibiotic resistance monitoring and reference laboratory, specialist and reference. Microbiology division, Health Protection Agency -Colindale, London. (2004).

[6]. Ben – Ami R., Rodriguez – Bafio J. Calbo Es. And Arslan H. A multinational survey of risk factors for infection with Extended spectrum beta lactamases producing *Enterobacteriaceae* in non hospitalized patients. 5(1): 49 – 51 (2009).

[7]. Lautenbach, E., Patel, J.B. and Fishman, P.H. Extended spectrum beta lactamases producing *Escherichia coli* and *Klebsiella pneumoniae. Risk factors for infection and impact of resistance on outcomes. 32(3*): 162-171 (2001).

[8]. Paterson, D.L., KO, W.C. and Goossens, H. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia. Implication of production of Extended spectrum beta lactamases *J Clin Microbiol 39*(5): 50 – 57 (2004).

[9]. Laurent, C., Rogriguez, V. and Vincent, J.L. Intensive care unit outbreak of Extended spectrum beta lactamases – producing patients and reinforcing infection control measures. *Infect Control hospital epidemiol* 29(3): 517 – 524 (2008).

[10]. Manchanda, V. and Singh, N.P. Occurrence and detection of AmpC beta-lactamases Among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, *India J Antimicrob Chemother*. 51:415–418 (2003).

[11]. Gupta, A., Della, L. and Haaas, J. Outbreak of Extended spectrum beta lactamases producing *Klebsiella pneumoniae* in a antenatal intensive care unit. 15(3): 10 – 15 (2004).

[12]. Clinical and Laboratory Standards Institute/NCCLS. *Performance Standards for Antimicrobial Susceptibility Testing*. Nineteenth informational supplement. M100-S19. CLSI, Wayne, PA (2009).

[13]. Black, J.A., Moland, E.S. and Thomson, K.S. AmpC disk test for detection of plasmid-mediated AmpC- b lactamases in *Enterobacteriaceae* lacking chromosomal AmpC- beta- lactamases. *J Clin Microbiol 43* : 3110-3 (2005).

[14]. Yusha'u, M., Olonitola, S. O., and Aliyu, B. S. Prevalence of Extended – Spectrum Beta lactamases (ESBLs) Among members of the Enterobacteriaceae isolates obtained from Mohammed Abdullahi Wase Specialist Hospital, Kano, Nigeria. *International Journal of Pure and Applied Sciences* ***1*** **(3):** 42 – 48 (2007).

[15]. Spanu, T.F., Luzzaru, A., Tonioto, P. and G. Feeddo (2002). Occurrence of Extended spectrum beat lactamases in members of the family *Enterobacteriaceae* in Italy. Implication of resistance to beta lactamases and other antimicrobial drugs. *Antimicrobial Agent of Chemotherapy. 46:* 196-202.

[16]. Gangoue-Pieboji, J., Benedic, B., Koulla-Shiro, S., Randegger, C., Adiogo, D., Ngassam, P., Ndumbe, P. and Hachler, H. ESBL producing *Enterobacteriaceae* in Yaounde, Cameroon. *J Clin Microbiol* 43 (7): 3237-7 (2005).

[17]. Aibinu, I., Odugbemi, P. and Brian, J.M. Extended-spectrum β-lactamase in isolates of *Klebsiella* spp and *Escherichia coli* from Lagos. *Nig. J. Health Biomed. Sci*., 2: 53-60 (2003).

[18]. El-Khizzi, N.A. and Bakeshwain, S.M. Prevalence of extended spectrum beta-lactamases among *Enterobacteriacea* isolated from blood culture in a tertiary care hospital. *Saudi med J. 27* (1): 37-40 (2006).

[19]. Yusuf, I., Arzai, A.H., Umar, A., Magaji, N., Salisu, N., Tukur, A. Prevalence of Extended Spectrum ß-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* in Tuberculosis patients in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences* 4(2):182-185 (2011).

[20]. Yusha'u, M., Aliyu, H. M., Kumurya, A.S. and Suleiman, K. Prevalence of extended spectrum β-lactamases (esbls) among *Enterobacteriaceae* in

Murtala Mohammed Specialist Hospital, Kano, Nigeria. *Bayero Journal of Pure and Applied sciences*, *3*(1): 169- 172 (2010).

[21]. Singhal, S., Mathur, T., Khan, S., Upadhyay, D.J., Chugh, S., Gaind, R. and Rattan, A. Evaluation of Methods for AmpC Beta-Lactamase in Gram Negative Clinical Isolates from Tertiary Care Hospitals. *Indian J Med Microbiol* 23:120-4 (2005).

[22]. Gazouli, M., Tzouvelekis, L.S., Vatopoulos, A.C. and Tzelepi, E. Transferable class C beta-lactamases in *Escherichia coli* strains isolated in Greek hospitals and characterization of two enzyme variants (LAT-3 and LAT-4) closely related to *Citrobacter freundii* AmpC beta-lactamase. *J Antimicrob Chemother 42*:419–425 (1998).

[23]. Eyitayo, O., Adenipekun, I., Aibinu, E., Oluwole, D., Afolabi, A., Aderemi, T., Ajekigbe, A. and Adelowotan, O. Occurrence of beta -lactamase resistance among isolates from cancer patients in Lagos Nigeria. *Academia Arena*,1(5), ISSN 1553-992 (2009).

[24]. Pangon, B., Bizet, C., Bure, A., Pichon, F., Philippon, A., Ragnier, B. and Gutmann, L. In vivo selection of a cephamycin-resistant, porin-deficient mutant of *K. pneumoniae* producing a TEM-3 beta-lactamase. *J Infect Dis. 159*:1005–1006 (1989).

[25]. Thomson, K.S. Controversies about extended-spectrum and AmpC beta-lactamases. *Emerg Infect Dis. 7*:333–336 (2001).

[26]. Sinha, P., Rajni, S., Suman, R., Raman, S., Smita, S. and Deepali, P. Prevalence of extended spectrum beta lactamase and AmpC beta lactamase producers among *E. coli* isolates in a tertiary care hospital in jaipur. *Indian J of pathol and Microbiol*. 51 (3): 367-9 (2008).

[27]. Mathur, P., Kapil, A., Das, B. and Dhawan, B. Prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *India J Med Res 115*:153-7 (2002).