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BETA-LACTAMASE PRODUCTION BY BACTERIA ISOLATED FROM THE GASTROINTESTINAL, UROGENITAL AND URINARY TRACTS OF PATIENTS AT MURTALA MUHAMMED SPECIALIST HOSPITAL AND DECENT LABORATORY, KANO.

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ABSTRACT
A total of two hundred and thirteen (213) bacterial isolates were obtained from the gastrointestinal, urogenital and urinary tract specimens from patients at Murtala Muhammmed Specialist Hospital and Decent Laboratory in Kofar Mata, Kano and identified by biochemical tests. The isolates were screened for the production of β-lactamase enzyme using the acidometric test method. β-lactamase production was detected in 146 (68.54%) isolates. The rate of incidence of β-lactamase production among Gram negative and Gram positive pathogenic bacterial isolates was found to be 141(70.15%) and 5 (41.67%) respectively. The Gram negative bacterial isolates of Haemophilus spp. 1(100%), Salmonella paratyphi 4(100%), Citrobacter spp. 4(100%) and Salmonella typhi 9(90%) were β-lactamase positive but only a few were isolated, while E.coli 102 (75.4%) and Klebsiella spp. 51 (66.67%) had higher rate of β-lactamase production and high frequency of occurence. Among the Gram positive organisms only Staphylococcus spp. 4(57.14%) and Enterococcus feacalis 1 (33.33%) showed evidence of β-lactamase production.
Keywords: β-lactamase, Gram negative, Gram positive, Hospital, E. coli.

INTRODUCTION

β-lactamases are enzymes produced by some bacteria and are responsible for their resistance to β-lactam antibiotics like penicillins, cephalosporins, cephamycine, ertapenems and carbapenems. These antibiotics have a common element in their molecular structure, a four atom ring known as a β-lactam. The β-lactamase enzyme breaks that ring open, deactivating the antibacterial properties of the molecules (Abraham *et al.,* 1940).

β-lactamases are the commonest single cause of bacterial resistance to β-lactam antibiotics. Numerous chromosomal and plasmid-mediated types are known and may be classified by their sequences or phenotypic properties. The ability of a β-lactamase to cause resistance varies with its activity, quantity and cellular location for Gram negative organisms and the permeability of the producer strain (Livermore, 1995).

β-lactamases have been described for many species of Gram positive and Gram negative bacteria. Some β-lactamases are plasmid-mediated (e.g., penicillinase of *Staphylococcus aureus*), while others are chromosomally mediated as in many species of Gram negative bacteria (Brooks *et al.,* 2004). β-lactamase producing bacteria may have an important clinical role in infections. These organisms can be pathogenic in causing the infection as well as have an indirect effect through their ability to produce the β-lactamase enzyme into their environment (Itzhak, 2009).

More than 340 β-lactamases have been described, unfortunately β-lactamases nomenclature is complex (Bush *et al.,* 1995). Many of the newer β-lactamase enzymes exhibit broad-spectrum hydrolytic activity against most classes of β-lactams (Bush, 2010).

This rudimentary study on the Prevalence of β-lactamase in bacteria isolated from the gastrointestinal, urogenital and urinary tracts, is of fundamental importance for the study of the means of resistance of pathogenic bacteria, mainly Gram negative bacteria that are resistant to β-lactam antibiotics due to the production of the β-lactamase enzyme which is supported by an earlier report from Olowe *et al.,* (2007). And also through the relevant data gained by this work, it could bring about better management of diseases in the study area.

MATERIALS AND METHODS

Study Area and Sample Collection

The research project was carried out in Kano metropolis at the pathology department of Murtala Muhammed Specialist Hospital and Decent laboratory. A total of 181 pathogenic bacterial isolates were collected after

isolation from urine, stool, High vaginal swab (HVS) and Urethral swab specimens from patients with urogenital, gastrointestinal and urinary tract infections at Murtala Muhammed Specialist Hospital, while 32 pathogenic bacteria isolates were collected from Decent laboratory from the aforementioned sources at Decent Laboratory and tested for β-lactamase production.

SAMPLE PROCESSING

Before carrying out tests for β-lactamase production on the bacterial isolates, controls were prepared. Known β-lactamase positive *E.coli* and β-lactamase negative *Klebsiella* spp. were tested as positive and negative controls respectively. The positve and negative controls were cultured using a sterile wire loop on slants of nutrient agar in bijour bottles and used as controls throughout the tests. The specimen from which the desired bacterial isolates were gotten were cultured onto suitable growth media such as Mackconkey agar, blood agar, CLED agar, as described by Cheesbrough, (2000) using a sterile wire loop and then incubated at 35℃-37℃ overnight. Afterwards, the colonies of desired bacterial pathogens were observed and identified as described by Cowan and Steel (1993).

TEST PROCEDURE

Buffered crystalline penicillin bromocresol purple solution was prepared as described by Cheesbrough (2000).

A strip of Whatman No.1 filter paper was placed into a petridish and a few drops of buffered crystalline penicillin bromocresol purple solution was added using a pipette until the paper was almost saturated, using a sterile wire loop, the growth from 10-20 colonies of the test organism on nutrient agar was spread on the moistened filter paper, covering an area of approximately 5mm in diameter. The lid of the petri dish was replaced and allowed to stand at room temperature for upto 30 minutes. The detection of the β-lactamase enzyme is made posssible due to a change in colour of the indicator bromocresol purple, from purple to yellow due to penicilloic acid produced by the breakdown of penicillin in the solution by the β-lactamase enzyme. The colour change is brought about by a change in pH. This change in pH is from alkaline (pH of the reagent) purple to acidic, which is yellow.

RESULTS

From the results obtained, urine had the highest number of bacterial isolates which was 126, stool had 64 isolates, high vaginal swab 18 and urethral swab 5 isolates as indicated in table 1.

Urine had the most number of bacterial isolates that were β-lactamase positive from the entire study area, which was 84 (39.44%), followed by stool 52 (24.41%), high vaginal swab 7 (3.29%) and least of all was urethral swab 3 (1.41%) as indicated in table 2.

Among the 32 Gram negative bacterial pathogens screened for β-lactamase production in Decent laboratory which is a private clinical laboratory, 27 (84.38%) were β-lactamase positive, 12 (85.71%) *E. coli* isolates, 11 (78.57%) *Klebsiella* spp. isolates while 1(100%) *Proteus* and *Shigella* spp. were β-lactamase positive and the least isolated as indicated in table 3.

A total of 169 Gram negative pathogenic bacterial isolates were screened at Murtala Muhammed Specialist Hospital from which 114(67.46%) were β-lactamase positive. *E.coli* had the highest occurrence of β-lactamase positive 65 (73.86%). The least number of β-lactamase positive isolates was found in *Haemophilus* spp. 1(100%) as indicated in table 4.

Twelve (12) Gram positive isolates were screened for β-lactamase production from Murtala Muhammed Specialist Hospital, Kano among which 5 (41.67%) were β-lactamase positive with *Staphylococcus* spp. having 4 (57.14%) positive isolates as indicated in table 5.

Prevalence of β-lactamase producers among the Gram negative clinical bacterial isolates in the entire study showed that 141 (70.15%) isolates were β-lactamase positive including *E. coli* 77 (75.49%), *Klebsiella* spp. 34 (66.67%) while *Neisseria gonorrhea* 1(0%) and *Haemophilus* spp. 1 (100%) had the least number of isolates that were β-lactamase positive as indicated in table 6.

Table 1: Distribution of bacterial isolates based on their source (Specimen)

Bacterial isolates	Specimen Samples			
	Urine	Stool	High vaginal Swab	Urethral swab
E.coli	58	37	6	1
Klebsiella spp.	39	5	6	1
Proteus spp.	14	1	3	1
Pseudomonas spp.	5	0	0	0
Staphylococcus spp.	4	0	3	0
Non- haemolytic *Streptococci*	1	0	0	0
α-haemolytic *Streptococci*	1	0	0	0
Salmonella typhi	2	8	0	0
Enterococcus faecalis	2	1	0	0
Salmonella paratyphi	0	4	0	0
Citrobacter spp.	0	4	0	0
Neisseria gonorrhea	0	0	0	1
Haemophilus spp.	0	0	0	1
Shigella spp.	0	4	0	0
Total	126	64	18	5

Table 2: Distribution of β-lactamase positive bacterial isolates in relation to their source.

Bacterial isolates	Specimen samples			
	Urine	Stool	High vaginal swab	Urethal swab
E.coli	43 (20.19%)	30(14.08%)	3(1.41%)	1(0.47%)
Klebsiella spp.	28 (13.15%)	3 (1.41%)	2 (0.94%)	1(0.47%)
Proteus spp.	7 (3.29%)	0	1(0.47%)	0
Pseudomonas spp.	2 (0.94%)	0	0	0
Staphylococcus spp.	3 (1.41%)	0	1 (0.47%)	0
Non-haemolytic *Streptococci*	-	-	-	-
α-haemolytic *Streptococci*	0	-	-	-
Salmonella typhi	1 (0.47%)	8 (3.76%)	-	-
Salmonella paratyphi	-	4 (1.88%)	-	-
Citrobacter spp.	-	4 (1.88%)	-	-
Neisseria gonorrhoea	-	-	-	0
Enterococcus faecalis	0	1 (0.47%)	-	-
Haemophilus spp.	-	-	-	1 (0.47%)
Shigella spp.	-	2 (0.94%)	-	-
Total	84 (39.44%)	52 (24.41%)	7 (3.29%)	3 (1.41%)

Table 3: β-lactamase production among Gram- negative bacterial isolates from Decent lab.

Organism tested	**Number of isolates screened**	**Number positive**	**Percentage (%) Positive**
E.coli	14	12	85.71
Klebsiella spp.	14	11	78.57
Salmonella typhi	2	2	100
Shigella spp.	1	1	100
Proteus spp.	1	1	100
Total	32	27	84.38

Table 4: β-lactamase production among Gram-negative bacterial isolates from Murtala Muhammed Specialist Hospital.

Organism tested	**Number of isolates screened**	**Number positive**	**Percentage (%) Positive**
E. coli	88	65	73.86
Klebsiella spp.	37	23	62.16
Salmonella typhi	8	7	87.5
Shigella spp.	3	1	33.33
Proteus spp.	18	7	38.89
Pseudomonas spp.	5	2	40
Haemophilus spp.	1	1	100
Citrobacter spp.	4	4	100
Salmonella paratyphi	4	4	100
Total	169	114	67.46

Table 5: β-lactamase production among Gram-positive bacterial isolates from Murtala Muhammed Specialist Hospital, kano.

Organism tested	Number of isolates screened	Number positive	Percentage (%) Positive
Staphylococcus spp.	7	4	57.14
Non-haemolytic *Streptococcus*	1	0	0
Enterococcus faecalis	3	1	3.33
α-haemolytic *Streptococci*	1	0	0
Total	12	5	41.67

Table 6: β-lactamase production among Gram-negative bacterial isolates from Murtala Muhammed Specialist Hospital and Decent laboratory Kano.

Organism tested	Number of isolates screened	Number positive	Percentage Positive (%)
E. coli	102	77	75.49
Proteus spp.	19	8	42.11
Klebsiella spp.	51	34	66.67
Pseudomonas spp.	5	2	40
Salmonella typhi	10	9	90
Shigella spp.	4	2	50
Haemophilus spp.	1	1	100
Neisseria gonorrhea	1	0	0
Salmonella paratyphi	4	4	100
Citrobacter spp.	4	4	100
Total	201	141	70.15

DISCUSSION

Out of the two hundred and thirteen isolates screened for β-lactamase production 146(68.54%) were β-lactamase positive. The highest prevalence of β-lactamase enzyme among bacterial isolates was found among E. coli 102(75.4%) and Klebsiella spp. 51 (66.67%). Only a few gram positive bacteria were isolated from specimens with *Staphylococcus* spp. 4(57.14%) showing the most isolates that were β-lactamase positive.

For the Gram negative bacterial isolates obtained from Murtala Muhammed Specialist Hospital alone there is relatively high prevalence of β-lactamase production (67.46%) was recorded which indicates β-lactamase producers among pathogenic bacterial isolates from patients attending Murtala Muhammed Specialist Hospital is high.

The high number of Gram negative pathogenic bacterial isolates recovered from the specimens and relatively high rate of production of the β-lactamase enzyme indicates that the Gram negative organisms are the major causes of gastrointestinal, urogenital and urinary tract infections and can have a high rate of resistance to commonly used drugs like β-lactam antibiotics. Upon that, the prevalence of β-lactamase enzyme in the isolates from various sources in the entire study area is 68.54% which is a good reflection of β-lactamase production by pathogenic bacteria in the entire Kano state, as shown by Yusuf *et al.,* (2012), which provides supplementary information for existing researches in Kano state.

The prevalence of β-lactamase production by the previously mentioned clinical isolates; 68.54% is quite higher than that reported from other parts of Nigeria such as Lagos (37.9%), Oshogbo (37.3%) and Jos (54.7%) but lower than Nsukka (89.7%) (Kesah *et al.,* 1996; Olowe *et al.,* 2007, Bello *et al.,* 1996; Oguike *et al.,* 1991), which indicates that β-lactamase enzyme producing bacteria may be endemic in Nsukka and Kano metropolis in comparison to other places as at the time the studies were conducted.

In this study urine 84(39.44%) and stool 52(24.41%) clinical specimens had the highest number of bacterial isolates which were β-lactamase positive and consisted of *E. coli* 43(20.19%) and *Klebsiella* spp. 28(13.15%) for urine specimen and *E. coli* 30(14.08%) and *Klebsiella* spp 3(1.41%) for stool specimen. This is similar to recent study conducted in Nsukka metropolis by Eze *et al.,* (2015), in which distribution of β-lactamase was determined among clinical specmens and urine had the highest prevalence of 86.7% followed by stool with prevalence of 84.6%, this study is also in agreement with a report by Doughari and Akafa (2009) who reported a prevalence rate of 91% of β-lactamase enzyme from bacterial isolates from urine specimen and Iroha *et al., (*2010) who reported 60.3% of of β-lactamase in urine specimen.

The high prevalence of β-lactamase in urine clinical isolates may be attributed factors like extreme age, sexual activity, contraception, pregancy, urinary tract obstruction, neurological dysfunction, antimicrobial use and poor hand washing techniques among health care practitioners as reported by Eze *et al.,* (2015). Due to the widespread urinary and gastrointestinal tract infections observed in this study, overcrowding, poor hygiene and sanitation among some in the populace may have promoted the spread of these infections and high prevalence of β-lactamase enzyme in the bacterial isolates.

β-lactamases are a major defense of Gram negative bacteria against β-lactam antbiotics as reported by Jacoby *et al.,* (2005). *Staphylococcus* spp. which is one of the few Gram positive isolates obtained in this study is resistant to penicllin G by producing a β-lactamase (penicllinase) that destroys the drug. Other β-lactamases are produced by gram negative rods like extended spectrum β-lactamases (ESBLs). β-lactamases open the β-lactam ring of penicllins and cephalosporins and abolish their antimicrobial activity (Brooks *et al.,* 2004). β-lactamases are now ubiquitous and occur at an alarming rate in both developing and developed countries of the world.

Abuse of antibiotics, non observance of personal hygiene and sanitation, increase in international travels, genetic exchange between bacteria and unsatisfactory procedures in hospitals are most likely to account for the emergence and spread of β-lactamase enzyme worldwide (Arzai and adamu, 2008).

The results of this research work correlates with other researches though with lower β-lactamase prevalence rates but shows a similar trend, in Cotonou, Benin on β-lactamase production in bacterial isolates by Anago *et al*., (2015) with prevalence rate of 35.5% of extended spectrum β-lactamases (ESBLs) and Kano, Nigeria by Yusuf *et al.,* (2012) with prevalence rate of 33.5% for carbapenemase and 24.5% for

metalobetalactamases (MBLs) in clinical isolates with urine samples having the highest prevalence of 38.3%. This confirms observations by earlier research work on β-lactamase production in bacterial isolates by Olowe *et al.,* (2007) with β-lactamases prevalence rate of 37.3% in Osogbo, Nigeria that β-lactamase producing gram negative bacilli are a problem in the tropics, and treatment is more difficult due to high prevalence with high multi-drug resistant strains.

CONCLUSION

There is a high prevalence of β-lactamase enzyme production by bacterial pathogens in the study area, especially the gram negative bacteria isolated from urine and stool specimens.

RECOMMENDATIONS

It is suggested that antibiotic sensitivity tests should be carried out on bacterial pathogens by laboratory scientists routinely to determine the suitable antibiotics to be prescribed to patients by doctors and good public health measures should be put in place by health authorities in order to reduce the spread of contagious diseases within the study area like ensuring proper disposal of waste, good environmental sanitation and good drainage. Further studies should be carried out so that the genes responsible for the trait of β-lactamase production in bacterial pathogens in the study area can be identified.

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CONTRIBUTIONS OF AUTHORS

This research was designed by Prof. Auwalu Uba while laboratory work, interpretation and analysis of data was carried out by Abdul-wahid Bello.

CONFLICT OF INTEREST

There is no conflict of interest in this research work.

REFERENCES

Abraham, E.P. and Chain E (1940). An enzyme from bacteria able to destroy penicillin *Nature 1940; 146:837-42.*

Arzai, A.H., and Adamu, D.J.M. (2008). Prevalence of β-lactamase producers among randomly selected bacterial pathogens in Kano, Nigeria. *Biological and Environmental Sciences Journal for the tropics 5(3): 218-223.*

Anago, E., Ayi-Fanou, L., Akpovi, C.D., Hounkpe, W.B., Tchbozo, M, A-D, Bankole, H.S. and Sanni, A. (2015). Antibiotic resistance and genotype of beta-lactamase producing Escherichia coli in nosocomial infections in Cotonou, Benin. *Annals of Clinical. Microbiology and Antimicrobials*, 14:5

Bello, C.S., Abdulrahman, A., Tanyigna, K.B. and Ughile, D. (1996). Relative prevalence of β-lactamase producers among bacterial pathogens isolated from clinical specimens in jos. *The Nigerian Medical Practitioner,* 31(5/6):83-84.

Brooks, G.F., Butel, J.S., Morse, S.A. (2004). Jawetz, Melnick and Adelbergs Medical Microbiology, 23rd edition, McGRaw-Hill companies Inc. *pp*. 162,164

Bush, K. (2010). Bench-to-bedside review: The role of β-lactamases in antibiotic-resistant Gram-negative infections. *Critical Care*, *14*(3): 224.

Bush, K., Jacoby, G.A. and Medeiros A.A. (1995). A functional classification sceme for β-lactamases and its correlation with molecular structures. *Antimicrobial agents and chemother*apy, 39:1211-1233.

Cowan, S.T and Steel, K.J. (1993),Cowan and Steel's manual for identification of Medical bacteria, 3rd edition Cambridge University press, London, pp. 191-241.

Cheesbrough, M., (2000), District laboratory practice in tropical countires, part 2, low price edition, press syndicate of the University of Cambridge. P. 90.

Doughari J.H and Akafa M.D (2009): Screening of some Clinical and Environmental samples for β-lactamase producing bacteria. *International Journal of Biochemistry.* volume 1(35): 1-14

Eze E. A., Agbo E. C., Eze C. N. (2015). Occurrence of Beta-Lactamases and the Antibiogram Pattern of Clinical Isolates of Escherichia coli and Klebsiella Species in Nsukka Metropolis. *American Journal of Microbiology and Biotechnology,* 2 (5): 69-74.

Iroha I.R, Amadi, E.S Nwazo A.C and Ejike-ugwu P.C. (2010): Detection of Plasmid borne ESBLs from blood and urine isolates of Gram Negative Bacteria from University Teaching Hospital in Nigeria. *Current Research in Bacteriology,* 3:77-83.

Itzhak, B. (2009). The role of beta-lactamase-producing-bacteria in mixed infections. *BioMed Central infectious Diseases, 9:202.*

Jacoby, G.A., Munoz-price, L.S. (2005). The new β-lactamases.The *New England Journal of Medicine* 353; 380-391.

Kesah, C.N., Coker, A.O., Alabi, S.A., and Olukoya, D.K. (1996). Prevalence, antimicrobial properties and β-lactamase production of haemolytic enterobacteria in patients with diarrhoea and urinary tract infections in Lagos, Nigeria.The *Central African Journal of Medicine,* 42 (5):147-50

Livermore, D.M. (1995), β-lactamase in laboratory and Clinical resistance. *Clinical Microbiology Rev*iews 8(4): 557-584.

Oguike, J.U., Gugnani, H.C. and Osuagwu (1991). A note on penicillin resistance and β-lactamase production in strains of *Stapylococcus aureus* recovered from different clinical materials in Nsukka, Nigeria. *Journal of communicable Diseases*. 23 (3): 200- 201.

Olowe, O.A., Eniola, K.I., Olowe, R.A. and Olayemi, A.B. (2007). Starch paper technique iseasy to detect β-lactamases from case of diarrheagenic *Escherichia coli* in Oshogbo, Nigeria., *Life Science Journal* 4(4):72-74.

Yusuf, I.,Yusha'u, M., Sharif, A.A., Getso, M.I., Yahaya, H., Bala, J.A., Aliyu, I.A and Haruna, M. (2012). Detection of metallo betalactamases among gram negative bacterial isolates from Murtala Muhammed Specialist Hospital, Kano and Almadina Hospital Kaduna, Nigeria. *Bayero Journal of Pure and applied Sciences*, 5(2): 84-88.