



Beta-Lactamase Production Among Uropathogens in Patients Attending Some Hospitals in Kano, Nigeria

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To cite this article:

Hamza Sule, Abdulhadi Sale Kumurya. Beta-Lactamase Production Among Uropathogens in Patients Attending Some Hospitals in Kano, Nigeria. *International Journal of Biomedical Materials Research*. Vol. 4, No. 3, 2016, pp. 58-62. doi: 10.11648/j.ijbmr.20160403.18

Received: August 19, 2016; **Accepted:** December 2, 2016; **Published:** January 4, 2017

Abstract: Ability of bacteria to withstand antibiotic therapy is often associated with resistance. Certain bacteria have the ability to produce some enzymes, like Beta-Lactamase, which confers resistance to some groups of antibiotics like penicillins and cephalosporins. The study focused on producers of these enzymes among uropathogens. A total of one hundred and fourteen isolates (114) were screened, out of which seventy one (62.3%) were found positive for the enzymes. *Escherichia coli* had the highest positivity rate 40 (56.3%). Followed by *Klebsiella* spp., *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* with 15 (21.1%), 7 (10.0%), 5 (7.0%), 3 (4.2%), and 1 (1.4%) respectively. In the same vein, it was discovered that, isolates of females' origin, produced more of the enzymes than those of their males counterpart. This was indicated by the production of the enzyme by at least one isolate in each of the species isolated from female samples while in males, some species did not even produce any of the enzymes. Age-wise distribution of the enzymes showed that, 21-30, and 31-40 age groups had all the isolates producing the enzymes in varying frequencies. Only *Pseudomonas aeruginosa* that produced its enzyme outside these age groups. Isolates of females origin had more of the enzymes produced in all the species compared to those from males, with ratios of; 26:14, 11:4, 5:2, 3:2, 2:1, and 1:0 for, *E coli*, *Klebsiella* species, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* respectively.

Keywords: Urinary Tract Infections, Uropathogens, Beta-Lactamase, Kano

1. Introduction

Urinary tract infections have been one of the major causes of diseases in human history. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars (Foxman *et al.*, 2000). It was thought that, with the introduction of antibiotics, these problems and related others are to be over. However, it was, and still remains surprising that, bacteria has been able to evolved mechanisms that helps them inactivate and/or bypass some of the activities of antibiotics. There is therefore the need for concerted efforts to prevent the emergence of new resistant strains and the spread of already existing ones (Senka džidić *et al.* 2008). Among the part of armory in the arsenal of these bacteria, are the enzymes beta-lactamases. Beta-lactamase are enzymes produced by some bacteria and are responsible for their resistance to certain groups of antibiotics, like penicillins, cephalosporins and carbapenem (Bush, *et al.*, 1995) β -lactamase

deactivates β -lactam antibiotics, by breaking and opening their common molecular structure (β - lactam ring). Some of these enzymes include extended spectrum β -lactamase (ESBL), and carbapenemase. The first β -lactamase was detected during the 1960s (Datta *et al.*, 1965). Urinary tract infection (UTI) is the second most common infection after respiratory tract infections in many parts of the world (Mansour, *et al.*, 2009). UTI is most commonly caused by various species of *Escherichia*, *Klebsiella*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Enterococcus faecalis* and *Candida albicans* (Abigail and Dixie, 2005; Rai *et al.*, 2008). Up to 70% of pregnant women develop glucosuria, which encourage bacterial growth in the urine, hence aggravating the scourge of urinary tract infection (Al-Issa, 2009). Uropathogens have shown a slow but steady increase in resistance to several antibiotics over decades. Extended-spectrum β -lactamases (ESBL) producing

Enterobacteriaceae, are among the most problematic multidrug resistance (MDR) bacteria worldwide (Romero *et al.*, 2005).

ESBLs are the beta-lactamases capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams, and are generally derived from TEM and SHV-type enzymes but, may not affect cefamycins and carbapenems. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species (Rupp and Fey 2003). Extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* have become widespread in hospitals and are increasing in community settings where they cause a variety of infections including UTI. In addition to hydrolyzing most β -lactam agents, bacteria harboring these enzymes display resistance to other unrelated antimicrobial agents and thus often poses a therapeutic dilemma (Maina *et al.*, 2013). Extended-spectrum β -lactamases (ESBLs) are a rapidly evolving group of β -lactamases which also have the ability to hydrolyze third-generation cephalosporins and aztreonam, yet, are inhibited by clavulanic acid (Bush *et al.*, 1995).

The persistent exposure of the bacterial strains to β -lactam related antibiotics has induced dynamics, continuous production and mutations of β -lactamase among uropathogens, these expands their activity even against the third and fourth generation cephalosporins. These new β -lactamases are called extended spectrum β -lactamases (Samaha-Kfoury *et al.*, 2003). Antimicrobial resistance among bacterial strains is an emerging problem worldwide. Urinary tract infections (UTIs) are one of the high ranked bacterial infections in humans, both in the community and hospital settings (Cox, 1988; Gonzalez, 1999; Ullah *et al.*, 2009). Class C Beta-lactamase 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*, and can result in high-level constitutive production resulting in resistance to all Beta-lactams except carbapenems (Livermore *et al.*, 2004). Gram negative *Enterobacteriaceae* expressing AmpC and Extended-Spectrum-Beta-Lactamases are among the most multi-drug-resistant pathogens in hospitals and they are spreading worldwide (Ben-Ami *et al.*, 2009).

2. Materials and Methods

2.1. Study Area

The study was conducted in three selected hospitals in Kano metropolis. The hospitals were: Murtala Mohammad Specialist Hospital, Kano, located near the amir's palace. Infectious Disease Hospital Kano, along France road which leads to Katsina road, and Sir Mohammad Sunusi Hospital, Kano, behind the Kano Eastern bypass, along Hadejia road, one of the largest town in Jigawa state. The major activity in the city is commercial in nature, but some are farmers and civil servants alike.

2.2. Specimen Collection

The patients were provided with universal bottles, into which they produced the required samples (i.e. clean catch midstream urine). Because, the first flow of urine usually wash-up the urethra, flushing anything that might be on the way including contaminants. This gives the subsequent urine that flow a true representation of what the bladder contained. Fifteen millilitre of urine samples were collected from each patient. All samples were immediately processed. But samples that could not be processed immediately were refrigerated at 4°C before processing.

2.3. Specimen Processing and Isolation of the Uropathogens

All samples, were examined macroscopically to check out for colour, turbidity and /or blood tinge as the case may be. The uncentrifuged urine samples were mixed by rotating the container before inoculating on to Cystein Lactose Electrolyte Deficient Agar (CLED) and Blood Agar by streak method (Cheesebrough, 2000), and later incubated at 37°C for 24 hours.

Some of the samples inoculated by streak method yielded colonies after 24 hours, which were subcultured on to MacConkey agar plate to obtain discrete colonies for further characterization and identification.

2.4. Identification and Characterization of the Isolates

Pure culture from purity plates (using MacConkey agar) containing discrete colonies of the isolates were used for the identification. The identification was based on colonial morphology and biochemical characteristics. Colonies produced after 24 hours (on purity plates) were physically examined for observable characters like size, shape, consistency, colour, opacity and effect on media i.e lactose or non lactose fermentation habit. Additionally motility, Gram staining reaction, and biochemical tests were used in characterizing the isolates from the purity plates (Mackey and McCartney, 1996).

2.5. Determination of Beta-Lactamase Production by the Isolates

Beta-lactamase production by isolates was determined using standard acidometric method, which was based on the detection of penicilloic acid produced due to the breakdown of penicillin by beta-lactamases. The penicilloic acid was detectable by change in colour from purple to yellow of the indicator, bromocresol purple (contained in the buffered crystalline penicillin bromocresol purple solution. (Cheesebrough, 2000).

Statistical analysis: The data obtained was analysed using ANOVA and Chi Square tests (X^2)

3. Results

Total of one hundred and fourteen (114) isolates were used to determined beta-lactamases producers among

uropathogens, of which seventy one 71 (62.3%) were found to be producers of the enzymes (Table 1). The results also revealed that among the isolates involved, those obtained from females subjects produced more of the enzymes than the ones received from males, with a total of 50 and 21 isolates respectively (Table 2). Distribution of the enzyme across the age groups involved indicated that, age limits of 21-30 and 30-41, had at least one isolate producing the

enzymes in all the screened species with the exception of *Peudomonas aeruginosa* while none of the isolates produce the enzymes in the age bracket of ≥ 71 (Table 3). Females to males ratio in terms of the enzymes production per species per gender confirmed isolates of females origin having the upper hand with regards to production of the enzymes throughout the work (Table 4).

Table 1. Beta-lactamase production by the isolates.

	E. coli	K. pneu.	S. aur.	S. sapr.	P. mir.	Ps. aer	Total
Total No.	58	27	8	11	8	2	114
B-L Producers	40	15	7	5	3	1	71
Percentage	56.3	21.1	10.0	7.0	4.2	1.4	100

KEY:

B-L Beta Lactamase

NE. coli=*Escherichia coli*

K. pneu.=*Klebsiella pneumoniae*

S. aur.=*Staphylococcus aureus*

S. sapr.=*Staphylococcus saprophyticus*

P. mir.=*proteus mirabilis*

Ps. aer.=*pseudomonas aeruginosa*

Table 2. Distribution of the enzymes with respect to gender.

	<i>Eschericia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus saprophyticus</i>	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>	TOTAL
No. of isolates	58	27	8	11	8	2	114
B/L (+) isolates	40	15	7	5	3	1	71
Females	26	11	5	4	3	1	50
Males	14	4	2	1	0	0	21

Key:

B/L=Beta-lactamase

(+)=Positive

Table 3. Distribution of beta-lactamase production across the age groups.

Age Group	<i>Eschericia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus saprophyticus</i>	<i>Proteus mirabilis</i>	<i>Pseudomonas Aeruginosa</i>
≤ 10	4	1	0	0	0	0
11-20	5	3	2	1	0	0
21-30	10	5	3	2	1	0
31-40	6	1	1	1	1	0
41-50	5	3	1	1	1	0
51-60	6	2	0	0	0	1
61-70	4	0	0	0	0	0
≥ 71	0	0	0	0	0	0
Total	40	15	7	5	3	1

Table 4. Distribution of beta-lactamase among the isolates in relation to gender.

Age Group	<i>Eschericia E. coli</i>		<i>Klebsiella pneumoniae</i>		<i>Staphylococcus aureus</i>		<i>Staphylococcus saprophyticus</i>		<i>Proteus mirabilis</i>		<i>Pseudomonas aeruginosa</i>	
	F	M	F	M	F	M	F	M	F	M	F	M
≤ 10	3	1	1	0	0	0	0	0	0	0	0	0
11-20	3	2	2	1	2	0	0	1	0	0	0	0
21-30	7	3	5	0	3	0	2	0	1	0	0	0
31-40	4	2	1	0	1	0	0	1	0	1	0	0
41-50	3	2	2	1	0	1	1	0	1	0	0	0
51-60	3	3	1	1	0	0	0	0	0	0	1	0
61-70	3	1	0	0	0	0	0	0	0	0	0	0
≥ 71	0	0	0	0	0	0	0	0	0	0	0	0
Total	26	14	12	3	6	1	3	2	2	1	1	0

4. Discussion

Seventy one 71 (62.3%) of the one hundred and fourteen (114) screened isolates produced the enzymes Beta-Lactamases. *Escherichia coli* was found to be the heights producer of the enzyme 40 (56.3%), followed by *Klebsiella pneumoniae* 15 (21.1%), *Staphylococcus aureus*, 7 (10.0%) *Staphylococcus saprophyticus*, 5 (7.0%), *Proteus mirabilis* 3 (4.2), and *Pseudomonas aeruginosa*. 1 (1.4%). Chander and Shrestha (2013), also found, in a tertiary care hospital in Kathmandu, Nepal, that, *E. coli* has the highest frequency of the enzyme production, in which 60 out of 444 isolates produce the enzymes followed by *Klebsiella pneumoniae* with 24 out of 145 isolates producing the enzyme. These proved these two uropathogens to be among the front runners as far as the production of these enzymes is concern. A similar pattern was observed Gaurav, (2012) in clinical isolates of a tertiary hospital, Jhalawar, Rajasthan, India. But with higher percentages than found in this study, where *E. coli*, led in the production rate (73%), *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas* followed in that order (58%), (50%) and (37%) respectively. These relative differences in terms of higher percentages, especially, that of *Pseudomonas aeruginosa* could be due to locality differences. More so, *Pseudomonas* is a well known or prominent nosocomial pathogen, therefore, hospitalized patients may be the source of its higher percentage in the clinical samples they reported to have used.

Another study on uropathogens which involved pregnant women, Thapa *et al.* (2015), also found similar trend, were the beta-lactamases enzymes production per isolates of the uropathogens revealed 78, 26, 24, and 1, isolate (s) for; *E. coli*, *Klebsiella* species, *Proteus mirabilis* and *Pseudomonas aeruginosa* respectively.

Beta-lactamase production with respect to gender revealed higher production of the enzymes by the isolates from female patients 50 than those isolates from male patients 21. A study in 2013, Chander and Shrestha also favoured this outcome, were 51 and 33 were the beta-lactamase production rates for isolates from females and males in their study respectively. Similar findings was also reported, Alabi *et al.* (2014), in a study titled Prevalence of extended-spectrum β -lactamase producing isolates from asymptomatic bacteriuria among students in a tertiary institution in Ibadan, Nigeria. Where beta-lactamases production by isolates of males origin were 34 while those from females samples were 40 respectively.

On the enzyme gender correlation also, production of beta-lactamases by uropathogens, was reported in 2013, in research conducted, in a Tertiary-care hospital in Kathmandu, Nepal, on two of the most important uropathogens that normally produces the enzymes with significant rate (*E. coli* and *Klebsiella* species). The work revealed 36 and 24 for *E. coli* and *Klebsiella* isolates that produced the enzymes from female urine samples while 15, and 9 were the *E. coli* and *Klebsiella* spp isolates that produce the enzyme from urine samples of male subjects respectively (Chander *et al.*, 2013).

According to age groups, it was discovered in this study that, age bracket of 21-30, a highly active group (sexually) who are also most likely to toy with risk factors of UTI, had the highest frequency of isolation and corresponding beta-lactamases production, because the group had 10 *E. coli*, 5 *Klebsiella pneumoniae*, 3 *Staphylococcus aureus*, 2 *Staphylococcus saprophyticus* and 1 *Proteus mirabilis* as beta-lactamase producers. This compares favourably (Kapur, *et al.*, 2015), in which 30-45, also sexually active age range had the highest production rate of the enzymes among the age groups used in their study, with up to (43.75%) of the Beta-lactamases producing uropathogens belonging to the group. Beta-lactamase production by the uropathogens in relation to gender, revealed isolates of females origin having much of the enzymes compared to those from males. It also indicated *E. coli* and *Klebsiella pneumoniae* as the predominant uropathogens among the screened species 26:14 as female to male ratio, total (40), and 11:4 as female to male ratio, total (15), for *E. coli* and *Klebsiella* isolates respectively. This is in line with the findings in Nepal (Chander and Shrestha, 2013) in which *E. coli* had a sub total of (36) and (24) isolates for females and males subjects with (60) as the total and *Klebsiella pneumoniae* isolates (15) and (9) for females and males with corresponding (24) as the total in the same manner.

5. Recommendations

Screening of beta-lactamases (enzymes) is highly recommended because of the growing evidence of its production among conventional and nonconventional Uropathogens. It is also important that, screening for the enzymes becomes a research priority if not a routine activity in private and public hospitals, especially in the study area.

6. Conclusion

Rampant display of resistant factors like beta-lactame among uropathogens is alarming and is sending strong signals to health care managers for urgent action. It can therefore be concluded that recurrent and persistent cases of UTI especially in women are attributable to these factors including beta-lactamase production by the uropathogens.

Acknowledgement

We thank the management of Wudil General Hospital, Kano, for allowing us to carry out the research in their facility. Our sincere gratitude also goes to the Head of the Laboratory Department of the Hospital, and all other staff.

Ethical Issues

Permission was obtained from the ethical committee of Wudil General Hospital before embarking on the research.

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