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Prevalence of Extended Spectrum Beta Lactamase Producing *Klebsiella Pneumoniae* in Urine Samples From Patients Attending Benue State University Teaching Hospital, Makurdi

Awua Yuana [1], Ogbole Giftan Apeh [2], Odo Joel Inya [3]

[1, 2] Department of Biological Sciences, Benue State University, Makurdi, Nigeria

[3] Department of Fisheries and Aquaculture University of Agriculture, Makurdi, Nigeria

Abstract: *Klebsiella pneumoniae* is one of the most predominant pathogens commonly isolated in urine. This uropathogen has developed resistance to antibiotics over the years and is increasingly becoming a public health problem. The aim of this study was to determine the prevalence of extended spectrum producing *Klebsiella pneumoniae* from clinical isolates obtained from Benue State University Teaching Hospital Makurdi.50 clinical urine isolates were obtained from in-patients from the study area and analysed for the presence of extended spectrum *K. pneumoniae* using standard diagnostic procedures. There were more ESBL positive cases (60.0%) than ESBL negative cases (40.0%) in this study. There was no statistically significant relationship between ESBL producing *K. pneumoniae* and Age (χ^2=1.857; DF=3;p=0.395); and ESBL cases and sex (χ^2=2.130; df=1;P=0.144). All the risk factors including difficulty in urination, Painful urination, Unpleasant urination, Use of catheter to aid urination, Frequent urination, Duration of admission in the hospital, Use of antibiotics for treatment, and use of antibiotics in the last 3 months (P>0.05) were not statistically related to *K. pneumoniae* infection in the study. Isolates such as *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. coli*, and *P. vulgaris* were identified from the urine samples using biochemical tests. Patients exhibiting symptoms and those without symptoms should be screened for the presence of ESBL producing *K. pneumonia*.

Keywords: Extended Spectrum Beta-Lactamases (ESBLs), Klebsiella pneumonia, Urine sample, Prevalence, Benue state.

Introduction

Antimicrobial resistance among bacterial strains is an emerging problem, worldwide. Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and the hospital settings. *Klebsiella pneumoniae* is one of the most pre-dominant pathogens commonly isolated in urine. These uropathogens have also developed resistance to commonly prescribed antimicrobial agents, this severely limits the treatment options of an effective therapy (Olowe *et al*., 2012).

Primarily, this *K. pneumoniae* exerts its antimicrobial resistance against beta-lactams by producing extended spectrum beta-lactamases (ESBLs), enzymes that confers bacterial resistance to all beta-lactams except carbapenems, cephamycins and clavulanic acid. ESBLs are class A beta-lactamases, and are plasmid-mediated enzymes that hydrolyze oxyimino-cephalosporins and monobactams, with various genotypes such as SHV (Spectrum Hydrolysing Enzymes), TEM (Transmission Electron Microscopy), CTX-M (Cefotaxime-Munich), and VEB (Vietnamese extended-spectrum β-lactamase)(Livermore, 2012).

ESBLs are clinically significant and when detected, indicate the need for the use of appropriate antibacterial agents. The mortality rate in misdiagnosed patients with ESBLs producing UTIs have

ranged from 42-100% over the years. Antibacterial choice is often complicated by multi-resistance. There is an increasing association between ESBL production and fluoroquinolone resistance and aminoglycoside resistance (Akinyemi *et al*., 2021).

Multi-drug resistance (MDR) among ESBL expressing strains is complex and is influenced by the location of resistance genes on integrons that possess promoters that drive the co-ordinated expression of downstream resistance cassettes. Thus, multi-resistance has severely limited the treatment options for ESBLs producing strains of Enterobacteriaceae. More recently, the emergence of carbapenem resistance has been reported among ESBL producing organisms (Akinyemi *et al*., 2021).

The prevalence of bacteria producing ESBLs varies world-wide, with reports from North America, Europe, South America, Africa and Asia. There is ample evidence to suggest the spread of ESBL infections is higher in resource poor countries. Limited number of studies had been reported in this regard from Nigeria. This study was thus designed to ascertain the prevalence of extended spectrum B-lactamase producing *Klebsiella Pneumoniae* from clinical isolates obtained from Benue State University Teaching Hospital Makurdi.

3.0. Materials and Method

3.1. Study Area

This study was conducted at Charis Rhema laboratory, Benue State, Makurdi between November to December 2021. Makurdi is the capital of Benue State in Nigeria. The state covers an area of about 34,059 square kilometers with a population of 4.2 million people according to the national census 2006. Temperature range from 26^{o}C to 29^{o}C in dry season and 19.5-24^{o}C in the rainy season with mean latitude 7° 30 ^{1} to 8° 00 ^{1} N and longitude 8° 30 ^{1} to 9 $^{\circ}$ 00 ^{1} E and is situated in the Guinea Savannah area of Nigeria.

3.2. Ethical Approval

Ethical approval was obtained from the hospital's management board on ethics relating to health issues. The purpose of the research was explained to them and their consent sought.

3.3. Collection of Samples

Fifty (50) urine samples were obtained from inpatients of Benue State University Teaching Hospital Makurdi, using wide mouth sterile urine containers. Data on demographic and risk factors of the patients were collected using a structured questionnaire.

3.4. Isolation of Bacteria from Urine Samples

All urine samples collected were cultured on Eosin Methylene Blue Agar (EMBA) and Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^{o}C for 18-24hours. All suspected colonies were identified by Gram staining, colony characteristics, motility and biochemical reactions

3.5. Identification of Bacteria

3.5.1. Cultural Characteristic

Klebsiella pneumoniae is a gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shape bacterium. It appears as a mucoid pink colonies on EMBA and mucoid yellow colonies on CLED agar. They are shorter and thicker when compared to others in the family of Enterobacteriaceae. They can be found singly, in pairs, in chains or linked end to end.

3.5.2. Gram Staining

Gram staining was carried out to identify *klebsiella pnuemoniae*. A clean and grease free slide was used for making a smear. About 0.85% normal saline was placed on the center of the slide and a loopful of well-isolated colony was taken from the solid media using a wire-loop, and it was emulsified in the saline drop forming a thick film. The smear was air-dried for five to ten seconds and heat-fixed by quickly passing the smear over the flame of the Bunsen burner. The smear was

covered with crystal violet and allowed to stand for 1 minute and rinsed gently under tap water. The smear was covered with Gram's iodine and allowed to stand for another one minute and rinsed gently under tap water. Ninety-five (95%) percent alcohol was used to decolorize the smear for 20 – 30 seconds and rinsed again gently under tap water. The smear was covered with safranin for 1 minute and rinsed gently under tap water and air dried. The smear was first observed under the (10x) objective, and then under the oil immersion (100x) objective. The result was a red color with a rod-like shape.

3.5.3. Motility Testing

Motility testing was done by preparing motility test medium in a test tube. Organism was inoculated into the test tube by stabbing the center of the medium to a depth of ½ inch. The tubes were incubated at 37^{o}C for 18-24hrs. The result was a reddish-pink color along the stab line without diffusion, which meant the organism, was non-motile.

3.5.4 Biochemical Identification

The biochemical tests identified were indole negative (-), citrate utilization test positive (+), urease test positive (+), triple sugar iron test positive (+) for lactose, sucrose positive (+), glucose positive (+), yellow slant, yellow butt, gas positive (+) and no H_2S produced.

3.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was determined by Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) recommendations. Antibiotic discs used were ampicillin (10µg), amoxicillin clavulanic acid (30µg) and ceftazidime (10µg). The discs were placed on the inoculated Muller – Hinton agar at a distance of 15mm from the edge of an inoculated plate and about 25mm apart from each disc. The plates were inverted and incubated at 37^{o}C for 18-24jhrs. Zone of inhibition were recorded with a meter rule and results were interpreted according to CLSI standard. (Olowe *et al*, 2012)

3.7. Extended Spectrum B-Lactamase Detection

Inoculum was prepared by suspending the test organism in normal saline inside a sterile test tube and adjusting its turbidity to that of 0.5 Mcfarland standards. Inoculation was done by rolling the cotton swabs, previously dipped into the inoculum suspension with the excess fluid removed by compressing the swab against the inside wall of the container and used to evenly inoculate on the surface of the Muller- Hinton agar plate.

ESBL production was assayed by double disc synergy test (DDST). 10µg of ceftazidime disc (Oxoid, UK) was placed at a distance of 15mm from an amoxicillin-clavulanic acid disc 30µg Oxoid, UK) and 10µg of amoxicillin disc (Oxoid UK) at a distance of 15mm on Muller- Hinton agar plate already inoculated with the test organism. This setup was incubated at 37^{o}C for 18-24hrs. A positive test result was defined as a 5mm or greater increase in the size of the zone diameter for either ceftazidime tested in combination with clavulanic acid vs. the zone for either antibiotic tested alone.

4.0. Results

From this study, ten *Klebsiella pneumoniae* isolates were identified and examined for the *Extended Spectrum Beta-Lactamase* producing potential. 6 (60%) out of the 10 isolates tested were observed to produce ESBL while 4 (40%) did not display the potential for ESBL production as shown in Figure 1.

Table 1 shows the prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing *K. pneumoniae* among the patients attending Benue State University Teaching Hospital, Makurdi based on age group. The highest rate of infection was observed among patients between 26 – 35 years 2 (13.33%) and 46 years and above 1 (11.11%) respectively. No infection was observed among patients between 16 – 25 years of age. No significant relationship was also observed between the rate of infection with ESBL producing *K. pneumoniae* and age group (χ^2=1.857; df=3; P=0.395).

Table 2 shows the prevalence of ESBL producing *K. pneumoniae* among BSUTH patients based on sex. The result shows that females were slightly more infected (15.39%) than the males (8.33%). No significant relationship was however observed between the rate of infection and sex (χ^2=2.130; df=1; P=0.144).

Table 3 shows the prevalence of ESBL producing *K. pneumoniae* in relation to some associated risk factors. The prevalence in relation to difficulty in urination showed an equal rate of infection between those who have difficulty in urination 3(12%) and those without difficulty in urination 3(12%) (χ^2=0.000; df=1; P=1.000).

The prevalence in relation to painful urination showed that the rate of infection was slightly higher among those who had painful urination 3(15.0%) than those without it 3(10%). However, no significant relationship was observed between the infection rate and painful urination (χ^2=1.000; df=1; P=0.317). No significant relationship was also observed in relation to unpleasant urine odour (χ^2=0.391; df=1; P=0.532) as those without unpleasant urine odour had slightly higher infection rate 5(12.50%) than those positive for the infection 1(10%).

The prevalence of infection in relation to the use of catheter to aid urination was also evaluated and the result showed that those who use catheter to aid urination had slightly higher infection rate 2(14.29%) than those who do not use them 4(11.11%). However, no significant relationship was observed in relation to the use of catheter (χ^2=0.360; df=1; P=0.549).

On the frequency of urination, the rate of infection was slightly higher among those who urinate more than two times a day 4(13.33%) compared to those who urinate twice a day 2(10%) and once a day in which had no infection case 0(0.0%). No significant relationship was observed in relation to frequency of urination (χ^2=4.171; df=2; P=0.124).

On the use of antibiotics for the treatment of UTI, those who do not use antibiotics were slightly more infected 4(13.33%) than those who use them 2(10%). No significant association was however observed in relation to the use of antibiotics (P=0.532). in the same vein, those who did not use antibiotics in the last 3 months had slightly higher infection rate 5(12.50%) than those who used them 1(10%). No significant relationship was however observed in relation to the use of antibiotics in the last 3 months (P=0.532).

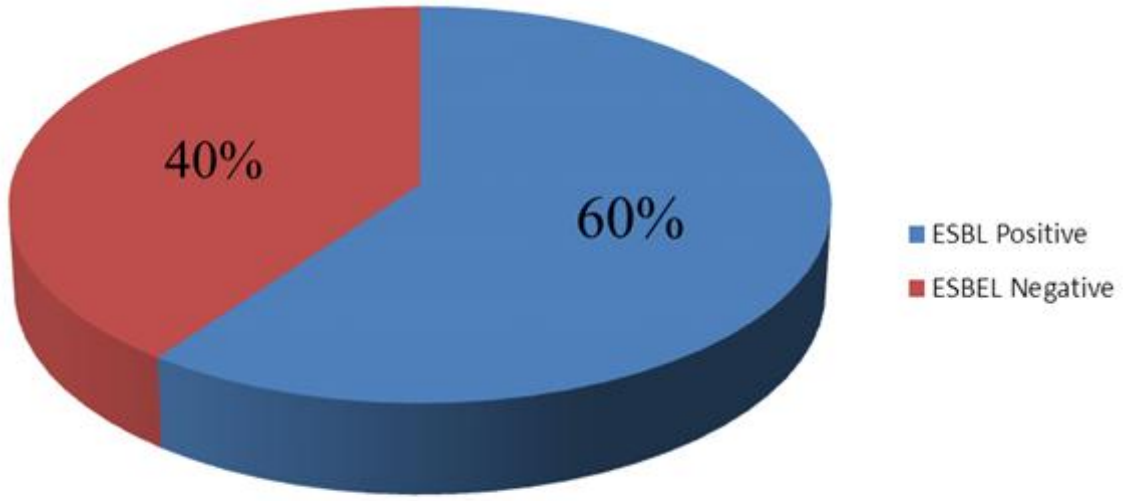

Figure 4.1. ESBL Status of *Klebsiella pneumoniae* isolated from the samples

Table 4.1: the Prevalence of Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* among BSUTH Patients Based on Age

Age Group	No examined	ESBL Positive (%)
16 – 25	9	0 (0.00)
26 – 35	15	2 (13.33)
36 – 45	17	3 (17.65)
46 and above	9	1 (11.11)
Total	50	6 (12.00)

X^2=1.857; df=3; P=0.395

Table 4.2: The Prevalence of Extended Spectrum Beta-Lactamase Producing *K. pneumoniae* Among BSUTH Patients Based on Sex

Sex	No. Examined	ESBL Positive (%)
Male	24	2 (8.33)
Female	26	4 (15.39)
Total	50	6 (12.00)

χ^2=2.130; df=1; P=0.144

Table 4.3: Prevalence of ESBL Producing *K. pneumoniae* in Relation to Some Associated Risk Factors

Risk Factors	No. examined	ESBL Positive (%)	P-value
Difficulty in urination			
Yes	25	3 (12.00)	1.000
No	25	3 (12.00)	
Painful Urination			
Yes	20	3 (15.00)	0.317
No	30	3 (10.00)	
Unpleasant urination			
Yes	10	1 (10.00)	0.532
No	40	5 (12.50)	
Use of catheter to aid urination			
Yes	14	2 (14.29)	0.549
No	36	4 (11.11)	
Frequency of urination			
Once a day	0	0 (0.00)	0.532
Twice a day	20	2 (10.00)	
More than two times daily	30	4 (13.33)	
Duration of admission in the hospital			
<1 week	20	3 (15.00)	0.124
1 wee	14	2 (14.29)	
>1 week	16	1 (6.25)	
Use of antibiotics for the treatment of UTI			
Yes	20	2 (10.00)	0.532
No	30	4 (13.33)	
Use of antibiotics in the last 3 months			
Yes	10	1 (10.00)	0.532
No	40	5 (12.50)	

Table 4.4: Biochemical Characteristics of Isolates

Gram	Motility	Urea	Indole	Cit	Lac	Suc	Glu	St	Butt	H_2S	Gas	Cultural and	Isolates

reaction												Morphological characteristic of isolates		
												CLED	**EMBA**	
-	-	+	-	+	+	+	+	+	A	A	+	Mucoid Yellow colonies	Mucoid pink colonies	***Klebsiella pneumoniae***
-	+	-	-	+	-	-	-	-	K	A	+	Flat Yellow Colonies	Greenish Metallic sheen	***Pseudomonas aeruginosa***
-	+	+	-	+	-	+	+	+	K	A	+	Blue-grey colonies	Colourless	***Proteus mirabilis***
-	+	-	+	-	+	+	+	+	A	A	+	Greenish colonies	Colourless	***Escherichia coli***
-	+	+	+	+	+	+	+	+	K	A	+	Yellow	Purple	***Proteus vulgaris***

Key

+ = Positive

- = Negative

K = Alkaline

A = Acidic

ESBL = Extended Spectrum Beta Lactamases

Discussion

From this study, a prevalence of 12% ESBL producing *K. pneumoniae* was reported in the study area. The higher ESBL negative cases reported in this study could be due to low resistance to antibiotics, or other associated risk factors. The prevalence of 12% ESBL producing *K. pneumoniae* reported in this is lower than the 26.3% reported by Fadeyi *et al*. (2016). The study however corresponds with the work of Olowe *et al*. (2012) who reported a prevalence of 16% for ESBL producing *K. pneumoniae* in urine samples from clinical patients. The varying prevalence rates reported in these studies could be due to the varying sample sizes/populations used by the different researchers.

Age group could not be linked to the positive cases reported in this study. The lack of statistical relationship between age group and ESBL positive cases in this study suggests that ESBL producing *K. pneumoniae* could occur in any age group. However, the higher proportion of positive cases isolated from ages 36-45 could indicate that this group is at higher risk of infection than any other age group in this study. The findings in this study corroborate the work of Mohammed *et al*. (2016) who reported higher cases in older age groups.

Urine isolates from female patients were more likely to be infected with ESBL producing *K. pneumoniae* than males in this study. Although there was no statistical relationship between gender and ESBL positive cases, females were twice as likely to contract the infection than males. This could be because the urinary tract of females is much more susceptible to infection than males, since the male urinary tract is narrow and usually has a self-cleaning mechanism which occurs during urination. This finding conforms with the work of Ugbo *et al*. (2020) who reported higher infection rates in females than males in their study.

The lack of statistical relationship between the associated risk factors and ESBL positive *K. pneumoniae* cases in this study suggests that the population contacted the infection randomly and not due to some associated metric. However, the higher number of ESBL producing *K. pneumoniae* cases reported in patients that observed painful urination suggests that the infection could cause such symptoms. The presence of ESBL positive cases in patients who did not experience any symptoms at

all also suggests that the infection could be asymptomatic. The findings in this study correspond with the work of Akinyemi *et al*. (2021) who reported higher ESBL producing *K. pneumoniae* cases in patients who reported painful urination.

While the use of catheter was not significantly related to infection, the higher infection rate observed suggests that it could be a possible risk factor for the transmission of infection. This is because use and reuse of infected catheter could introduce the organism into the urinary tract and lead to colonization of the system with the organism. The findings in this study disagrees with the work of Azekhueme *et al*. (2015) who reported higher ESBL positive cases in patients who used catheter to aid urination.

The higher infection rates in patients who urinated more than twice daily could be due to several reasons. The use of contaminated toilets, use of infected catheter, and cleaning of the urinary tract (with contaminated hands) could be some of the reasons for the higher infection noticed. The higher infection rates noted in patients who had been admitted for less than a week in the hospital suggests that the infection could not be nosocomial after all.

The findings from this study suggest that the use of antibiotics reduced the likelihood of contracting ESBL producing *K. pneumoniae* as compared with individuals who did not. While modern literature has reported the resistance of bacteria to antibiotics hence leading to higher infection and reinfection rates, the findings in this study show otherwise. This finding disagrees with the work of Akanbi *et al*. (2013) who stated that the use of antibiotics was a major predisposing factor for the higher infection rates of ESBL producing *K. pneumoniae* in urine samples of sampled respondents.

Conclusion

The prevalence reported in this study is moderately low compared to the works of other authors in the country. There was no statistical relationship between socio-demographic factors (age and sex) and infection, although higher infection was noted in females than males, and in patients between ages 36 – 45 than any other group. There was also no statistical relationship between ESBL producing *K. pneumoniae* and the associated risk factors in this study. Hence, the ESBL producing *K. pneumoniae* can be contracted by patients irrespective of age, gender or risk factors in this study.

Recommendations

From the finding in this study, it is recommended that:

1. Patients exhibiting symptoms and those without symptoms be screened for the presence of ESBL producing *K. pneumoniae*.
2. Patients be screened routinely for the infection since it could be asymptomatic as reported in this study.
3. More research be carried out on the prevalence of the infection in the study area as there is little or no information to serve as reference material for future studies.