ejpmr, 2018,5(1), 13-17

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DETECTION OF COMMUNITY-ACQUIRED AND EXTENDED SPECTRUM BETA LACTAMASE-PRODUCING UROPATHOGENS IN A SUB-URBAN COMMUNITY IN RIVERS STATE, NIGERIA.

Easter Godwin Nwokah*[1] and Smart Enoch Amala[1]

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Easter Godwin Nwokah**

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

Article Received on 25/10/2017 **Article Revised on 15/11/2017** **Article Accepted on 05/12/2017**

ABSTRACT

Extended-spectrum β-lactamase-producing (ESBLs) organisms are a major challenge today in hospital and community-acquired infections. These plasmid-mediated enzymes hydrolyze extended spectrum Cephalosporins like Ceftazidime, Cefotaxime which are used in the treatment of urinary tract infections (UTI) and rendering them ineffective. This study was undertaken to determine the prevalence of extended spectrum beta lactamase-producing uropathogens in a sub-urban setting. 250 mid-stream clean catch urine samples were collected randomly from consenting subjects at Nchia, Eleme, Rivers State. Samples were analysed microbiologically for significant bacteriuria. Significant asymptomatic bacteriuria was detected in 92 (36.8%) of the total study population of 250 subjects. Distribution of the isolates showed *Escherichia coli* as the highest occurring isolate (40.2%), followed by *Klebsiella pneumoniae* (26.1%). The least isolated is *Pseudomonas* spp (1.1%). Study also showed 26 (28.3%) of the isolates as ESBL producers with *Escherichia coli and Klebsiella pneumoniae* accounting for 57.7% and 26.9% respectively. Furthermore, based on species, 15 (40.5%) of 37 *Escherichia coli and* 7 (29.2%) of 24 *Klebsiella pneumoniae were* ESBL producers. On antibiotic susceptibility testing, isolates showed highest susceptibility to the Carbapenems- Meropenem (100%) and Imipenem (96.7%). All the ESBL isolates were susceptible to the carbapenems emphasizing their high therapeutic value. Susceptibility to Ciprofloxacin was 82.6% while the Cephalosporins- Ceftazidime, Cefotaxime and Ceftriaxone – showed 66.3%, 65.2% and 63.0% susceptibilities respectively. Highest level of resistant was observed in ampicillin (6.5%). Most of the ESBL producing isolates were multidrug resistant. Besides antimicrobial susceptibility testing, it has become very necessary, in our setting, to include routine screening for ESBL to guide therapy for UTI.

KEYWORDS: Urinary tract infection, Antimicrobial Resistance, Extended spectrum β-lactamases.

INTRODUCTION

Resistance of pathogenic microorganisms to many antimicrobial agents hitherto used for the treatment of indicated infectious diseases has become a global problem with far-reaching consequences. There is an alarming increase of antibiotic resistance in bacteria that cause either community-acquired infections or hospital-acquired infections, including Urinary tract infections and Asymptomatic bacteriuria, and of particular interest are the multidrug resistant pathogens of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumanni, Methicillin-Resistant Staphylococcus aureus, Vancomycin-Resistant Enterococcus* and Extensively Resistant *Mycobacterium tuberculosis.*[1] The increased use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributory to the phenomenon.

The beta lactamases are the collective name of enzymes that open the β-lactam ring by adding a water molecule to the common β-lactam bond form and this inactivates the beta-lactam. This hydrolyzation was first observed in 1940 by Abraham and Chain in a strain of *E. coli.*[2] However, the clinical effect of such hydrolyzation was not noted until the beginning of the 1950s, when the first β-lactam resistant *S. aureus* isolates appeared in hospitals.[3]

Beta-lactam antimicrobial agents have been the common treatment options for bacterial infections but also continue to be the promoting cause of resistance to β-lactam antibiotics among Gram-negative bacteria worldwide. The frequent exposure of bacterial strains to a multitude of β-lactams induces dynamic and continuous production and mutation of β-lactamases in these bacteria, thereby expanding their activity even against the newly developed β–lactam antibiotics. These enzymes are known as extended-spectrum betalactamases (ESBLs).[4,5]

Extended-spectrum β-lactamases (ESBLs) have the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid and the carbapenems.[6,7] Various classes of *β*-lactamases have been described and typically, they derive from genes for TEM-1, TEM-2, or SHV-1 and CTX-M, which are the most extensive and clinically relevant.[8-10] The ESBLs are frequently plasmid encoded[11] and these plasmids usually carry genes encoding resistance to other antimicrobial agents. The presence of ESBLs carries tremendous clinical significance.[12] Incidents of Extended spectrum betalactamase-producing organisms-related infections, first reported in 1983 from Germany in isolates of *Klebsiella pneumonia*[13], has continued to increase globally.[14] Production of extended-spectrum β-lactamases (ESBLs) is a significant resistant mechanism that impedes the antimicrobial treatment of infections caused by enterobacteriaceae and a serious threat to the currently available antibiotic armory.[15,16]

Inspite of the growing data on ESBL-related uropathogenic infections at cities and urban centers and their obvious consequences, there appears to be paucity of information on this subject in our local setting. Knowledge of local epidemiology is required to define the practical treatment for patients with related infections.

The aim of this study was to determine the prevalence of Community-acquired and ESBL-producing gram-negative uropathogens in Nchia, Eleme- a sub-urban setting in Rivers State of Nigeria.

METHODOLOGY

Study Area

This cross sectional study was carried out between January and June, 2015 in Nchia, a sub-urban community covering an area of 138km^2 and located in Eleme Local Government Area of Rivers State in the Niger Delta region of Nigeria. It is located east of Port Harcourt Local Government Area. Nchia, with a population of about 190,884 is predominated with mostly farmers and fishermen.

Specimen collection and processing

A total of two hundred and fifty (250) apparently healthy consenting adults at Nchia Eleme, Rivers State were used as subjects. The criteria for selection of volunteers included no recent history of antibiotic intake in the previous three (3) weeks and no history of hospitalization or visit to a health facility in the past six months. Short structured questionnaires were also administered for demographic and other relevant information.

Clean-catch, mid-stream urine collected aseptically from two hundred and fifty consenting subjects, were processed for detection of significant asymptomatic bacteriuria following standard laboratory protocol.[17] All significant isolates, following identification, were preserved and stored at +4^{O}C until used for antimicrobial susceptibility and ESBL testing.

Antimicrobial Susceptibility Testing

This was done using disc diffusion method of Bauer-Kirby[18] and the interpretative criteria set by Clinical and Laboratory standard institute.[19] Briefly, inocula of bacteria were prepared to 0.5 McFarland standards. Sterile swab stick was dipped into the bacteria suspension and used to streak the Mueller Hinton agar (Oxoid, UK). After seeding the agar plate with the test organism, the disc was placed and plate incubated at 37^{O}C for 18-24 hours. The following third generation Cephalosporins- Ceftriazone (CRO), Ceftazidime (30µg), Cefotaxime (30µg) were tested. Other antimicrobial agents tested included Meropenem (10µg), Imipenem (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Augmentin (30µg), Gentamicin (10µg), Nitrofurantoin (300µg), Cotrimoxazole (25µg), Tetracycline (30µg) and Ampicillin.

Detection of extended-spectrum beta lactamase production

All Gram-negative isolates were tested for ESBL production by two phenotypic confirmation methods as per CLSI guidelines.

Screening by Modified Double disc diffusion approximation/ synergy test (MDDST)

Using this method, all the isolates which showed a diameter less than 27mm for cefotaxime and less than 25mm for ceftazidime were selected for detection of ESBL production. The ESBL production was tested by modified double disc synergy test (MDDST) by using a disk of amoxicillin-clavulanic acid (20/10µg) along with cephalosporins. A culture of Mueller-Hinton agar was used as recommended by CLSI. A disc which contains amoxicillin – clavulanic acid (20/10µg) was placed in the centre of the plate. Then discs of ceftazidime (30µg) and cefotaxime (30µg) were placed 15mm and 20mm apart respectively, centre to centre to that of amoxicillin–clavulanic acid (20/10µg). Plates were incubated at 37°C for 18-24 hrs. A clear distortion or increase in the zone about 5mm or more towards the discs of amoxicillin–clavulanic acids was considered as positive for ESBL production. Known *Klebsiella and E.coli* was used as a negative and positive control respectively for ESBL production.

Combined Disk (Double Disc Synergy) method

Phenotypic confirmatory test (Combination disc method)[20] was carried out. On a Mueller Hinton Agar was inoculated suspect test organisms matched to 0.5 MacFarland turbidity standards. Ceftazidime (30µg) alone and in combination with clavulanic acid (30 µg/10 µg) and cefotaxime alone and in combination with clavulanic acid (30 µg/10 µg) were placed at a distance of 20 mm apart. The plates were incubated at 37°C for 18-24 hrs. The test organism was considered to produce ESBL if the zone size around the combined

ceftazidime/clavulanic acid or cefotaxime/clavulanic acid increased by ≥5mm in comparison to ceftazidime or cefotaxime disks alone. This increase occurred because the β lactamases produced by the isolates were inactivated by clavulanic acid. *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922) were used as positive and negative controls respectively.

RESULTS

Significant asymptomatic bacteriuria was detected in 92 (36.8%) of the total study population of 250 subjects. Table 1 shows the distribution of the isolates. *Escherichia coli* ranked the highest occurring isolate (40.2%), followed by *Klebsiella pneumoniae* (26.1%). The least isolated is *Pseudomonas* spp.

Table 2 shows 26 (28.3%) of the isolates as ESBL producers with *Escherichia coli* and *Klebsiella pneumoniae* accounting for 57.7% and 26.9% respectively. Furthermore, based on species, 15 (40.5%) of 37 *Escherichia coli* and 7 (29.2%) of 24 *Klebsiella pneumoniae* were ESBL producers.

Table 3 shows Antimicrobial Susceptibility profile of 92 Isolates. Isolates showed highest susceptibility to the Carbapenems- Meropenem (100%) and Imipenem (96.7%). Susceptibility to Ciprofloxacin was 82.6% while the Cephalosporins- Ceftazidime, Cefotaxime and Ceftriaxone- showed 66.3%, 65.2% and 63.0% susceptibilities respectively. Highest level of resistant was observed in ampicillin (6.5%).

Table 1: Frequency and Percentage occurrence of the isolates.

Isolates	Frequency	Percentage of Occurrence (%)
Staphylococcus aureus	9	9.8
Coagulase-Negative Staphylococcus	3	3.3
Klebsiella pneumoniae	24	26.1
Escherichia coli	37	40.2
Proteus spp	18	19.6
Pseudomonas aeruginosa	1	1.1
Total	**92**	**100%**

Table 2: Occurrence of ESBL-Producing Organisms.

No of Organism Tested	No. of ESBL Producers (%)	Percentage of Total ESBL Producers (%)
Gram-Negative Organisms:		
Escherichia coli (37)	15 (40.5)	57.7
Klebsiella pneumoniae (24)	7 (29.2)	26.9
Proteus spp (18)	3 (16.7)	11.5
Pseudomonas aeruginosa (1)	1(100)	3.8
Gram-Positive Organisms:		
Staphylococcus aureus (9)	0	-
Coagulase-Negative *Staphylococcus (3)*	0	-
Total (92)	26 (28.3)	100

Table 3: Antimicrobial Susceptibility Profile of 92 Isolates.

Antibiotic tested	No. of isolates susceptible	Percentage Susceptible (%)
Meropenem (10µg)	92	100
Imipenem (10µg)	89	96.7
Ciprofloxacin (5µg)	76	82.6
Levofloxacin (5µg)	71	77.2
Augmentin (30µg)	64	70.0
Ceftazidime (30µg)	61	66.3
Cefotaxime (30µg)	60	65.2
Ceftriaxone (30µg)	58	63.0
Gentamicin (10µg)	50	54.3
Nitrofurantoin (300µg)	38	41.3
Cotrimoxazole (25µg)	29	31.5
Tetracycline (30µg)	15	16.3
Ampicillin	6	6.5

DISCUSSION

Urine samples from 250 Subjects in a community setting were analyzed for the isolation of organisms which produce ESBL. Significant asymptomatic bacteriuria was detected in 92 (36.8%). Sule and Kumurya[21] in a study of UTI in Kano, Nigeria reported a 30.5% significant

culture. Prevalence of asymptomatic bacteriuria varies from one geographical region to another as well as from one facility to another, some of the factors being hygiene and other risk factors.

Of the uropathogens isolated, *Escherichia coli* ranked the highest occurring isolate (40.2%), followed by *Klebsiella pneumoniae* (26.1%) while the least isolated was *Pseudomonas aeruginosa* (1.1%). The ranking in occurrence of organisms in this study concurs with reports of Sule and Kumurya,[21] that *Escherichia coli* was the most frequent organism responsible for UTI with 29(14.5%), followed by *Klebsiella* species with 16 (8.0%). Again occurrence varies from institution to institution.

The prevalence of ESBL producers varies across continents and countries and also within hospitals. In Nigeria, the prevalence rate varies in different institutions with some findings ranging from 26% in Enugu,[22] 41.7% in Port Harcourt[16] to 80.0% in Ebonyi.[23] This study revealed that 28.3% of isolates were ESBL-producing uropathogens and this is a cause for concern and therefore, poses a significant threat to the current antibiotic armory. This is moreso against the backdrop of the pathogens being community-acquired. ESBL detection is not routinely carried out in many laboratories in our setting, largely due to lack of awareness or due to limited resources to conduct ESBL detection. Therapeutic options against infections due to ESBL producers have also become increasingly limited. The spread of ESBL-producing bacteria has been expanding rapidly worldwide, underscoring the need for continuous monitoring and effective infection control measures.

E. coli accounted for 57.7% of the ESBL-producing uropathogens in this study while rate of recovery for *K. pneumoniae and Proteus spp* were 26.9% and 11.5% respectively. In a study at a tertiary hospital in Mwanza, Tanzania, Mshana *et al*.,[24] reported a growing rate of ESBL prevalence: 64% for *K. pneumoniae* and *24%* for *E.coli*. In another related study in India, Subitha and Sornajeyanthi,[25] reported 43% ESBL producers among uropathogens with *E.coli* and *Klebsiella pneumoniae* accounting for 50% and 32% respectively.

On antibiotic susceptibility testing, isolates showed highest susceptibility to the Carbapenems- Meropenem (100%) and Imipenem (96.7%). All the ESBL isolates were susceptible to the carbapenems, emphasizing their high therapeutic value. Susceptibility to Ciprofloxacin was 82.6% while the Cephalosporins- Ceftazidime, Cefotaxime and Ceftriaxone – showed 66.3%, 65.2% and 63.0% susceptibilities respectively. Highest level of resistant was observed in ampicillin (6.5%). The therapeutic options for EBSL-producing organisms are very limited. The carbapenems are still the first choice of treatment for serious infections with ESBL-producing organisms as exemplified in our findings and this also corroborates the reports of Perez *et al.*[26] Unfortunately, the costs of the more effective agents are prohibitive in our setting, leaving Patients with options that may portend some serious consequences and ultimately more selective pressure on organisms.

CONCLUSION

Significant bacteriuria and the prevalence of ESBL were found to be 36.8% and 28.3% respectively in our study setting and this is of great concern. ESBLs constitute a serious threat to currently available antibiotics. Thus, adequate sensitization on the use/misuse of antibiotics in the community and its underlying consequences is needful. Clinical laboratories in our setting should include routine screening for ESBL in their protocols to guide therapy for UTI. Enhanced infection control practices and improved personal and environmental hygiene are instructive to preventing the spreading and outbreaks of ESBL producing organisms.

REFERENCES

1. Alekshun, M. N. and Lay, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 128: 1037 – 1050.
2. Abraham, E.P. and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Nature*, 146-837.
3. Jacoby, G. A. (2009). AmpC beta-lactamases. *Clinical Microbiology Review*, 22(1): 161 -182.
4. Pitout, J. D. and Laupland, K. B. (2008). Extended-spectrum β-lactamase-producing enterbactenaceaes an emerging public health concern. *The Lancet Infectious Disease*, 8(3): 159 – 166.
5. Paterson, D. L. and Bonomo, R. A. (2005). Extended spectrum beta-lactamases, a clinical update. *Clinical Microbiology Review*, 18: 657–686.
6. De Champs, C., Sirot, D., Chanal, R., Bonnet, M. J. and J. Sirot (2000). A 1998 survey of extended-spectrum beta-lactamases in *Enterobacteriaceae* in France. The French Study Group. *Antimicrobial Agents Chemotherapy*, 44: 3177-3179.
7. Du, B., Long, H., Liu, D., Chen, D., Liu, Y., Xu, U. and Xie, X. (2002). Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bloodstream infection: Risk factors and clinical outcome. *Intensive Care Medicine*, 28: 1718-1723.
8. Dumarche, P., DeChamps, D., Siroit, C., Chanal, R., Bonnet, E. and Sirot, J. (2002). TEM derivative-Producing Enterobacter aerogenes strains: Dissemination of a prevalent clone. *Antimicrobial agents Chemotherapy*, 46: 1128 – 1131.
9. AitMhand, R. A., Soukri, N. Moustaoui, H. Amarouch, N. ElMdaghri, D. Sirot, M. and Benbachir, A. (2002). Plasmid- mediated TEM-3 extended-spectrum beta-lactamase production in *Salmonella typhimuium* in Casablanca. *Journal of Antimicrobial Chemotherapy*, 49: 169-172.

10. Philippon, A. (2013) Les bêta-lactamases à spectre élargi ou étendu (BLSE). *Immuno-analyse & Biologie Spécialisée*, 28: 287-296.
11. Babini, G. S. and Livermore, D. M. (2000). Are SHV beta- lactamases universal in *Klebsiella pneumoniae*? *Antimicrobial Agents Chemotherapy*, 44: 2230.
12. Bell, J. M., Turnidge, J. D., Gales, M. A., Pfaller, A. C. and Jones, R. N. (2002). Prevalence of extended spectrum beta-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998- 99). Diagnostic Microbiology. *Infectious Disease*, 42: 193-198.
13. Shukla, I., Tiwari, M. and Agrawai, M. (2004). Prevalence of extended-spectrum-lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital, *Indian Journal of Medical Microbiology*. 22(2): 87-91.
14. Rupinder, B., Gecta, W. and Shikha, J. (2013). Prevalence of extended spectrum β-lactamases in multidrug resistant strains of gram negative bacilli. *Journal of Academic Industrial Reserves*, 1(9): 558–560.
15. Sibhghatulla, S., Jamale, F., Shazi, S. and Syed, M. (2014). Antibiotic resistance and extended-spectrum beta lactamases types epidemiology and treatment. *Saudi Journal of Biological Sciences*, 12(3): 1920–1923.
16. Horsfall S. J, Abbey S. D, Nwokah E. G and Okonko I.O. (2017). Prevalence of Extended-Spectrum Beta-lactamases (ESBLs) and Plasmid status of *Eschericha coli* and *Klebsiella pneumoniae* isolates from clinical sources in UPTH, Port-Harcourt, Nigeria. *New York Science Journal*, 10(3): 29-39.
17. Cheesebrough M (2006). District laboratorypractice in tropical countries. Part 2. Cambridge University Press, p. 357.
18. Bauer, A. W., Kriby, W. M., Sherris, W. M. and Turk, J. C. (1966). Bauer-Kirby standardized, single disc susceptibility, test for rapid growing pathogens. *American Journal of Clinical Pathology*, 45: 493-498.
19. Clinical Laboratory Standard Institute (2012). Performance standards for antimicrobial susceptibility testing; twenty- second informational supplement. Clinical Laboratory Standard Institute. Wayne, Pennsylvania, USA. 32: 70–71.
20. Paul, C. Schreckenberger, 2010. Changes in the CLSI standards for antibiotic susceptibility testing for, 2010.
21. Subha, A., V. Renuka Devi and S. Ananthan, Amp, C. 2003. B lactamase producing multidrug resistant strains of Klebsiella spp. & Escherichia coli isolated from children under five in Chennai, *Indian J Med Res*, 117: 13-18.
22. Sule H. and Kumurya A. S. The Prevalence of Klebsiella Species Causing Urinary Tract Infections in Murtala Muhammad Specialist Hospital, Kano, Nigeria. *American Journal of Biomedical and Life Sciences*. 2016; 4(2): 11-15.
23. Ejikeugwu C, Ikegbunam M, Ugwu C, Eze P, Iroha I, Esimone C. Phenotypic Detection of *Klebsiella pneumoniae* Strains – Producing Extended Spectrum β-Lactamase (ESBL) Enzymes. Scholarly Academic Journal of Biosciences. 2013; 1: 20-23.
24. Nwakaeze EA, Anyim C, Ngwu NJ, Nwankwo C (2013). "Extended-Spectrum β-Lactamase – Producing *Klebsiella pneumonia* and *Escherichia coli* from Blood Cultures of Hospitalized Patients in Abakaliki Metropolis." *American Journal of Infectious Diseases and Microbiology*, 1.4: 75-78.
25. Mshana, S. E., Kamugisha, E., Mirambo, M., Chakraborthy, T. and Lyamuya, E. F. (2009). Prevalence of multi resistant gram negative organisms in a tertiary hospital in Tanzania. *Journal of Antimicrobial Chemotherapy*, 26(2): 49 – 53.
26. Subitha, B. and Sornajeyanthi, P (2015). Evaluation of Extended Spectrum Beta Lactamase in Gram Negative Urinary Isolates. *International Journal of Current Research in Life Sciences, 4(7): 269-271.*
27. Perez, F., Endimiani, A., Hujer, K.M. and Bonomo, R.A. (2007). The continuing challenge of ESBLs. *Current Opinion of Pharmacol*ogy, 7: 459-469.