Asian Journal of Research in Medicine and Medical Science

Volume 6, Issue 1, Page 120-127, 2024; Article no.AJRMMS.1620

Prevalence and Pattern of Extended Spectrum Beta-Lactamases (ESBL) Producing *Klebsiella pneumoniae* in Biological Specimen at a Tertiary Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.globalpresshub.com/review-history/1620

Original Research Article

Received: 22/04/2024
Accepted: 26/06/2024
Published: 29/06/2024

ABSTRACT

Aim: The study was carried out to assess the distribution and pattern of ESBL producing *Klesbsiella pneumoniae* in specimen analysed at the Microbiology laboratory of a tertiary healthcare center.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Medical Microbiology, University of Port Harcourt Teaching Hospital, Rivers state, Nigeria carried out between January 2019 to June 2019

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Cite as: *Jonah, A. A., O. K. Obunge, and K. T. Wariso. 2024. "Prevalence and Pattern of Extended Spectrum Beta-Lactamases (ESBL) Producing Klebsiella Pneumoniae in Biological Specimen at a Tertiary Hospital". Asian Journal of Research in Medicine and Medical Science 6 (1):120-27. https://jofmedical.com/index.php/AJRMMS/article/view/75.*

Methods: Clinical specimen including blood, wound biopsy/aspirate, urine and sputum specimens were collected and processed according to standard methods. Blood agar, Cysteine Lactose Electrolyte Deficient agar (CLED) and MacConkey agar were used to culture the specimens to isolate *K. pneumoniae.* A confirmatory test was carried out on all suspected ESBL isolates using combination disks according to the CLSI guidelines.
Results: The study reports a prevalence rate of 34.7% (118/340) for ESBL-production among *K. pneumoniae* isolates in UPTH, Port Harcourt. Blood culture was found to have the highest proportion of *ESBL K. pneumoniae* (57.1%) while urine samples had the least proportion of *ESBL K. pneumoniae* growth (32.5%). There was no significant distribution of *ESBL K. pneumoniae* growth by age groups or gender among the patients sampled. However, the occurrence of *ESBL K. pneumoniae* was higher among in-patients (37.0%) compared to outpatients (28.7%).
Conclusion: The growth of *ESBL K. pneumoniae* was observed to be higher among inpatient subject indicative of the likelihood of high prevalence *ESBL K. pneumoniae* in hospital acquired infection. Therefore, there is a great need for urgent interventions in the areas of antimicrobial usage and infection prevention and control in our settings.

Keywords: Antibiotics; prevalence; extended spectrum beta-lactamase; klebsiella pneumoniae.

1. INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are a heterogeneous group of plasmid-mediated bacterial enzymes that confer significant resistance to oxyimino-cephalosporin and monobactam antimicrobials [1]. They are however inhibited by beta-lactamase inhibitors such as clavulanic acid [2]. There is a global variation even in closely related regions of the prevalence of ESBLs. Reports have shown that ESBL-producing bacteria are prevalent in clinical and community settings with serious public health consequences [3,4]. A study conducted by Mohammed et al. in Maiduguri Nigeria, recorded a prevalence of 23.8% and 30.0% ESBL-producing *Escherichia coli* and *Klebsiella* spp, respectively [5]. *Klebsiella pneumoniae,* a gram negative bacillus, is one of the common pathogens associated with multiple drug resistance and an important source of transferable antibiotic resistance [6]. There has been a significant increase of ESBLs among clinical isolates caused by the Enterobacteriaceae in the last three decades [7–9]. Consequently, the occurrence of ESBL has been a major challenge in both the healthcare settings and local communities. The consequences of ESBL (Extended-Spectrum Beta-Lactamase) Klebsiella infections in hospital settings can be significant and potentially life-threatening and are associated with treatment difficulties, spread of infection, especially in high-risk areas such as intensive care units, Increased morbidity and mortality, while increasing the Length of stay and costs and exacerbating the current challenge of antimicrobial resistance [1,3,7,10]. Therefore, it is important to implement effective infection control measures, use antibiotics judiciously, and promote the development of new treatments for ESBL Klebsiella infections to reduce their impact in hospital settings. The current study was carried out to assess the distribution and pattern of ESBL producing *Klesbsiella pneumoniae* in specimen analysed at the Microbiology laboratory of a tertiary healthcare center [11].

2. METHODS

2.1 Study Area

This study was conducted at the University of Port Harcourt Teaching Hospital (UPTH) located at 4°53’59,4N, 6° 55’45.6E in Rivers State, Nigeria. The State is a cosmopolitan area full of industrial activities especially in the oil and gas sector. The State has a population of 5 million people and is bounded on the South by the Atlantic Ocean, to the North by Imo, Abia and Anambra States, the East by Akwa-Ibom State and to the West by Bayelsa and Delta States. It is home to 3 major indigenous ethnic groups: Ijaw, Ikwerre and Ogoni. People from diverse cultural and ethnic backgrounds also live and work in the State. The University of Port Harcourt Teaching Hospital has an estimated bed capacity of 830 and an estimated 200,000 patients are seen annually. It is a tertiary healthcare institution with specialist consultants in various medical specialties that serves as a referral centre in Rivers State and neighbouring States including Bayelsa, Imo and Abia.

2.2 Study Population

The study population included all individuals attending the outpatient clinic and those on

admission at the different wards of the study centre from whom various clinical specimens were collected. The individuals selected for the study included those that were recommended for microbiological investigations by the attending physicians.

2.3 Sample Size

The sample size was determined using the Fischer's formula [12], n= (Z^2pq)/d^2, where Z is standard deviation corresponding to a specific confidence interval = 1.96 (at confidence interval 95%), p= the prevalence of ESBLs from a similar study [1] = 28.0% (0.331), q= 1.0-p, and d is degree of accuracy desired (usually set at 0.05). Therefore, n **=** 3.84 x 0.28 x 0.72/0.0025 = 309.78 + 10% attrition for incomplete data = 340.76.

2.4 Sampling Method

Patients from whom *Klebsiella pneumoniae* were isolated from their clinical specimens (urine, wound biopsies/aspirates, blood and sputum) at the UPTH Medical Microbiology and Parasitology Laboratory between January and June 2019, were selected by systematic random sampling based on sampling interval k = N/n where N= 1600 (Estimated number of individuals referred for microbiological examination at the study center) and n = 340 (required sample size).

2.5 Specimen Collection and Analysis

Clinical and basic demographic information were obtained from selected patients using a PROFORMA data collection sheet. The information collected included age, gender, ward/clinic and specimen types. The specimen collected included wound aspirate, urine, blood and sputum specimen and all specimen were cultured on processed according to standard procedures as previously described. For blood samples were inoculated medium was aerobically incubated at 35 – 37^{o}C for 7 days, urine samples were inoculated on Blood agar and Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated aerobically at 35 – 37^{o}C for 18-24hours, sputum samples and wound/aspirate samples were cultured on MacConkey agar plate and incubated aerobically at 35 – 37^{o}C for 18-24hours [13] The ESBL confirmatory test was carried out on all the suspected ESBL isolates using the combination disks according to the CLSI guidelines [14].

2.6 Data Analysis

The data were analyzed using the Epi Info v 7 software and presented in tables or charts as appropriate. Variables such as sex, age group, wards, and departments were expressed as frequencies and proportions. The Chi-square statistic was used to compare the distribution of ESBL and non ESBL- producing isolates by gender, age groups and patient types. All analyses were done at a 95% confidence interval and a p-value of less than 0.05 was considered significant.

3. RESULTS

A total of 340 isolates were obtained from specimen collected from patients within a six-month period (January to June 2019). Table 1 shows the demographic distribution of the patients with *Klebsiella pneumoniae* positive specimen. Of the 340 *K. pneumoniae*, 127 (37.4%) were from male subjects and 213 (62.6%) were from female subjects. The age distribution showed that 6 (1.8%) were between 40 – 49 years old, 10 (2.9%) were at least 60 years old, 14(4.1%) were at least 50 – 59 years old, 26 (7.6%) were less than 20 years old, 127 (37.4%) were between 30 – 39 years old and 157 (4.2%) were between 20 – 29 years old.

Table 1. Demographic characteristics of subjects with confirmed *Klebsiella pneumoniae* isolates

Variable	Frequency (n = 340)	Percentage
Gender	127	37.4
Male		
Female	213	62.6
Age Groups (years)		
< 20	26	7.6
20 – 29	157	46.2
30 – 39	127	37.4
40 – 49	6	1.8
50 – 59	14	4.1
>60	10	2.9

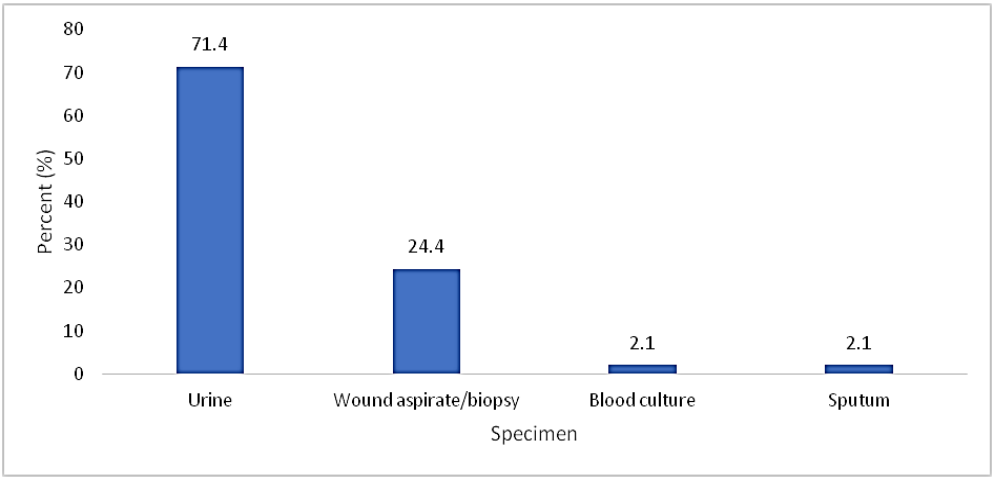

Fig. 1. Distribution of specimen positive for K. pneumoniae growth

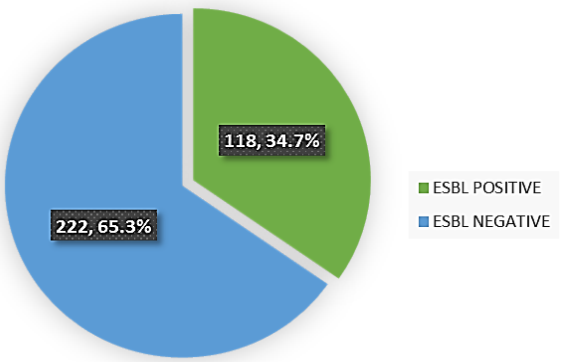

Fig. 2. Prevalence of ESBL -producing *Klebsiella pneumoniae*

Table 2. Distribution of ESBL producing *Klebsiella pneumoniae* from different specimen

Specimen	ESBL positive (n, %)	ESBL negative (n, %)	Total (n, %)	Chi-square (p-value)
Sputum	3(42.9)	4(57.1)	7(100.0)	
Blood Culture	4(57.1)	3(42.9)	7(100.0)	
Wound aspirate/biopsies	32(38.6)	51(61.4)	83(100.0)	2.80 (0.4203)
Urine	79(32.5)	164(67.5)	243(100.0)	

Table 3. Gender and Age-related distribution of *Klebsiella pneumonia*

Variable	ESBL-positive (n, %)	ESBL-negative (n, %)	Total (n, %)	Chi-square (p-value)
Gender				
Male	42(33.1)	85(66.9)	127(100.0)	0.23 (0.6248)
Female	76(35.7)	137(64.3)	213(100.0)	
Age Groups (years)				
< 20	11(42.3)	15(57.7)	26(100.0)	
20 – 29	54(34.4)	103(65.6)	157(100.0)	
30 – 39	46(36.2)	81(63.8)	127(100.0)	4.31 (0.546)
40 – 49	0(0.0)	6(100.0)	6(100.0)	
50 – 59	4(28.6)	10(71.4)	14(100.0)	
>60	3(30.0)	7(70.0)	10(100.0)	

Table 4. Distribution of ESBL and Patient Types

Source	ESBL Positive n, (%)	ESBL Negative n, (%)	Total (n, %)	Chi-Square (p-value)
Inpatient	91(37.0)	155(63.0)	246(100.0)	2.05 (0.152)
Outpatient	27(28.7)	67(71.3)	94(100.0)	

Fig. 1 shows the distribution of the different specimen positive for *Klebsiella pneumoniae* growth. There were 7 (2.1%) sputum, 7 (2.1%) Blood culture, 83 (24.4%) wound aspirate/biopsies, and 243 (71.5%) urine samples.

Fig. 2 shows that a 34.7% (n = 118) prevalence of ESBL producing *K. pneumoniae* isolates among the 340 *K. pneumoniae* isolates identified in the study.

Table 2 shows the distribution of ESBL Klebsiella pneumoniae producing isolates by the different specimen was not statistically significant in the different samples (χ^2 = 2.80, *p* = 0.4203). The table showed that the highest proportion of ESBL producers was found in blood culture specimen (57.1%), followed by sputum samples (42.9%), wound aspirates/biopsies (38.6%) and urine samples (32.5%)

The distribution of the ESBL-producers by gender was not statistically significant ($\square^2$ = 0.23, *p* = 0.6248). the data showed that ESBL *K. pneumoniae* distribution was higher in female subjects (35.75) compared to male subjects (33.1%). The findings also showed that the distribution of ESBL *K. pneumoniae* was highest in persons less than 20 years (42.3%), followed by persons between 30 – 39 years (36.2%), while the age group with the least distribution of ESBL *K. pneumoniae* was the persons between 50 – 59 years (28.6%), with persons between 40-49 years not having any growth of ESBL *K. pneumoniae*. However, The distribution of ESBL producing *K. pneumoniae* in the different age groups was not statistically significant (χ^2 = 4.31, *p* = 0.546).

Table 4 shows the distribution of the ESBL-producing isolates and patient types, 37% of the inpatient subjects had ESBL *K. pneumoniae* compared to 28.7% in the outpatient subjects. However, the distribution of ESBL *K. pneumoniae* was not statistically significant (χ^2 = 2.05, *p* = 0.152).

4. DISCUSSION

Urine specimen had the highest proportion of *Klebsiella* growth (71.5%), followed by wounds/aspirates (24.4%), while sputum samples (2.1%) had the least proportion of *Klebsiella* growth. This is consistent with reports of studies that isolated *Klebsiella pneumoniae* in clinical settings, as similar studies report that urine samples and wound/aspirates were the most common sources of *Klebsiella pneumoniae* isolated in a variety of clinical settings [2,9,15,16].

There was a 34.7% prevalence of ESBL *klebsiella* among the 340 *klebsiella* isolates observed in the current study. The prevalence of ESBL producing *K. pneumoniae* was observed to be 34.7%. This was consistent with the findings of previous studies done by Adeyankinnu *et. al*, [17] and Kiratisin *et. al*. [18] The clinical samples were varied in distribution with urine as the highest in frequency 216 (63.5%) and blood culture as the least 3 (0.9%). This is not surprising because previous work done by Kiratisin *et. al* observed that ESBL- producing isolates were recovered majorly from urine specimens. The prevalence of ESBL-production among *K. pneumoniae* isolates in this study is 34.7%. This is comparable to the rates of 39.8% reported in Enugu,[19] 33.6% in Abuja,[20] 31.6% in Lagos, 35.3% in Zaria [21] and 30.0% in Maiduguri. Studies however have shown higher prevalence of ESBL producers as reported by Ogefere et al*.* [19] and Egbebi et al., in Benin, Southwest Nigeria, Saraswathi et al., [22] Hyderabad in India with rates as high as 44.3%, 56.0%, and 50.0%, respectively among Gram-negative bacteria isolated in clinical settings. The observed difference may be attributed to the different study population and specimen used such as urine and wound swabs by Ogefere et al*.* The study by Egbebi et al., used a double-disk synergy testing method for ESBL testing, while Saraswathi et al., assessed only post-operative infected wounds. The comparability of high prevalence in many developing nations throughout the world compared to industrialized countries in Europe and North America is especially remarkable. *Klebsiella* spp. are of significant medical value among other Enterobacteriaceae because they exhibit the ESBL mechanism and are commonly identified in hospital and community infections that require medications.

Blood culture was found to have the highest proportion of ESBL *K. pneumoniae* while urine samples had the least proportion of ESBL *K. pneumoniae* growth. This is in agreement with reports of similar studies showing a relatively high occurrence of *ESBL* producing *K. pneumoniae* in clinical specimen ranging from 24.6% - 60% [2,23–25].

Similarly, the findings of the current study showed the occurrence of *ESBL K. pneumoniae* was higher among in-patients (37.0%) compared to outpatients (28.7%). A comparison of studies in developed nations report a 10% - 30% occurrence of ESBL *K. pneumoniae* growth in blood specimen [2,15,26,27] compared to developing countries like Nigeria which report between 40% - 55% occurrence of ESBL *K. pneumoniae* growth in blood culture in hospital settings [1,28–30]. The contrasting reports of ESBL *K. pneumoniae* growth in developed nations compared to developing nations is an indication of the effectiveness of antimicrobial prescription policies in developed countries compared to developing nations. Similarly, many hospital settings in developed nations tend to have better management and control of antimicrobial resistance in comparison to developing countries.

5. CONCLUSION

The study showed that blood culture specimens had the highest burden of *ESBL K. pneumoniae* growth. It was also observed that *ESBL K. pneumoniae* growth was not significantly associated with gender of age groups. The growth of *ESBL K. pneumoniae* was observed to be higher among inpatient subject indicative of the likelihood of high prevalence *ESBL K. pneumoniae* in hospital acquired infection. Therefore, there is a great need for urgent interventions in the areas of antimicrobial usage and infection prevention and control in hospital-based settings.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

Ethical approval to carry out the study was obtained from the Ethical committee of University of Port-Harcourt teaching hospital. Willing informed consent was obtained from each of the patients before they were included into the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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