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ORIGINAL RESEARCH ARTICLE

OCCURRENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *Klebsiella pneumoniae* ISOLATES IN A TERTIARY CARE HOSPITAL IN LALITPUR

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ABSTRACT

Background: Bacteria have been ever-evolving and conferring resistance to advanced and more powerful antibacterial drugs. With the increase in multidrug resistance and extended spectrum beta lactamase (ESBL) producing isolates worldwide, Nepal too faces diagnostic and therapeutic challenges. The dilemma to choose right empirical therapy leads to delayed and inappropriate treatment resulting in increased morbidity, mortality and increased hospital stay. The objective of our study was to determine the occurrence of ESBL producing *Klebsiella pneumoniae* (ESBLK) in clinical samples of patients attending KIST Medical College and Teaching Hospital (KISTMCTH) and determine their antimicrobial susceptibility pattern.

Methods: A total of 212 clinical samples in which *Klebsiella pneumoniae* was isolated were studied. Isolates were identified by standard microbiological methods and tested for in vitro antibiotic susceptibility by modified Kirby-Bauer disc diffusion method. Phenotypic confirmatory disc diffusion test was performed for confirmation of ESBL production. The obtained data was entered and analyzed in WHONET 2023 program.

Results: A total of 212 *Klebsiella pneumoniae* isolates were isolated in our study. Among these, 41 (19.33%) of *K. pneumoniae* were found to be ESBL producers. The ESBLK isolates were 100% sensitive to Colistin and Polymyxin B. Least sensitivity was seen against Ciprofloxacin and Cotrimoxazole. Meropenem resistance was seen in 26.8% ESBLK isolates.

Conclusions: Our study shows increased prevalence of ESBLK resistant to commonly used antibiotics Therefore, judicious use of antimicrobials, active infection control practices, stringent antibiotic policy and regular surveillance should be conducted to keep antibiotic resistance at bay.

INTRODUCTION

Bacteria have been ever-evolving and conferring resistance to advanced and more powerful antibacterial drugs. Owing to the limited reserve of drugs, antibiotic resistance is widely considered to be the next global pandemic.[1] Extended-spectrum β-lactamases (ESBLs)are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes which share the ability to hydrolyze penicillins, first and third generation cephalosporins except aztreonam but are inhibited by clavulanic acid.[2]Ever since Extended Spectrum Beta Lactamase producing *Klebsiella pneumoniae* (ESBLK) were first recognized in 1983, they have been rapidly evolving.[3] The prevalence of ESBL isolates may vary among group of patients, type of infection and geographic location.[4]They frequently carry genes responsible for resistance to other group of antibiotics and even newly developed beta lactam drugs leading to multidrug resistance (MDR).[5]

As the MDR and ESBL isolates increase worldwide,[6] a developing country like Nepal too faces diagnostic and therapeutic challenges. The dilemma to choose right empirical therapy leads to delayed and inappropriate treatment with increased morbidity, mortality and length of hospital stay. This increases the financial burden and threats the patient to hospital acquired infections. Carbapenems are used to treat ESBL positive *Enterobacteriaceae* infections. Nevertheless, with frequent and irrational use, there is an increasing concern over the emergence of carbapenem resistance.[7,8] Our study therefore intends to present the local epidemiology and guide the clinicians to follow evidence based antibiotic policies.
The objective of the study is to determine the occurrence of ESBLK in clinical samples of patients attending KIST Medical College and Teaching Hospital (KISTMCTH) and determine their antimicrobial susceptibility pattern.

METHODS

This hospital based cross-sectional study was conducted in the Department of Microbiology, KISTMCTH Nepal from 8th December 2022 to 8th December 2023 after obtaining ethical clearance from the Institutional Review Committee of KISTMCTH.(IRC REF NO: 2079/80/25)

A total of 212 clinical samples from patients attending different

departments of KISTMCTH, including the wards and Out Patient Departments that reported growth of *Klebsiella pneumoniae* were included in the study. Unlabeled and mislabeled samples, inadequate volume of samples, samples received after 24 hours of collection, repeated samples were excluded from the study. Sample Size was calculated using the formula:

Sample size (n) = Z^2 X p X q/e^2

= $(1.96)^2$ X 0.085 X (1-0.085)/ $(0.05)^2$

= 120

Where,

n= required sample size

Z=1.96 at 95% confidence interval

p= prevalence of ESBL *Klebsiella pneumoniae i.e.* 8.57%[5]

q= 1-p

e= margin of error, 5%

The minimum sample size calculated was 120. Nevertheless, 212 *Klebsiella pneumoniae* isolated during the study period were included.

The samples included blood, urine and body fluids which include sputum, tracheal aspirates, pleural fluid, throat swab, wound swab, pus, bile, eye swab, catheter (central and peripheral lines), high vaginal swabs, cerebrospinal fluid, peritoneal fluid and tissue.

Automated blood culture was done using BACT/ALERT. Those blood samples in which growth was indicated was subcultured in Blood agar, Chocolate agar and Mac Conkey agar and incubated aerobically at 37°C for 48 hours. Urine samples were inoculated into Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated aerobically at 37°C for 24 hours. Body fluid were inoculated into Blood agar, Chocolate agar and Mac Conkey agar respectively and incubated aerobically at 37°C for 48 hours. *Klebsiella pneumoniae* were identified based on standard microbiological methods based on colony morphology, Gram staining and standard biochemical tests.

Antimicrobial susceptibility was determined by modified Kirby Bauer disk diffusion method following the criteria designed by the Clinical and Laboratory Standards Institute (CLSI 2023). The antimicrobial agents used were: Cefotaxime (CTX 30 µg), Ceftriaxone (CTR 30 µg), Cotrimoxazole (COT 25 µg), Gentamicin (GEN 10 µg), Amikacin (AK 30µg), Ciprofloxacin (CIP 5 µg), Ampicillin (AMP 10 µg), Polymyxin B (PB 300µg), Colistin (CL 25 µg), Tigecycline (TGC 15 µg), Ceftriaxone (CTR 30 µg). Meropenem (MRP 10 µg), Amoxycillin clavulanic acid (AMC 20/10µg), Ceftazidime (CAZ 30 µg) and Ofloxacin (OF 5 µg).

Klebsiella pneumoniae isolates showing zone of inhibition of ≤ 25 mm for Ceftriaxone were selected for screening of ESBL production. Phenotypic confirmatory disc diffusion test was performed for confirmation of ESBL production.

A lawn culture of *K. pneumoniae* on Mueller Hinton agar was made and Ceftazidime (30 µg) and the combination disc Ceftazidime + Clavulanic acid (30 µg + 10 µg) was placed 25 mm apart. An increase of ≥ 5 mm in zone of inhibition for Ceftazidime + Clavulanic acid compared to Ceftazidime alone was confirmed as ESBL producing strains, as recommendation of CLSI.

All data were entered and analyzed using WHONET 2023 program.

RESULTS

A total of 212 *Klebsiella pneumoniae* isolates were obtained during the study period. Among these, 41 (19.33%) of *K. pneumoniae* were found to be ESBL producers (Fig 1). Out of these, 22 (53.65%) samples belonged to males and 19 (46.34%) samples belonged to female (Fig2).Seventy three percent of samples where growth of ESBLK was observed belonged to the age group above 40 years (Table1). Extended Spectrum Beta Lactamase producing *K. pneumoniae* was isolated most frequently from urine sample (17; 41.4%) (Fig 3).

Table 1: Age wise distribution of the ESBLK isolates

Age group	Number of ESBLK isolated
0- 20years	4
21-40 years	7
41-60 years	14
61-80 years	11
>80 years	5
Total	**41**

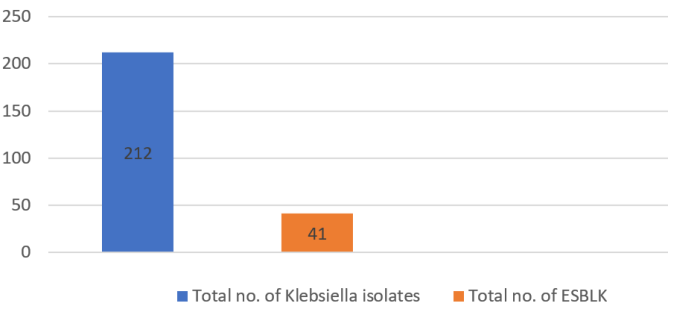

Figure 1: Total number of ESBLK isolates

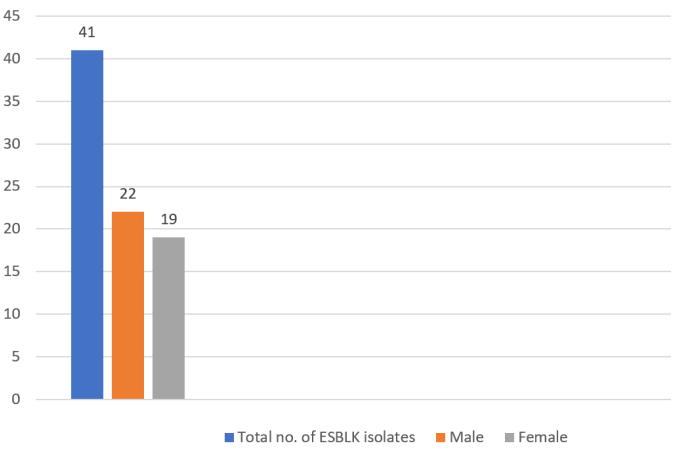

Figure 2: Gender wise distribution of ESBLK isolates

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Table 2: Antibiotic resistance pattern of *Klebsiella pneumoniae* isolates

Name of the isolates	No. of isolates	Antibiotic resistance pattern														
		CIP	GEN	COT	AK	CTR	NIT	NX	NA	PB	CL	TGC	AMC	CAZ	OF	MRP
Klebsiella pneumoniae (Non ESBL producing)	171	60 (35)	43 (25.1)	48 (28)	33 (19.2)	81 (47.3)	43 (25.1)	16 (9.3)	20 (11.6)	0	0	15 (0)	46 (26.9)	49 (28.6)	37 (21.6)	40 (23.3)
ESBL Klebsiella pneumoniae	41	25 (60.9)	12 (29.2)	22 (53.6)	8 (19.5)	30 (73.1)	13 (31.7)	9 (21.9)	10 (24.3)	0	0	10 (0)	15 (36.5)	26 (63.4)	15 (34.1)	11 (26.8)
TOTAL	212	85 (40)	55 (25.9)	70 (33)	41 (19.3)	111 (52.3)	56 (26.4)	25 (11.7)	30 (14.1)	0	0	25 (11.7)	61 (28.7)	75 (35.3)	52 (24.5)	51 (24)

Numbers in parenthesis indicate percent

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Antibiotic susceptibility test showed that *Klebsiella pneumoniae* isolates were least sensitive to Ceftriaxone and Ciprofloxacin (Table 2). Thirty three percent of isolates were resistant to Cotrimoxazole. Among ESBLK least sensitivity was seen against Ciprofloxacin, Cotrimoxazole and Amoxycillin Clavulanic acid respectively. All ESBLK were 100% sensitive to Colistin and Polymyxin B. Among the 1st line antibiotics, the urinary isolates were most sensitive to Norfloxacin. Meropenem resistance was seen in 26.8% of ESBLK isolates. Fifty two percent of *K. pneumoniae* isolates were resistant to Ceftriaxone. Twenty six percent of ESBLK isolates showed in vitro sensitivity to Ceftriaxone. Ninety (42.4%) isolates showed multidrug resistance. Extended spectrum β-Lactamase producing *K. pneumoniae* isolates were most resistant to Ciprofloxacin and least resistant to Colistin and Polymyxin B.

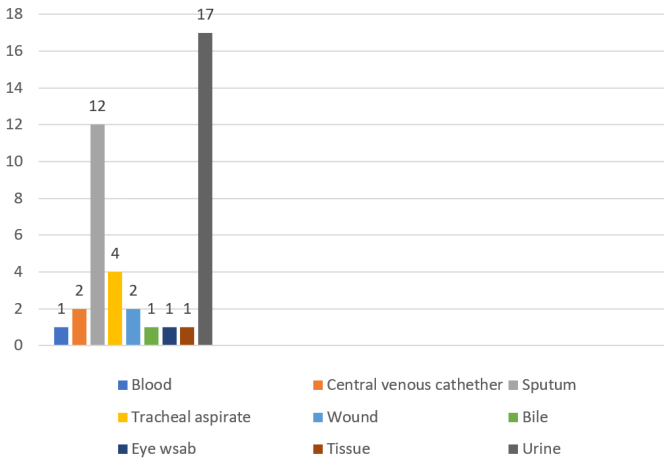

Figure 3: Samples with ESBLK isolation

DISCUSSION

Antimicrobial resistance is a global crisis.[9] Globally, the emergence of AMR has compromised the treatment of infectious diseases worldwide, leading to severe diseases and complications.

Our study shows the prevalence rate of ESBLK to be 19.33% which was similar to a study conducted at National Public Health Laboratory[10]and a meta analytic study by Shyaula M *et al*[11] which reported the prevalence rate of16% and 23% respectively. However, other studies conducted in Kathmandu reported a higher prevalence rate of 36.3%[12] and 33%[13]. Lower prevalence of ESBLK (8.5%) has been reported by Shilpakar *et al*[5] in a study conducted at MMH. A study conducted in India,[14] reported the prevalence of ESBLK to be 17%. A meta-analytic study carried out by Salawudeen *et al* reveals significant difference in prevalence of ESBLK among South Asian countries.[15]However, the overall prevalence of ESBL producers among clinical samples yielding Enterobacteriaceae was only 1% in an observational study conducted in United Kingdom.[16] Various factors, such as study location, type of sample, seasonal variation and environmental conditions, including temperature, p^H and humidity of the study site, may be responsible for the difference in the rate of prevalence in other studies.[10] Geographical variations, antimicrobial stewardship programs, infection control practices and nationwide policies and programs also play an important role.[12]

Extended Spectrum Beta Lactamase producing *Klebsiella pneumoniae* isolates was most prevalent in urine samples similar to study conducted by Shilpakar *et al*.[5] Urinary tract infections are one of the most common infections encountered in the community and hospital settings. Urine sample is therefore the most common sample obtained in our study where *Klebsiella pneumonaie* was isolated, which justifies the presence of highest number of ESBLK in this study. ESBLK were isolated mostly from age group between 31-45 years [5,12] which was in contrast to the finding of our study.

Our study revealed multidrug resistance in 42.4% of *Klebsiella pneumoniae* isolates which was found to be lesser than other studies conducted in India and Nepal where the prevalence of MDR *Klebsiella pneumoniae* ranged from 88.5%[5,13] to 55%.[11, 15]Our study also showed reduced susceptibility to most of the antibiotics as reported by a study conducted by Aslam B *et al.* The increase in resistance may be due to the inappropriate use of antibiotics without medical supervision, improper administration and insufficient therapy. Studies conducted at NPHL and MMH reported 100% Polymyxin B sensitivity of *Klebsiella pneumoniae* isolates [10,12] which is similar to our study. In most of the studies, [11,12,13,14] Carbapenems were the most susceptible drug (99-100%) whereas in our study, only 26.8% of *Klebsiella pneumoniae* were sensitive to Carbapenems. Carbapenem resistance in *K. pneumoniae* seen in our study is concerning as they are the drug of choice for the treatment of ESBL infections.

Molecular confirmation of ESBL production could not be done due to limitation in the study setting and funding.

CONCLUSION

High prevalence of ESBL producers is a threat to the healthcare facilities. With the ESBLs becoming increasingly complex and diverse, their detection is challenging. Our study shows increased prevalence of ESBLK which were resistant to commonly used antibiotics with marked resistance to Carbapenems too. Therefore, judicious use of antimicrobials, active infection control practices, stringent antibiotic policy and regular surveillance should be conducted with strict adherence to the concept of "reserve drugs" to prevent the alarming scenario of antibiotic apocalypse.

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CONFLICT OF INTEREST: None

FINANCIAL DISCLOSURE: None

REFERENCES:

1. Bush K, Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harbor Perspectives in Medicine.2016 Aug; 6(8): a025247. [DOI]
2. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases. 2010 Sep-Dec; 2(3): 263–74. [DOI]
3. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella* spp. Causing Urinary Tract Infection: A Hospital Based Study. The Open Microbiology Journal. 2017; 11: 23–30. [DOI]
4. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G et al. CTX-M: changing the face of ESBLs in Europe. The Journal of Antimicrobial Chemotherapy. 2007;59(2):165–174. [DOI]
5. Shilpakar A, Rai KR, Rai G, Rai SK. Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal. BMC Tropical Medicine and Health. 2021; 49 (1): 1-9. [DOI]
6. Gajic I, Jovicevic M, Popadic V, Trudic A, Kabic J, Kekic D, et al. The emergence of multidrug resistant bacteria causing healthcare associated infections in COVID-19 patients: a retrospective multicentric study. The Journal of Hospital Infection. 2023 Jul;137:1-7. [DOI]
7. Lee N,Lee CWH, Tsui K,Hsueh P, Ko W. Carbapenem Therapy for Bacteremia Due to Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* or *Klebsiella pneumoniae*: Implications of Ertapenem Susceptibility. Antimicrobial agents and Chemotherapy. 2012 Jun;56(6):2888-93. [DOI]
8. Nepal K, Pant ND, Neupane B, Belbase A, Baidhya R, Shrestha RK, et al. Extended spectrum beta-lactamase and metallo beta-lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. Annals of Clinical Microbiology and Antimicrobials. 2017 Sep; 16: 62. [DOI]
9. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. Infection and Drug Resistance. 2018 Oct;11:1645-1658. [DOI]
10. Kuinkel S, Acharya J, Dhungel B, Adhikari S, Adhikari N, Shrestha UT, et al. Biofilm Formation and Phenotypic Detection of ESBL, MBL, KPC and AmpC Enzymes and Their Coexistence in *Klebsiella* spp. Isolated at the National Reference Laboratory, Kathmandu, Nepal. Microbiology Research. 2021; 12: 683-697. [DOI]
11. Shyaula M, Khadka C, Dawadi P, Banjara MR. Systematic Review and Meta-analysis on Extended-Spectrum β-lactamases Producing *Klebsiella pneumoniae* in Nepal. Microbiology Insights. 2023 Jan; 12(16) :11786361221145179. [DOI]
12. Parajuli NP, Maharjan P, Joshi G,Khanal PR. Emerging Perils of Extended Spectrum β-Lactamase Producing Enterobacteriaceae Clinical Isolates in a Teaching Hospital of Nepal. BioMed Research International. 2016 Dec;1782835. [DOI]
13. Kayastha K, Dhungel B, Karki S, Adhikari B, Banjara MR, Rijal KR, et al. Extended-Spectrum β-Lactamase-Producing *Escherichia coli* and Klebsiella Species in Pediatric Patients Visiting International Friendship Children's Hospital, Kathmandu, Nepal. Infectious Diseases. 2020 Feb;13:1178633720909798. [DOI]
14. Sarojamma V, Ramakrishna V. Prevalence of ESBL-Producing *Klebsiella pneumoniae* Isolates in Tertiary Care Hospital. ISRN Microbiology. 2011 Dec; 2011: 318348. [DOI]
15. Salawudeen, A, Raji YE, Jibo G. Epidemiology of multidrug-resistant *Klebsiella pneumoniae* infection in clinical setting in South-Eastern Asia: a systematic review and meta-analysis. Antimicrobial Resistance and Infection Control. 2023 Dec; 12;142. [DOI]
16. Enoch DA, Brown F, Sismey AW, Mlangeni DA, Curran MD, Karas JA, et al. Epidemiology of extended -spectrum beta lactamase producing Enterobacteriaceae in a UK district hospital; an observational study. Journal of Hospital Infection. 2012 Aug; 81(4): 270-77. [DOI]