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Microbiology of Klebsiella with extended-spectrum β-lactamases production

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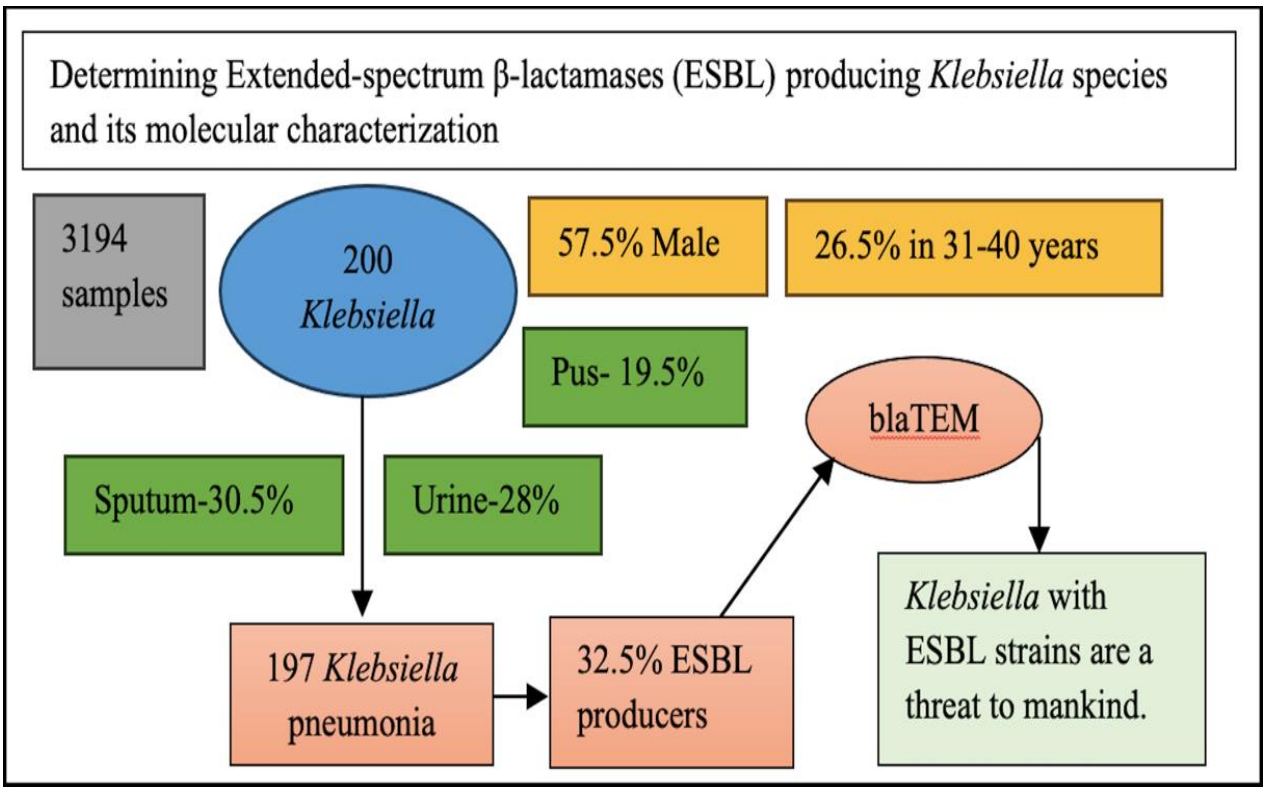
ABSTRACT

Extended-spectrum β-lactamases (ESBL) producing Klebsiella represents one of the greatest challenges in therapeutic management of the patients. Hence, the objective was to determine ESBL producing Klebsiella species and its molecular characterization in clinical samples of tertiary-care hospital. Klebsiella isolated from various samples were included and by using disc diffusion method ESBL detection amongst the Klebsiella isolates along with molecular detection of blaTEM, blaSHV, blaCTX-M genes which are associated with ESBL production was done. Results revealed that out of 3194 samples, 200 Klebsiella species were isolated. The ratio of Klebsiella species isolated from inpatient and outpatient departments was 75.5% and 24.5% respectively.



The maximum isolates were obtained from men consisting of 57.50% and aged between 31-40 years with 26.50%. Majorly Klebsiella species were isolated from sputum including 30.5%, followed by urine consisting of 28% and pus samples with 19.5%. From the 200 isolates, 197 were Klebsiella pneumonia and 32.5% were ESBL producers with majorly having the blaTEM gene incorporated. Being Multidrug and Pan-drug resistant, Klebsiella with ESBL strains are a threat to mankind. This can be overcome by adhering to strict hospital policies to avoid irrational use of antibiotics, strictly following institutional and National antibiotic policies and by prescribing antibiotics according to the culture and sensitivity reports.

Keywords: *Klebsiella* species, extended-spectrum β-lactamases, multi-drug resistant, extensively drug resistant, nosocomial infections, Toxicity.

INTRODUCTION

Enterobacteriaceae family is comprised of variety of organisms that forms the gastro-intestinal microbiota. These organisms can be either commensals or pathogenic like *Salmonella, Shigella*. One such organism is the *Klebsiella* species that has become a part of community acquired as well as nosocomial pathogens [1]. *Klebsiella* are characteristically gram negative, facultative anaerobic, non-sporing organism comprising thick polysaccharide capsule which contributes as a major virulence factor in pathogenesis [2]. Since the last two decades, *Klebsiella* has caused 14–20% of nosocomial infections, including pneumonia caused due to ventilators, primary bacteremia, urinary tract infections, intra-abdominal infections, and wound infections [2–4].

Multi-drug resistant (MDR) and Extensively Drug Resistant (XDR) *Klebsiella* species are caused due to the advent of antibiotic era and irrational use of antibiotics. A wide variety of β-lactam antibiotics, such as penicillin monobactam, carbapenem, and cephalosporins including both third- and fourth-generation are not effective against these MDR pathogens [4]. Producing plasmid-coded β-lactamases and other enzymes like carbapenamases, penicillinases, and cephalosporinases allows for the development of resistance against β-lactam antibiotics [5].

With emerging advancements in therapeutics, β-lactamase inhibitors were quite useful to fight against MDR strains until a mutation led to Extended-spectrum β-lactamases (ESBL) and Amp-C mediated β-lactamases strains of *Klebsiella* which became a major concern in health care system [6]. The ability to identify ESBL-resistant isolates on the basis of the amino acid sequence is mostly due to the presence of the genes blaTEM, blaSHV, and blaCTX-M [7].

Treatment of patients infected with such MDR strains becomes a challenge to health care systems. It increases morbidity and mortality rate of patients, affects quality of life, increases hospital stay and treatment becomes costly. Also, for patients having co-morbidities MDR infections lead to even skimming amongst the available therapeutic options. Although, carbapenems are still considered as drug of choice to treat patients infected with ESBL producing strains as they possess stability against hydrolyzing activity of ESBL [8]. In order to identify ESBL-producing *Klebsiella* species among the diverse clinical samples, phenotypic and molecular techniques were used in this study.

MATERIALS AND METHODS

After approval from the Institutional Ethics Committee (IEC) with reference number (2018/SC/1/1) clinical samples were collected from infected patients coming to different departments of the hospital with maintaining all the aseptic conditions. Within two hours after sample collection, samples were delivered to the microbiological lab.

Blood cultures, sputum, frank pus and pus swabs, urine, bodily fluids (bronchoalveolar lavage, cerebral fluid, pleural fluid, and peritoneal fluid), catheter tips, and endotracheal secretions were the clinical samples. Samples were accepted according to the acceptance criteria of laboratory. All samples were inoculated into Mac Conkey and blood agar for the purpose of isolating bacteria as illustrated in Figure 1, and for 16–18 hours at 37°C they were then incubated. The different species were determined using gram staining, biochemical processes, and colony characteristics. For biochemical characterisation, catalase, sugar fermentation tests (glucose, sucrose, maltose, and lactose), cytochrome oxidase, urease test, triple sugar iron (TSI), nitrate test, and Indole, Methyl Red, Voges Proskauer and Citrate (IMVIC) test were performed.

Figure 1: Mac Conkey Agar showing lactose fermenting mucoid colonies

The antibiotic susceptibility profile of both that produced ESBL strains and that did not produced ESBL *Klebsiella*

strains was examined using the Kirby-Bauer's disc diffusion method. The antibiotics used were Ceftriaxone (30µg), Cefotaxime (30µg), Cefoperazone (75µg), Cefixime (30 µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Ofloxacin (2µg), Norfloxacin (10µg), Levofloxacin (5µg), Imipenem (10µg), Meropenem (10µg).

Figure 2: Double disc diffusion method

The isolates screened for third-generation cephalosporin's with zone sizes of <27mm for cefotaxime and <22mm for ceftazidime were subsequently tested for ESBL status using the phenotypic confirmatory test by double disc diffusion. A double disc diffusion test was carried out with ceftazidime + clavulanic acid (30/10µg) and ceftazidime disc (30µg). Alternatively, cefotaxime + clavulanic acid, (30/10µg), and cefotaxime (30µg) disc can also be used as illustrated in Figure 2.

An overnight-grown isolation culture was calibrated to 0.5 Mc Farland's standard for this test. On Mueller Hinton Agar (MHA), a lawn culture was created using a disc of cephalosporin and its combination, which were placed 20 mm apart and for 18–24 hours at 37°C was cultured. For phenotypic marker of ESBL isolate a zone size of ≥ 5 mm diameter was considered.

Genotypic characterization of ESBL demonstrated that ESBL genes were detected using molecular methods. Using the proper primers in the PCR master mix, the genes blaSHV as demonstrated in Figure 3, blaTEM as shown in Figure 4, and blaCTX-M were detected using polymerase chain reaction (PCR). Initially bacterial DNA was extracted from the isolates followed by 35 cycles of PCR reaction. The PCR outcome was detected in polyacrylamide gel electrophoresis. Finally, the size of PCR products was identified using silver staining.

Figure 3: blaSHV gene amplification from ESBL-Klebsiella

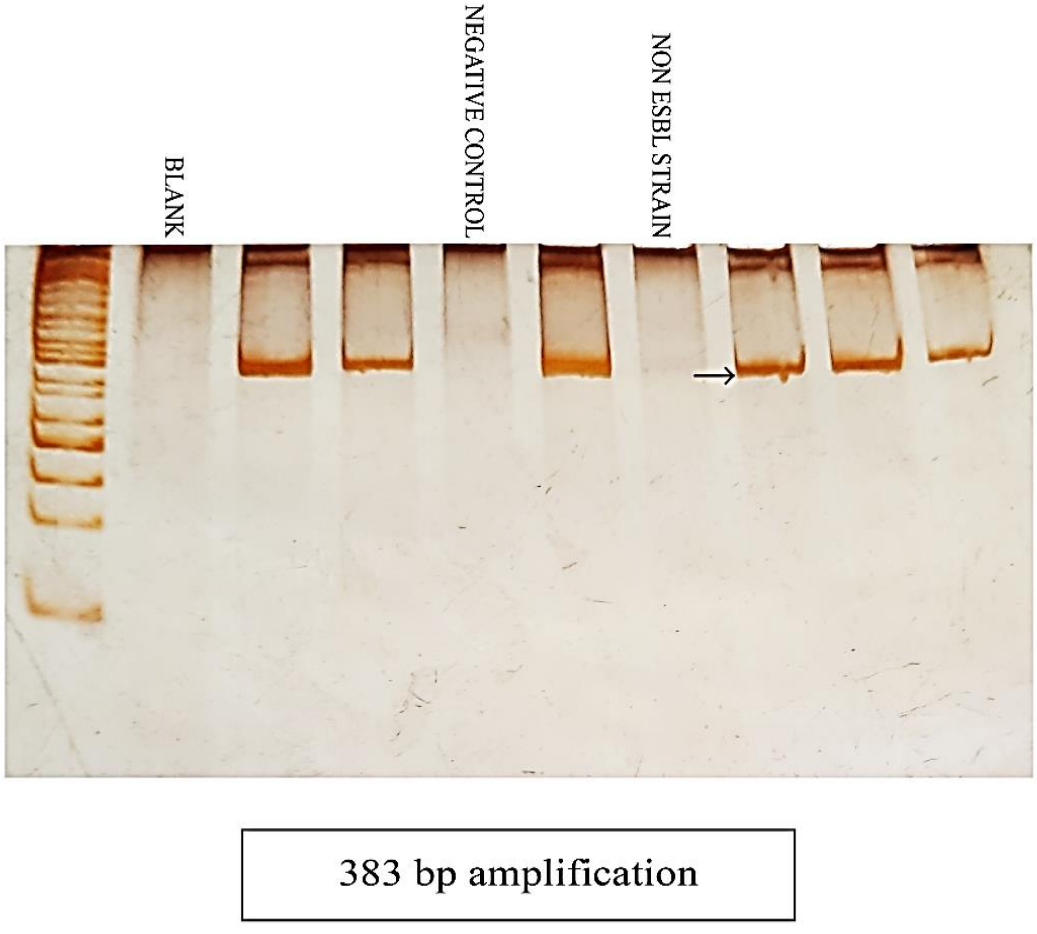
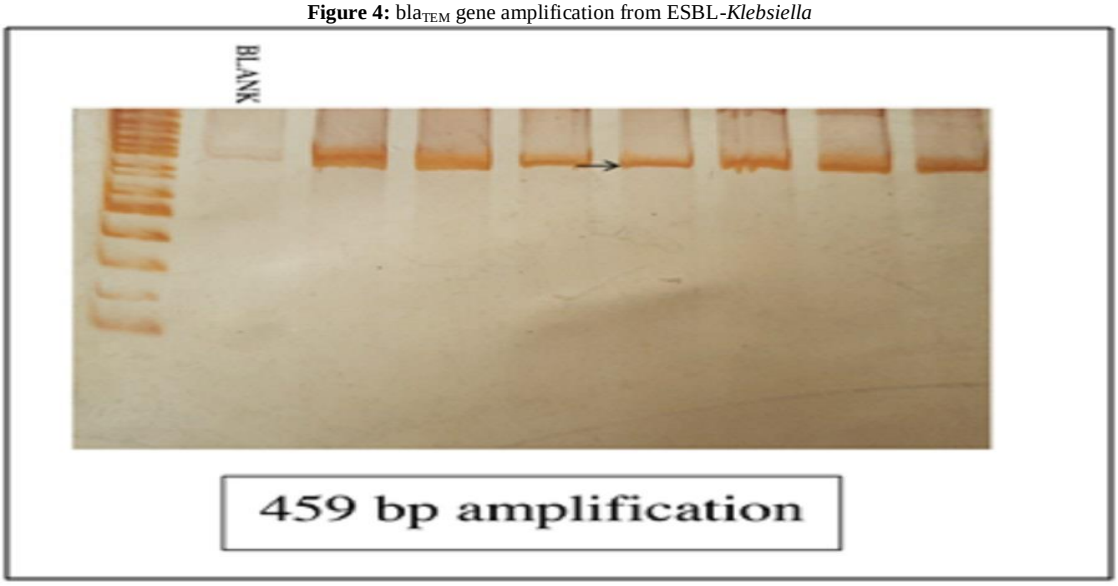


Figure 4: bla_{TEM} gene amplification from ESBL-*Klebsiella*



RESULTS

Out of 3194 clinical samples tested, 200 pathogenic isolates of *Klebsiella* species were obtained "between" (November 2017 to June 2019). Out of those 200 samples, 85 were obtained from female patients and 115 were obtained from male patients. The biochemical reactions of *Klebsiella* were positive for IMVIC reduction and all sugars with TSI showing A/A with gas while Indole and Methyl Red were negative as shown in Table 1.

The age group between 31 and 40 years had the highest prevalence of *Klebsiella* consisting of 26.5%, followed closely by the group ≥ 60 years as demonstrated in Table 2.

Table 1: Biochemical characteristics of Klebsiella isolate

Organism	Indole	MR	VP	Citrate	TSI	Urease	Sugars			
							G	S	L	M
Klebsiella pneumoniae	-	-	+	+	A/A with Gas	+	+	+	+	+

MR = Methyl Red, VP = Voges Proskauer, TSI = Triple sugar iron

Table 2: Age wise prevalence of Klebsiella species

Age (years)	Number of samples positive	Percentage
≤ 10 years	4	2%
11-20 years	14	7%
21-30 years	31	15.50%
31-40 years	53	26.50%
41-50 years	25	12.50%
51-60 years	28	14%
≥ 60 years	45	22.50%
Total	200	100%

In 200 Isolates of *Klebsiella* species, 197 were *Klebsiella pneumoniae* (98.5%) while 03 were *Klebsiella oxytoca* (1.5%). In clinical samples, sputum (30.5%) yielded highest number of *Klebsiella* isolates followed by urine (28%) and rest of the samples as shown in Table 3.

Table 3: Distribution of Klebsiella from different clinical samples

Type of sample	Number of samples	Percentage
Sputum	61	30.5%
Urine	56	28%
Pus	39	19.5%
Biomedical devices	19	9.5%
Blood	18	9%
Body fluids	5	2.5%
BAL	2	1%
Total	200	100%

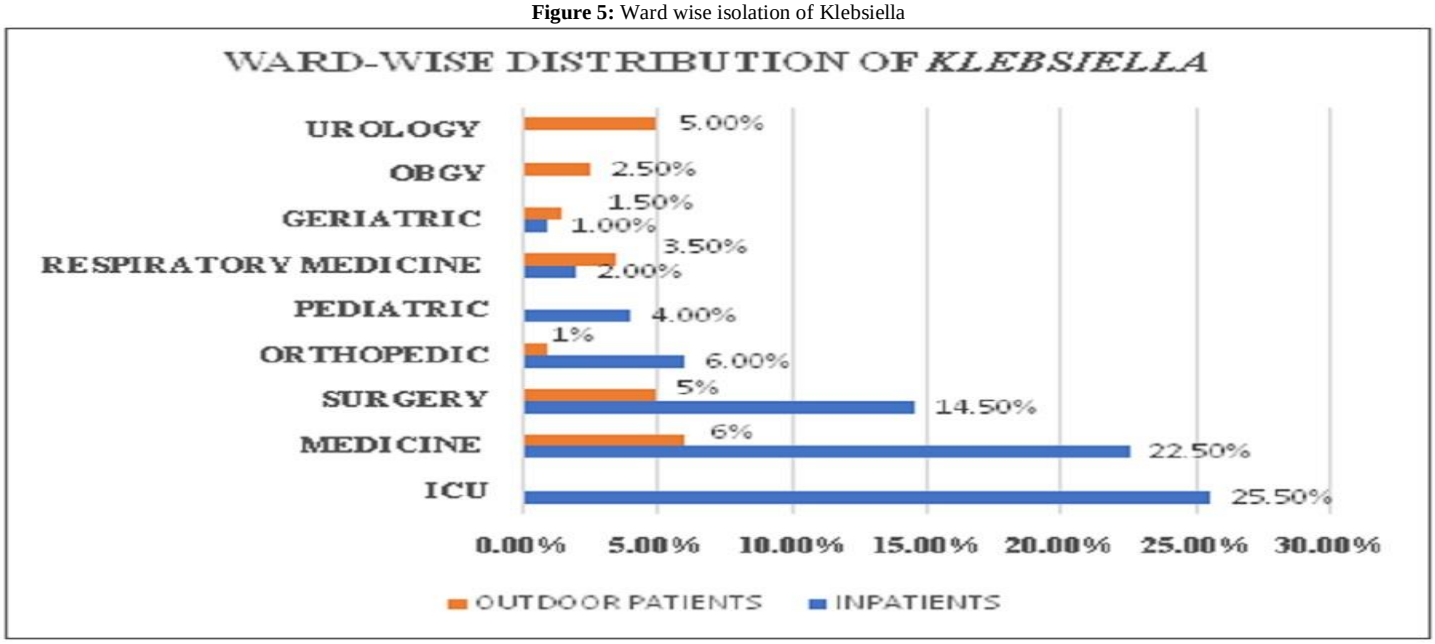
The antibiotics to test against Gram-negative bacteria were selected rationally according to Hospital Antibiotic Policy. The susceptibility pattern is given in Table 4.

Table 4: Antibiotic susceptibility pattern of *Klebsiella*

Antibiotic group	Antibiotic screened	Average/ Mean Resistance	Percentage resistance	Percentage sensitivity
Quinolones	Ciprofloxacin	64.4%	64.42 %	35.58%
Fluroquinolone	Norfloxacin	58.3%	62.5 %	37.5%
	Ofloxacin		53.85%	46.15%
	Levofloxacin		58.55%	41.45%
Aminoglycosides	Gentamycin	63.41%	54.81%	45.19%
	Amikacin		72%	28%
Cephalosporins	Cefaperazone	57.91%	58.65%	41.35%
	Ceftriaxone		59.62%	40.38
	Cefotaxime		65.38%	34.62
	Cefixime		48%	52%
Carbapenems	Meropenem	51.44%	37.5%	62.5
	Imipenem		65.38%	34.62

Out of total *Klebsiella* isolated, phenotypic testing using double disc diffusion method revealed 32.5% ESBL producers while 67.5% were non-ESBL producing strains. Out of all ESBL producing strains, genotypic characterization using molecular Techniques showed that 43 *Klebsiella* comprised blaSHV gene, 11 comprised blaTEM gene while rest comprised none of the molecular gene. Ward location-wise distribution revealed that prevalence of *Klebsiella* species was higher in inpatient department than outpatient department. Ward wise prevalence is mentioned in Figure 5.

Figure 5: Ward wise isolation of Klebsiella



DISCUSSION

Gram-negative bacteria that produce ESBLs, mainly consisting of Klebsiella pneumoniae and *Escherichia coli* have become important pathogens in both hospital and community-acquired infections globally over the past ten years. Infections like septicemia that are caused by ESBL-producing organisms have a much greater fatality rate than infections caused by non-ESBL isolates, according to recent investigations [9]. Clinical isolates' ESBL prevalence varies substantially globally and geographically, and it is rapidly changing over time [9].

Hospitals now have a large number of multidrug resistant infections due to the widespread and inappropriate use of antibiotics. Low virulence organisms like *Klebsiella* have potential to cause severe nosocomial and opportunistic infections [9]. Over the past few years, mutations have led to ESBL strains limiting the therapeutic options for the patients. This possesses a major threat to community as well as hospital settings [10]. In order to ascertain the frequency of *Klebsiella* species that produce ESBL in clinical samples was performed that found that 65 of 200 *Klebsiella* isolates were ESBL producers, which is remarkably comparable to a study by Shah et al. who found that 16 of 60 *Klebsiella* isolates were ESBL producers [11].

E. coli and *Klebsiella* isolate that cause urinary tract infections were researched by Shakya et al. and they reported that only 0.7% of the identified *Klebsiella* were *Klebsiella oxytoca*, and 3.8% were *K. pneumoniae*, according to their findings [12]. Chakraborty et al. [13] looked for prevalence of *K. pneumoniae* and *K. oxytoca* in hospitalized patients in Bangladesh and they reported 90% *K. pneumoniae* and 10% *K. oxytoca*. Both these organisms were surrendered for determining their antibiotic profiles and it was alarming to find that *K. oxytoca* showed 100% resistance to Ampicillin, a trait which is intrinsic to *Klebsiella* species. The prevalence of ESBL-producing *Klebsiella* species. was reported in the current study to be 32.5%, although investigations conducted by others showed prevalence's of 37% and 40%, which were comparably higher than this study [14,15].

Also, in this study genotypic characterization of ESBL producing *Klebsiella* strains showed presence of blaSHV and blaTEM gene while in a study done by Kazemianet al., showed that out of 90 *Klebsiella* isolates, 36 (40%) were ESBL producers having blaTEM (23.3%), blaSHV (21.1%), and blaCTX-M (11.1%) molecular genes [16].Additionally, a large rise in *Klebsiella* species that produce ESBL has been seen in recent years from the Turkey (78.6%) [17],China (51%) [18], Canada (4.9%), USA (42-44%) [19], Spain (20.8%) [18], Algeria (20%) [20], and Taiwan (28.4%) [21]. AmpC-lactamase expression which can be made by chromosomal plasmid genes can conceal ESBLs [9].

In a multi centric study conducted by Gautam et al. studied the genotypic characterization of ESBL using non-duplicate isolates, molecular profiling revealed bla_{TEM}, bla_{OXA-1} and $bla_{CTXM-15}$ to be

common in India. They put forth an important discrepancy in their study namely the combined disc test (CDT) could not phenotypically detect ESBL but gave a PCR positive ESBL finding of 40.8% [22].

K. pneumoniae is a serious pathogen that poses a risk to the public's health. Modern treatment, such as combination therapy and/or novel antimicrobial drugs, may be effective in combating such drug-resistant pathogens [16]. Multidrug resistance, difficulties in diagnosis and treatment, and higher patient mortality are issues related to ESBL-producing isolates. The most reliable and sensitive anti-microbial drugs for infections brought on by ESBL-producing isolates are carbapenem. However, excessive use of carbapenem can make other gram-negative bacteria resistant to it [10]. Therefore, it is crucial to identify ESBL-producing microbes in order to stop the spread of these diseases and develop an effective treatment plan. The phenotypic confirmatory disc diffusion test provides a quick, easy, reliable, and inexpensive technique of identification however, a larger-scale drug susceptibility surveillance program is also necessary.

CONCLUSION

This study demonstrates the emergence of *Klebsiella* towards multidrug and pan drug resistance. Additionally, ESBL detection will envisage the entire scope of the resistance pattern associated with this organism. Furthermore, hospital antibiotic policies should be followed to reduce irrational use of antibiotics and lastly there has been a rising trend of resistance seen even in outpatient department patients. This can be attributed to multiple courses of antibiotic regimes the patient takes before visiting a tertiary care hospital hence antibiotics should only be taken as per the prescription and culture and sensitivity testing.

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Conflict of interest

The author’s declare no conflict of interest.

Author’s Contribution

The final manuscript was reviewed and approved by all authors.

REFERENCES

1. Gorrie CL, Mirčeta M, Wick RR, et al, 2022. Genomic dissection of Klebsiella pneumoniae infections in hospital patients reveals insights into an opportunistic pathogen. Nat Commun. 13(1), Pages 3017-3020. Doi: 10.1038/s41467-022-30717-6.

2. Paczosa MK, Mecsas J, 2016. Klebsiella pneumoniae: going on the offense with a strong defense. Microbiol mol Biol Rev. 80(3), Pages 629–61. Doi: 10.1128/MMBR.00078-15.

3. Kang C I, Kim S H, Park WB, et al, 2004. Bloodstream infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob agents Chemother. 48(12), Pages 4574–4581. Doi: 10.1128/AAC.48.12.4574-4581.2004

4. Vuotto C, Longo F, Pascolini C, et al, 2017. Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. J Appl Microbiol. 123(4), Pages 1003–1018. Doi: 10.1111/jam.13533.

5. Rawat D, Nair D, 2010. Extended-Spectrum β-Lactamases in gram negative bacteria. J Glob Infect Dis. 2(3), Pages 263–274. Doi: 10.4103/0974-777X.68531.

6. Shaikh S, Fatima J, Shakil S, et al, 2015. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci. 22(1), Pages 90–101. Doi: 10.1016/j.sjbs.2014.08.002.

7. Bajpai T, Pandey M, Varma M, et al, 2017. Prevalence of TEM, SHV, and CTX-M Beta-lactamase genes in the urinary isolates of a tertiary care Hospital. Avicenna J Med. 7(1), Pages 12–16. Doi: 10.4103/2231-0770.197508.

8. Harada S, Ishii Y, Yamaguchi K, 2008. Extended-spectrum beta-lactamases: implications for the clinical laboratory and therapy. Korean J Lab Med. 28(6), Pages 401–412. Doi: 10.3343/kjlm.2008.28.6.401.

9. Ali Abdel Rahim KA, Ali Mohamed AM, 2014. Prevalence of extended spectrum β-lactamase-producing Klebsiella pneumoniae in clinical isolates. Jundishapur J Microbiol. 7(11), Pages 17114. Doi: 10.5812/jjm.17114.

10. Alipourfard I, Nili NY, 2010. Antibiogram of extended spectrum beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolated from hospital samples. Bangladesh J Med Microbiol. 4(1), Pages 32–36. Doi: 10.3329/bjmm.v4i1.8467.

11. Shah R, Singh Y, Sanjana R, et al, 2010. Study of extended spectrum beta-lactamases (ESBLs) producing Klebsiella species in various clinical specimens: a preliminary report. J Coll Med Sci Nepal. 6(3), Pages 19-23. Doi: 10.3126/jcmsn.v6i3.4070.

12. Shakya P, Shrestha D, Maharjan E, et al, 2017. ESBL Production among E. coli and Klebsiella spp. causing urinary tract infection: A Hospital Based Study. Open Microbiol J. 11, Pages 23–30. Doi: 10.2174/1874285801711010023.

13. Chakraborty S, Mohsina K, Sarker PK, et al, 2016. Prevalence, antibiotic susceptibility profiles and ESBL production in Klebsiella pneumoniae and Klebsiella oxytoca among hospitalized patients. Period Biol. 118(1), Pages 53-58. Doi: 10.18054/pb.v118i1.3160.

14. Padmini SB, Appalaraju B, 2004. Extended spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital. Indian j med Microbiol. 22(3), Pages 172-174. Doi: 10.1016/S0255-0857(21)02830-9.

15. Bindayna KM, Senok AC, Jamsheer AE, 2009. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Bahrain. J infect public health. 2(3), 129–35. Doi: 10.1016/j.jiph.2009.04.005.

16. Kazemian H, Heidari H, Ghanavati R, et al, 2019. Phenotypic and Genotypic Characterization of ESBL-, AmpC-, and Carbapenemase-Producing Klebsiella pneumoniae and Escherichia Coli Isolates. Med Princ Pract. 28(6), Pages 547–51. Doi: 10.1159/000500311.

17. Hoşoğlu S, Gündes S, Kolayli F, et al, 2007. Extended-spectrum beta-lactamases in ceftazidime-resistant Escherichia coli and

Klebsiella pneumoniae isolates in Turkish hospitals. Indian J Med Microbiol. 25(4), Pages 346–350. Doi: 10.4103/0255-0857.37336.

18. Romero EDV, Padilla TP, Hernández AH, et al, 2007. Prevalence of clinical isolates of Escherichia coli and Klebsiella spp. producing multiple extended-spectrum beta-lactamases. Diagn Microbiol infect dis. 59(4), Pages 433–437. Doi: 10.1016/j.diagmicrobio.2007.06.007.

19. Saurina G, Quale JM, Manikal VM, et al, 2000. Antimicrobial resistance in enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. J antimicrob chemother. 45, Pages 895–898. Doi: 10.1093/jac/45.6.895.

20. Messai Y, Iabadene H, Benhassine, et al, 2008. Prevalence and characterization of extended-spectrum beta-lactamases in Klebsiella pneumoniae in Algiers hospitals (Algeria). Pathol Biol (Paris). 56(5), Pages 319–25. Doi: 10.1016/j.patbio.2008.05.008.

21. Kuo KC, Shen YH, Hwang KP, 2007. Clinical implications and risk factors of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae infection in children: a case-control retrospective study in a medical center in southern Taiwan. J Microbiol Immunol infect. 40(3), Pages 248–254. PMID: 17639166.

22. Gautam V, Thakur A, Sharma M, et al, 2019. Molecular characterization of extended-spectrum β-lactamases among clinical isolates of Escherichia coli and Klebsiella pneumoniae: a multi-centric study from tertiary care hospitals in India. Indian j med res. 149(2), Pages 208–215. Doi: 10.4103/ ijmr.IJMR_ 172_18.