Kumaran et al. | Article 7861
J Pure Appl Microbiol. 2022;16(4):2659-2668. doi: 10.22207/JPAM.16.4.33
Received: 24 May 2022 | Accepted: 20 August 2022
Published Online: 01 November 2022

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Distribution of CTX-M, TEM, SHV Beta-lactamase Gene among the *Klebsiella pneumoniae* Clinical Isolates from Tertiary Care Centre in Palakkad, Kerala

Ramya Kumaran[1]*, R.V. Geetha[2] and Sabitha Baby[1]

[1]Department of Microbiology, Karuna Medical College, Chittur, Palakkad, Kerala, India.
[2]Department of Microbiology, Saveetha Dental College and Hospital, Chennai, India.

Abstract

Resistance against the routinely used antibiotics has reached a worrying level globally. Extended spectrum β- lactamases (ESBLs) production is the major mechanism of antimicrobial resistance. These ESBLs bacteria are resistance to penicillin, cephalosporins, monobactams. TEM1&2, CTX-M, SHV are the main ESBLs genes present in *Klebsiella pneumoniae*, which is produced by the alteration of amino acid in the active site. The aim of this study is to determine the prevalence of ESBL genes such as bla_{TEM} 1&2, bla_{CTX-M} and bla_{SHV}. The present study was carried out from April 2019 to September 2019, a total of 121 *K. pneumoniae* isolates were collected and subjected to phenotypic study. Among these 19 isolated was ESBL positive, genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) were detected by conventional PCR method. bla_{TEM} (100%) was the predominant gene detected flowed by CTX-M (68.42%) and SHV (57.89%). The highest level of antimicrobial resistance towards ampicillin (93.4%) followed by ceftriaxone (28.9%), cefotaxime (24.8%) and ciprofloxacin (22.3%). However, ESBL-producing isolates were showed resistance to ampicillin (100%) followed by ceftazidime (94.74%), cefotaxime (89.47%), amikacin and amoxicillin-clavulanic acid (68%). Antimicrobial resistance of bacteria is due to the genes, especially extended spectrum beta lactamase, which is widely found in members of Enterobacteriaceae. Nevertheless, there is a paucity of studies regarding the distribution of ESBL in *K. pneumoniae* in Palakkad Dist., Kerala. Hence the aim of the current study determines the distribution of ESBL genes in ESBL producing *K. pneumoniae* isolated from various clinical samples.

Keywords: *K. pneumoniae*, Antimicrobial Resistance, ESBL, Genotype

***Correspondence:** ramyyakumaran@gmail.com

Citation: Kumaran R, Geetha RV, Baby S. Distribution of CTX-M, TEM, SHV Beta- lactamase Gene among the *Klebsiella pneumoniae* Clinical Isolates from Tertiary Care Centre in Palakkad, Kerala. *J Pure Appl Microbiol*. 2022;16(4):2659-2668. doi: 10.22207/JPAM.16.4.33

INTRODUCTION

Beta-lactam antibiotics are versatile antibiotics used for a broad spectrum of infections. The emergence of antimicrobial resistance is a significant threat in today's society. The members of the *Enterobacteriaceae* family are commonly occurring bacteria in the environment. *Klebsiella pneumoniae* is one of the frequently isolating bacteria coming under this family. Resistance to various antibiotics is quite a concern because of the ESBL and carbapenem resistance of *K. pneumoniae*, which is repeatedly reported from different parts of the world. It is a causative agent of health care infections and community acquiring infections.[1] The most common diseases are Urinary tract infections, respiratory tract infections, and wound infections, which now act as a co-pathogen in Covid -19 cases.

K. pneumoniae is a well-studied organism due to its multidrug resistance. The ESBL is an enzyme produced by bacteria, acting on the beta-lactam ring. B-lactam antibiotics are widely used worldwide; they bind to the penicillin-binding protein and inhibit the biosynthesis of the bacterial cell wall. Nevertheless, these bacteria produce a β-lactamase enzyme, which acts on β- the lactam ring and develops resistance to beta-lactam antibiotics.[2] The β -lactamase is classified by Ambler into four classes, A, B, C & D in this. Class A, C & D have serine enzymes in the active site and Zinc –Meltallo enzyme in the case of class B.[3]

ESBL gene is the essential resistance present in *Enterobacteriaceae*; it is encoded by bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes, and of these, CTX-M is common. These plasmid-mediated genes are associated with other resistance genes, resulting in a co-resistance that includes aminoglycosides and tetracycline.[4] The first ESBL resistance in *K. pneumoniae* was detected in Europe in 1980 and later spread to other areas. The distribution of ESBL resistance varies from one institution to another, one place to another. The ESBL resistance is a global concern; it leads to treatment failure, increased hospital stay, and a financial burden on humans. So the early detection of ESBL will help the clinicians with therapy. Hence, this study aims to detect the ESBL *K. pneumoniae* and the distribution of ESBL genes present in *K. pneumoniae*, isolated from different clinical samples.

MATERIALS AND METHODS

The study was conducted from April 2019 to September 2019 in the Department of Microbiology, Karuna Medical College, Palakkad, Kerala. A total of 121 isolates of *K. pneumoniae* from different clinical samples were subjected to phenotypic studies.

The bacterial isolates were identified by biochemical reactions according to the standard protocol.[5] All the *K. pneumoniae* isolates were phenotypically tested for ESBL production by double disc synergy test.[6]

Antimicrobial Susceptibility Testing

All ESBL-positive isolates were tested for antimicrobial susceptibility by the Kirby Bauer disc diffusion method according to CLSI guidelines.[7] The discs used for susceptibility test, Ampicillin (10µg), cotrimoxazole (25µg), amoxyclave (10µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), ciproflaxacine (5 µg), amikacin (30µg), gentamicin (10µg), imipenem (10 µg) and tetracycline (30 µg), (Himedia, Mumbai).

ESBL Detection by Double Disc Synergy Test

The ESBL detection of all isolates is performed by double disc synergy test using cefotaxime (30 µl) and cefotaxime clavulanic acid (30 µl + 10 µl) combination according to CLSI guidelines.[8] The *K. pneumoniae* isolates were tested with cefotaxime and ceftazidime disc with and without clavulanic acid are placed 25 mm apart from each other in Muller-Hinton agar (Himedia). The zone of inhibition >5mm in clavulanic acid combination is interpreted as ESBL positive.

Genotypic Method for the Detection of ESBL Gene

Phenotypically confirmed ESBL producing *K. pneumoniae* isolated were tested genotypically by conventional PCR. CTX-M, TEM 1&2, and SHV gene specific primers were used for performing PCR test. The plasmid DNA isolation was performed by using Thermo scientific TM Gene JET Plasmid Kit, according to manufactures instruction. The

ESBL genes such as CTX-M Group 1, TEM 1 & 2, SHV were amplified individually by using specific primers (Table 1).

The amplification mixture final volume 15µl containing, Thermo master mix 8 µl, primer mix 1µl, deionized water 5 µl and DNA template 1 µl. The PCR amplification condition of each gene displayed in Table 2,3 & 4. After amplification the PCR product were loaded in 1.5% agarose gel. After the run the band visualized on gel documentation system (Bio Rad gel doc XR+) and took the photographs.

Statistical Analysis

All the data were analyzed using SPSS version 21(IBM corporation/ Armonk, Newyork/ USA). The chi-square test and Fisher's exact test evaluated the antimicrobial resistance patterns of ESBL and non-ESBL. 'P' value less than or equal to 0.01 was considered statistically significant.

RESULTS

In the present study, 121 *K. pneumoniae* were isolated from different clinical samples; among them, 58(47.9%) were females, and 63 (52.1%) were males (Table 5).

The distribution of isolates in different specimen, 56 (46.3%) urine, 37 (30.6%) sputum, 23(19%) pus, 2 (1.7%) blood and 1(0.8%) each from secretion tip, tissue and ascetic fluid (Table 6). Out of these 121 isolates, 19 were phenotypically positive for ESBL production (Figure 1). The distribution of ESBL positive isolates distribution among various clinical samples is shown in table 6, and among the clinical samples, ESBL prevalent in Urine 8 (14.3%), followed by sputum 5(13.51%) and pus 4 (17.4%).

The antimicrobial resistance of ESBL and non-ESBL are shown in Figure 2. The percentage of resistance to cephalosporins in ESBL producing *K. pneumoniae* and non-ESBL producing *K. pneumoniae* were 89% and 13% for cefotaxime, 95% and 8% for ceftazidime, 84% and 19% for ceftriaxone. However, the ESBL and non-ESBLs were susceptible to carbapenem such as imipenem (100%).

The comparison of the antimicrobial resistance pattern of ESBL and non-ESBL is shown in Table 7. The association between antimicrobial resistance and ESBL production was statistically significant for Cotrimoxazole (<0.001), cefotaxime (<0.001), Ceftazidime (<0.001), ceftriaxone (<0.001), amikacin (<0.001), amoxiclav (<0.001), gentamicin (<0.001), tetracycline (<0.001), ciprofloxacin (<0.001), cefoxitin (0.002). The antimicrobial susceptibility of ESBL isolates revealed that the highest resistance was against

Table 1. Primers used for PCR

Gene	Primers
TEM 1 & 2	Forward Primer: 5'-CATTTYCGTGTCGCCCTTATTC-3'
	Reverse Primer: 5'- CGTTCATCCATAGTTGCCTGAC-3'
SHV	Forward Primer: 5'- ATCCCGCAGATAAATCACCAC-3'
	Reverse Primer: 5'- AGCCGCTTGAGCAAATTAAAC-3'
CTX-M Group 1	Forward Primer: 5'- TTAGGAARTGTGCCGCTGYA-3'
	Reverse Primer: 5'- CGATATCGTTGGTGGTRCCCAT-3'

Table 2. Protocol for TEM 1 & TEM 2 Amplification

Step	Temp.	Time
Initial denaturation	95°C	5 minutes
Denaturation	94°C	30 seconds
Annealing	62°C	40 seconds
Extension	720C	1 minute
Go to step 2-4 for 35 times		
Elongation	72°C	10 minutes

Table 3. Protocol for SHV gene amplification

Step	Temp.	Time
Initial denaturation	95°C	5 minutes
Denaturation	94°C	30 seconds
Annealing	55°C	45seconds
Extension	72°C	1 minute
Go to step 2-4 for 35 times		
Elongation	72°C	10 minutes

ampicillin (100%), followed by ceftazidime (94.74%) and cefotaxime (89.47%). All isolates were susceptible to imipenem.

All phenotypically ESBL positive isolates were genotypically tested for the detection of resistance genes such as bla_{SHV}, bla_{TEM} 1 & 2, and bla_{CTX-M}. Out of this beta-lactamase (*bla*) genes, all 19 (100%) isolates showed bla_{TEM} 1 & 2, followed by bla_{CTX-M} 13 (68.42%) and bla_{SHV} 11(57.89%)(Figure 3-5). Among the nineteen *K. pneumoniae* isolates, 4 (21.05%) isolates possess only bla_{TEM} 1 & 2 ESBL gene. No isolates carry bla_{CTX-M} and bla_{SHV} genes alone (Table 8).

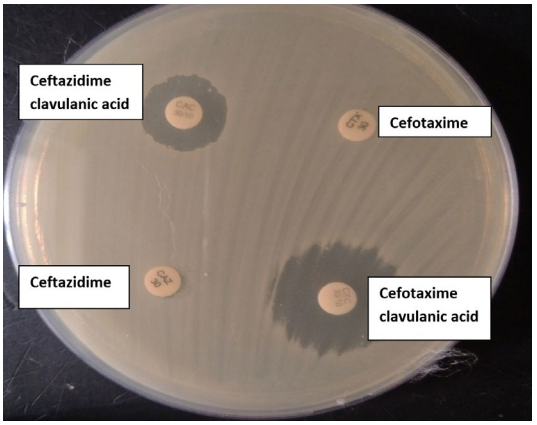

Figure 1. ESBL detection by double disc synergy test (CTX- Cefotaxime and CEC -Cefotaxime clavulanic acid; CAZ-Ceftazidime and CAC-Ceftazidime clavulanic acid)

DISCUSSION

Antimicrobial resistance is a significant concern in the health care system. ESBL production is considered one of the antimicrobial resistance mechanisms in the family *Enterobacteriaceae*.[9] ESBLs are the plasmid-coded genes, which provide resistance to third-generation cephalosporins, and monobactam (aztreonam)[10] is considered an essential factor for the emergence of antimicrobial resistance.

Antimicrobial resistance in K.pneumoniae was noted as an important problem in the health

Table 4. Protocol for CTX-M gene Amplification

Step	Temp.	Time
Initial denaturation	95°C	5 minutes
Denaturation	94°C	30 seconds
Annealing	58°C	45 seconds
Extension	72°C	1 minute
Go to step 2-4 for 35 times		
Elongation	72°C	10 minutes

Table 5. Gender wise distribution of *K. pneumoniae* isolates

Gender	Frequency	Percent
Female	58	47.9
Male	63	52.1
Total	121	100.0

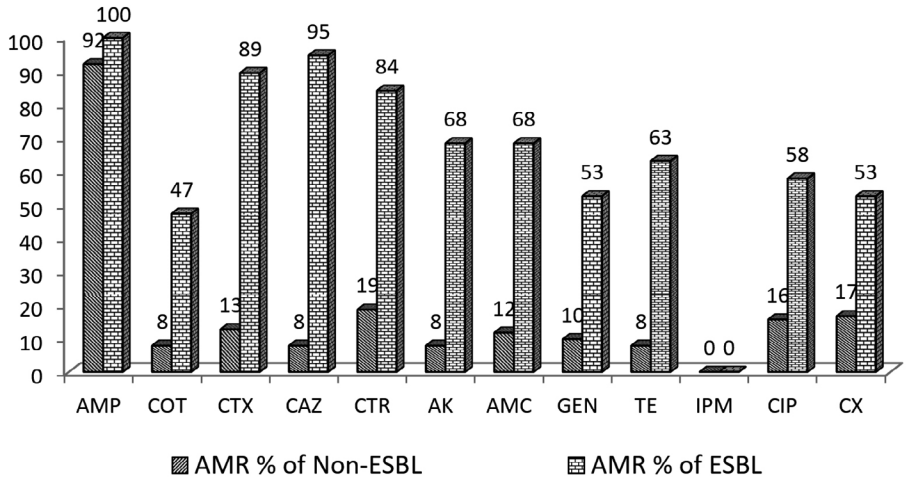

Figure 2. Antimicrobial resistance in ESBL and Non-ESBL producing *K. pneumoniae*
AMP-Ampicillin, COT- cotrimoxazole, CTX- cefotaxime, CAZ- ceftazidime, CTR- ceftriaxone, AK- amikacin, AMC-amoxicillin-clavulanic acid, GEN- gentamicin, TE- tetracycline, IPM- imipenem, CIP – ciprofloxacin, CX- cefoxitin

care setting worldwide. In the present study, all isolates showed low susceptibility to ampicillin (6.61%) and a high-level susceptibility to imipenem (100%). This suggests ampicillin is inadequate for empirical treatment unless combined with other suitable drugs. In the current study, ESBL *K. pneumoniae* are resistant to amikacin (68%), gentamicin (53%), cotrimoxazole (47%), and ciprofloxacin (58%). The leading cause of antimicrobial resistance is the overuse of antibiotics, which is one of the factors contributing to the emergence of resistant bacteria in the community.[11] Carbapenem is a high-level antibiotic used for the treatment of resistant organisms. The current study showed that all isolates are susceptible to imipenem. This follows Ghafourian et al.[12] and Uddin et al.[13]

The distribution of ESBL-producing isolates varied between the geographical regions. In the present study, 19 (15.45%) ESBL *K. pneumoniae* were isolated phenotypically. This report follows the study from Hyderabad, India, conducted by Kumar et al.,[14] showed 19% of ESBL in *K. pneumoniae*. A study from India reported that 25-84% was ESBL occurrence in different institutional studies.[15] Another study from North India reported 52.27% of ESBL *Klebsiella pneumoniae* isolated from various clinical samples. A Study from China reported that ESBL producers' isolation rate varies between 25-40%.[16] Another study from Israel and Spain showed 40% ESBL prevalence in *K. pneumoniae*.[17-18] Some other studies recorded a high incidence rate of ESBL-producing isolates, Feizabedi et al.,[19] reported 69.7% of *K. pneumoniae* were ESBL positive from Iran, and a study by Lal et al.,[20] noticed 97.1% of ESBL producers from India and another study from north India indicated 66.7% of *K. pneumoniae* were ESBL positive.[21] Turkey (60%), Brazil (45.4%), Western Pacific (24.6%), Netherlands (22.6%), and Iran (44 -74%) have reported the highest rate of ESBL prevalence.[22] The percentage of isolation of ESBL producers varies with different factors such as prolonged hospitalization, antibiotic use and policy, the hygiene of hospital personnel, and disinfection of ICU.[23] Some studies reported a low

Table 6. Distribution of *K. pneumoniae* among the clinical sample

No.	Samples	No. of isolates (N=121)	Percentage (%)	No. of ESBL positive isolates
1.	Urine	56	46.3	8 (14.3)
2.	Sputum	37	30.6	5 (13.51)
3.	Pus	23	19.0	4(17.4)
4.	Blood	2	1.7	0 (0)
5.	Secretion tip	1	0.8	1 (100)
6.	Tissue	1	0.8	1 (100)
7.	Ascitic fluid	1	0.8	0 (0)
8.	Total	121	(100)	19

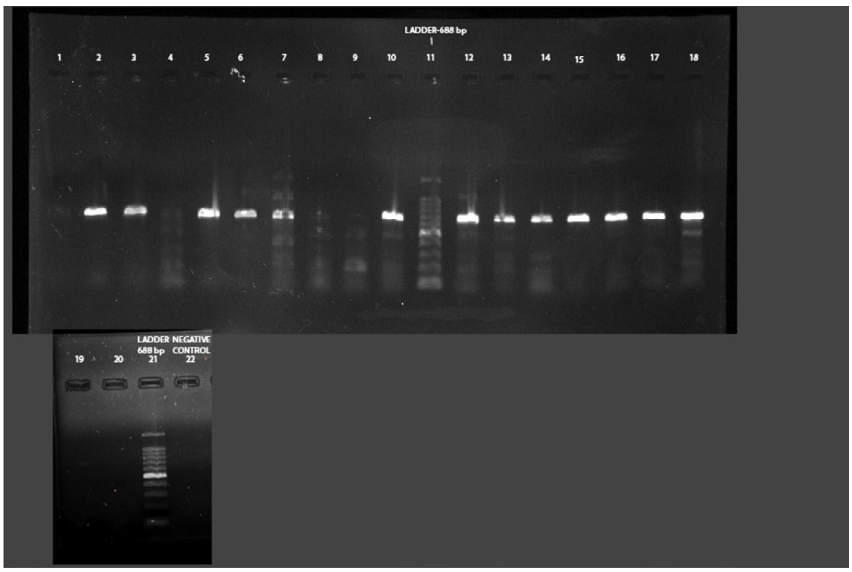

Figure 3. PCR amplification image of CTX-M gene in *K. pneumoniae* after 1.5% agarose gel electrophoresis

level of ESBL prevalence in *K. pneumoniae*, 17%24-25 and 12%.[26]

The ESBL production is due to the mutation in TEM-1 & 2 and SHV 1 genes.[27] In the current study, bla_{TEM} (100%) was the most prevalent gene in ESBL producers, followed by CTX-M (68.42%) and SHV (57.89%). A similar result was found in the study conducted by Ferreiral et al.[28] in this bla_{TEM} (100%), CTX-M (72%), and SHV (96%). The ESBL genes were present in all ESBL positive isolates, but the prevalence of this gene varies. The previous reports from north India showed that 73% of bla_{TEM} and 55% of $bla_{CTX\text{-}M}$ were the most dominant ESBL genes present in PGIMER and AIIMS, respectively. Another study from Lucknow also reported 75% of bla_{TEM} in ESBL *K. pneumoniae*.[29] Samanje et al.,[30], in their study, found that 95% of ESBL *K. pneumoniae* isolates harbor the bla_{TEM} gene, and 90% CTX-M were detected. On the other hand, over the past decade, CTX-M was the predominant gene reported in many Asian countries in *K. pneumoniae*.[31] A study

Table 7. Comparison of antibiotic resistance patterns of ESBL and Non ESBL producing *K. pneumoniae*

Antibiotic		ESBL		X^2	P
		Negative (n=102)	Positive (n=19)		
AMP	Resistant	94	19	1.596ns	0.354ns (Fisher's Exact test)
	Susceptible	8	0		
COT	Resistant	8	9	20.72**	<0.001
	Susceptible	94	10		
CTX	Resistant	13	17	46.54**	<0.001 (Yates correction)
	Susceptible	99	2		
CAZ	Resistant	8	18	66.63**	<0.001 (Yates correction)
	Susceptible	94	1		
CTR	Resistant	19	16	33.39**	<0.001
	Susceptible	83	3		
AK	Resistant	8	13	40.98**	<0.001
	Susceptible	94	6		
AMC	Resistant	12	13	31.36**	<0.001
	Susceptible	90	6		
GEN	Resistant	10	10	21.29**	<0.001
	Susceptible	92	9		
TE	Resistant	8	12	35.52**	<0.001
	Susceptible	94	7		
IMP	Resistant	0	0	-	-
	Susceptible	102	19		
CIP	Resistant	16	11	16.46**	<0.001
	Susceptible	86	8		
CX	Resistant	17	10	11.951**	0.002

** Significant at 0.01 level; ns Non significant
AMP-Ampicillin, COT- cotrimoxazole, CTX- cefotaxime, CAZ- ceftazidime, CTR- ceftriaxone, AK- amikacin, AMC- amoxicillin-clavulanic acid, GEN- gentamicin, TE- tetracycline, IPM- imipenem, CIP – ciprofloxacin, CX- cefoxitin

Table 8. Overview of ESBL genotype among *K. pneumoniae* isolates

A. Single ESBL gene	No. of positive	Percentage (%)
bla CTX-M	0	0
bla TEM 1&2	4	21.05%
bla SHV	0	0
B. Two ESBL genes		
bla CTX-M and bla SHV	0	0
bla CTX-M and bla TEM	4	21.05%
bla TEM and bla SHV	2	10.53%
C. Three ESBL genes		
bla CTX-M, bla TEM and bla SHV	9	47.37

from Malaysia reported that the most prevalent ESBL gene in *K. pneumoniae* isolates was CTX-M (91.3%).[32] A study from Nepal reported that 78.9% of *K. pneumoniae* contains the CTX-M gene.[33] However, a study from the middle east detected that 93.75% (30/32) TEM and 87.5% (28/32) SHV gene was found in ESBL *K. pneumoniae*.[34] The prevalence of the ESBL gene depends on various factors like the time of the study, the number of isolates, and geographical area.

The current study showed the presence of multiple genes in a single isolate. This follows the other studies.[35] Nevertheless, the combination of ESBL genes is different in the different studies.

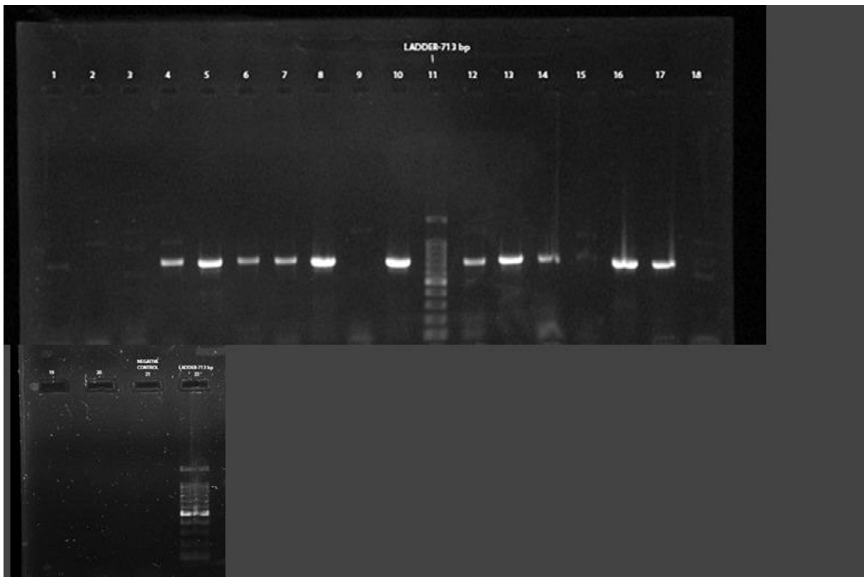

Figure 4. PCR amplification image of SHV gene in *K. pneumoniae* after 1.5% agarose gel electrophoresis

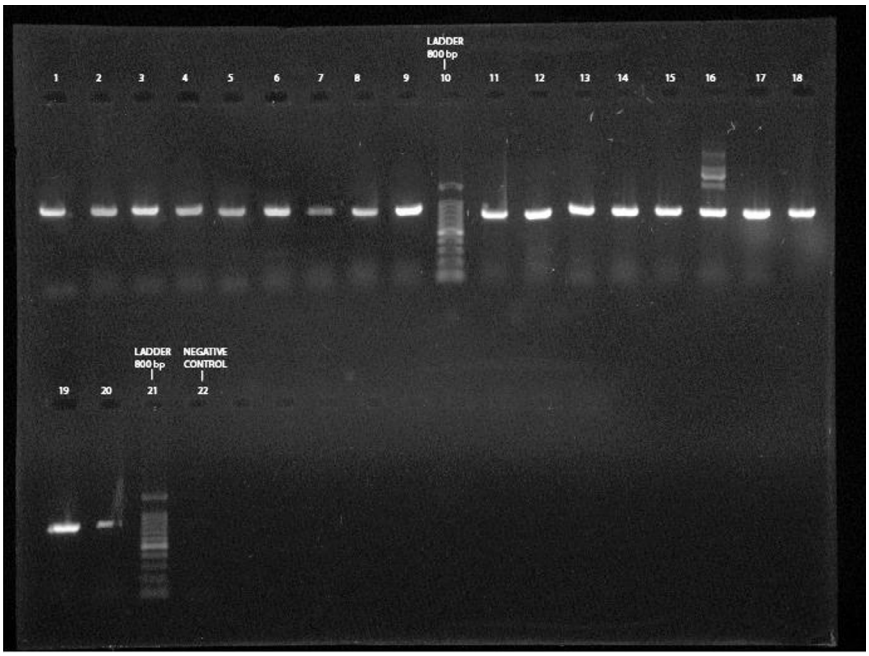

Figure 5. PCR amplification image of TEM 1 & TEM 2 gene in *K. pneumoniae* after 1.5% agarose gel electrophoresis

The current study reported that the co-existence of bla_{TEM} and bla_{CTX-M} was 21.05% (4/19), and bla_{TEM} and bla_{SHV} was 10.53% (2/19). A study conducted by Bora et al.[36] observed that 27.58% of *K. pneumoniae* isolates pose bla_{TEM} and bla_{CTX-M} together. In the present study, the presence of three genes was 47.37%. National Institute of hygiene in Lome, Togo, conducted a study from 2013-2015 that reported the presence of 3 genes in *K. pneumoniae* 59.26% (32/54).[37] However, another study conducted by Manoharan et al.[38] observed that 42.6% of *K. pneumoniae* isolates have all three genes TEM, SHV, and CTX-M. The co-existence of these three genes and some other antimicrobial resistance mechanisms may increase the antimicrobial resistance towards the last choice antibiotics like carbapenems and contribute to a high level of antimicrobial resistance.

CONCLUSION

ESBL is the most crucial antimicrobial resistant mechanism, and the high level of high resistance is consistent with the presence of the resistance gene in this study. Determining ESBL-producing bacterial isolates is essential in controlling infection and epidemiological surveillance studies. The current study reported that most ESBL-producing isolates are resistant to cephalosporins. The ESBL genes TEM 1 & 2, CTX-M, and SHV, these three genes are present in most cases. Only imipenem was active against the ESBL-producing *K. pneumoniae*. The knowledge, identification, and updating of the antimicrobial resistance among the common pathogens are helpful for the appropriate treatment of infections. Monitoring ESBL production using phenotypic and genotypic methods is recommended to benefit infection control.

ACKNOWLEDGMENTS

The authors would like to thank Principal, Karuna Medical College, Palakkad and Head, Department of Microbiology, Karuna Medical College for the facilities provided.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed in this study have made a substantial direct and intellectual contribution to the work and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated during the study are included in the manuscript.

ETHICS STATEMENT

Not applicable

REFERENCES

1. Nirwatil H, Sinanjung K, Fahrunissa F, et al. Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in tertiary care hospital, Indonesia. *BMC Proc.* 2019;13(Suppl 11):20. doi: 10.1186/s12919-019-0176-7
2. Umadevi S, Kandhakumari G, Joseph NM, et al. Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram negative bacilli. *J Clin Diagn Res*. 2011;5(2):236-239.
3. Bush K, Jacoby GA. Updated functional classification of β-lactamase. *Antimicrob Agents Chemother.* 2010;54(3):969-976. doi: 10.1128/AAC.01009-09
4. Tacao M, Moura A, Correia A, Henriques I. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic system. *Water Res.* 2014;48:100-107. doi: 10.1016/j.watres.2013.09.021
5. Collee JG, Fraser AG, Marmian BP, Simmons A, Mackie and McCartney practical medical microbiology. 14th Ed. New York.Churchill Livingstone, 1996.
6. Wayne PA. CLSI performance standard for antimicrobial susceptibility testing, Twenty-fifth informational supplement. Clinical and Laboratory Standards institute. CLSI document M100-S25; 2015.
7. Wayne PA. CLSI performance standard for antimicrobial susceptibility testing. Twenty- fourth informational supplement. Clinical and Laboratory standards institute. CLSI document MI00-S24. 2014.
8. Wayne PA. CLSI performance standard for antimicrobial susceptibility testing. Clinical and Laboratory standards institute. 30th ed. CLSI supplement MI00. 2020
9. Thomson KS. Extended-spectrum-β- lactamase, AmpC and carbapenemase issue. *J Clin Microbiol.* 2010;48(4):1019-1025. doi: 10.1128/JCM.00219-10
10. Pitout JDD. *Enterobacteriaceae* that produce Extended-spectrum β- lactamases and AmpC β- lactamases in the community: the tip of the Iceberg?. *Curr Pharm Des.* 2013;19:257-263. doi: 10.2174/138161213804070348
11. Prestinanci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health.* 2015;109(7):309-318. doi: 10.1179/2047773215Y.0000000030

12. Ghafourian S, Sekawi Z, Neela V, Khosravi A, Rhabar M, Sadeghifard N. Incidence of Extended- spectrum beta- lactamases- producing *Klebsiella pneumoniae* in patients with urinary tract infection. *Sao Paulo Med J.* 2012;130(1):37-43. doi: 10.1590/S1516-31802012000100007
13. Uddin MA, Motazzim-Haque HM, Noor R. Isolation and identification of pathogenic *Escherichia coli, Klebsiella* spp. and *Staphylococcus* spp. in raw milk samples collected from different areas of Dhaka city, Bangladesh. *Stamford Journal of Microbiology.* 2011;1(1):19-23. doi: 10.3329/sjm.v1i1.9098
14. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of Extended - spectrum beta- lactamases among *Enterobacteriaceae* spp isolated at a tertiary care institute. *Indian J Med Microbiol.* 2006;24(3):208-211. doi: 10.1016/S0255-0857(21)02352-5
15. Kaur M. Occurrence of the CTX- M, SHV and the TEM genes among the extended spectrum beta lactamase producing isolates of Enterobacteriaceae in a Tertiary Care Hospital of North India. *Journal of Clinical and Diagnostic Research*. 2013: 642- 645. doi.: 10.7860/jcdr/2013/5081.2872.
16. Yu Y, Zhou W, Chen Y, Ding Y, Ma Y. Epidemiological and antibiotic-resistant study on extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Zhejiang province. *Chin Med J* (Engl). 2002;115(10):1479-1482. PMID: 12490091
17. Navon- Venezia S, Hammer-Munz O, Schwartz D, Turner D, Kuzmenko B, Carmeli Y. Occurrence and phenotypic characteristics of extended-spectrum beta- lactamase among members of the family *Enterobacteriaceae* at the Tel-Aviv Medical Center (Israel) and evaluation of diagnostic tests. *J Clin Microbiol.* 2003;41(1):155-158. doi: 10.1128/JCM.41.1.155-158.2003
18. Canton R, Novias A, Valverde A, et al. Prevalence and spread of extended - spectrum beta- lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect*. 2008;14(1):144-53. doi: 10.1111/j.1469-0691.2007.01850.x
19. Feizabadi MM, Delfani S, Raji N, et al. Distribution of bla (TEM), bla (SHV) genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran. *Iran Microb Drug Resist.* 2010;16(1):49-53. doi: 10.1089/mdr.2009.0096
20. Lal P, Kapil A, Das BK, Sood S. Occurrence of TEM and SHV gene in extended - spectrum beta-lactamases (ESBLs) producing *Klebsiella* spp. Isolated from a tertiary care hospital. *Indian J Med Res.* 2007;125(2):173-178. PMID: 17431288.
21. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A. Extended spectrum beta - lactamases in Escherichia coli and Klebsiella pneumoniae and associated risk factors. *Indian J Med Res*. 2009; 129(6): 695- 700. PMID: 19692752.
22. Ghafourian S, Bin Sekawi Z, Sadeghifard N, et al. The prevalence of ESBLs producing *Klebsiella pneumoniae* isolates in some major hospitals, Iran. *Open Microbiol J.* 2011;5:91-95. doi: 10.2174/1874285801105010091
23. Ananthan S, Subha A. Cefoxitin resistance mediated by loss of porin in clinical strains of *Klebsiella pneumoniae* and *Escherichia coli. Indian J Med Microbiol*. 2005;23(1):20-23. doi: 10.4103/0255-0857.13867
24. Tola MA, Abera NA, Gebeyehu YM, Dinku SF, Tullu KD. High prevalence of extended- spectrum beta-lactamases- producing *Escherichia coli* and *Klebsiella pneumoniae* fecal carriage among children under five years in Addis Ababa, Ethiopia. *PloS ONE.* 2021;16(10):e0258117. doi: 10.1371/journal.pone.0258117
25. Riaz S, Faisal M, Hasnain S. Prevalence and comparison of beta-lactamase producing *Escherichia col*i and *Klebsiella* spp from clinical and environmental source in Lahore, Pakistan. *Afr J Microbiolo Res.* 2012;6(2):465-470. doi: 10.5897/AJMR11.1457
26. Behrooozi A, Rhabar M, Yousefi JV. Frequency of extended -spectrum beta- lactamase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine in an Iranian 1000 -bed tertiary care hospital. *Afr J Microbiol Res.* 2010;4:881-884. http://www.academicjournals.org/ajmr
27. Chaudhary U, Aggarwal R. Extended- spectrum beta-lactamases(ESBL)- an emerging threat to clinical therapeutics. *Indian J Med Microbiol.* 2004;22(2):75-80. doi: 10.1016/S0255-0857(21)02884-X
28. Ferreial RL, da Silva BCM, Rezende GS, et al. High prevalence of multidrug- resistant *Klebsiella pneumoniae* harbouring several virulence and β-lactamase encoding genes in a Brazilian Intensive Care unit. *Front Microbiol.* 2019;9:3189. doi: 10.3389/fmicb.2018.03198
29. Jain A, Mondal R. TEM & amp; SHV genes in Extended-spectrum β- lactamase producing *Klebsiella pneumoniae* species beta their antimicrobial resistance pattern. *Indian J Med Res.* 2008;128(6):759-64. PMID: 19246801
30. Samanje J, Mohammed AS, Al-Hamami MSS. Phenotypic and genotypic detection of Extended -spectrum β-lactamase production by *Klebsiella pneumoniae* isolated from different clinical sample in Baghdad, Iraq. *J Pure Appl Microbiol.* 2021;15(3):1681-1688. doi: 10.22207/JPAM.15.3.64
31. 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. *Open Microbiol J.* 2017;11(1):23-30. doi: 10.2174/1874285801711010023
32. Al-Marzooq F, Yusof MYM, Tay ST. Molecular analysis of antibiotic resistance determinants and plasmids in Malaysian isolates of multidrug resistant *Klebsiella pneumoniae*. *PLOS ONE.* 2015;10(7):133654. doi: 10.1371/journal.pone.0133654
33. 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 Res Int.* 2016;2016:1782835. doi: 10.1155/2016/1782835
34. Aljanaby AAJ, Alhasani AHA. Virulence factors and antibiotic susceptibility patterns of multidrug resistance *Klebsiella pneumoniae* isolated from different clinical infections. *Afr J Microbiol Res.* 2016;10(22):829-843. doi: 10.5897/AJMR2016.8051
35. Hassan H, Abdalhamid B. Molecular characterization

of Extended- spectrum β- lactamase producing *Enterobacteriaceae* in a Saudi Arabian tertiary hospital. *J Infect Ctries.* 2014;8:282-288. doi: 10.3855/jidc.3809

36. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. *Indian J Pathol Microbiol.* 2014;57(2):249-254. doi: 10.4103/0377-4929.134698

37. Diagbouga S, Salah FD, Sadji AY, et al. Detection of High Prevalence of TEM/SHV/CTX-M Genes in ESBL Producing and Multidrug Resistant *Klebsiella pneumoniae* and *Klebsiella Oxytoca. J Clin Diagn Res.* 2016;4(1):130. doi: 10.4172/2376-0311.1000130

38. Manoharan A, Premalatha K, Chatterjee S, Mathai D. Correlation of TEM, SHV and CTX-M extended spectrum beta-lactamases among *Enterobacteriaceae* with their *in vitro* antimicrobial susceptibility. *Indian J Med Microbiol.* 2011;29(2):161-164. doi: 10.4103/0255-0857.81799