Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL16, ISSUE 1, 2025

Molecular characterization of Extended Spectrum Beta Lactamases among Uropathogenic isolates of Klebsiella pneumoniae by Real time Multiplex PCR in a tertiary care hospital in Indore, Central India.

Deepak Chaudhary[1*], Kailash Jatav[2], Priyanka Chaudhary[3]

[1]PhD Scholar, Index Medical College Indore, MP
[2]Assistant Professor, Amaltas institute of medical sciences, Dewas, MP
[3]Tutor, Dept. of Microbiology, Govt. Medical College Haldwani, UK
[*1]Corresponding Author

Abstract-

Introduction- ESBL producing Klebsiella pneumoniae are globally responsible for an increasing number of UTIs with severe morbidity and death. Worldwide, ESBLs have become an important concern for hospitalised patients and have been linked to numerous outbreaks. By using molecular techniques to identify prevalent ESBL genes like TEM, SHV, and CTX-M in ESBL-producing bacteria and their pattern of antibiotic resistance, it is possible to gain insight into the epidemiology of ESBLs and support targeted antimicrobial therapy.

Aim- The purpose of this study was to determine the presence of TEM, SHV, and CTX-M genes in Extended Spectrum Beta Lactamse (ESBL) producing Klebsiella pneumoniae.

Material and Methods- A total of 282 Klebsiella pneumoniae isolates were identified and collected using conventional culture and biochemical tests. Using the disc-diffusion method, antibiotic susceptibility testing was conducted in compliance with CLSI standards. The phenotypic confirmatory disc diffusion test (PCDDT) was used to confirm isolates that had been screened for the production of ESBLs. Using specific primers for the identification of CTX-M, SHV, and TEM genes, a Real Time qPCR was then performed on 100 randomly selected isolates to check for the presence of ESBL-encoding genes.

Results- Imipenem was the most effective antibacterial agent against strains of K. pneumoniae that produced ESBL. Among ESBL-positive isolates, the prevalence of the TEM, SHV, and CTX-M genes was 68%, 35%, and 41%, respectively.

Conclusion- In our study, the prevalence of ESBL producing K. pneumoniae was significantly high. Common genes (TEM, SHV, and CTX-M) that produce extended spectrum beta lactamase in Klebsiella pneumoniae can be rapidly identified using Real Time multiplex PCR. It will be useful for monitoring and identifying the best course of action for combating drug-resistant pathogens, saving significant time and money. It is crucial to regulate these strains in order to stop and minimise the spread of infections brought on by these organisms because managing such infections is challenging.

Key words- UTI, ESBL, Real Time PCR, Klebsiella pneumoniae, TEM, SHV, CTX-M**.**

INTRODUCTION

Among the most prevalent infectious diseases seen in hospitals and the community, urinary tract infections (UTIs) have high rates of morbidity as well as substantial treatment expenses. [1–3] Urinary tract infections, out of all the nosocomial infections, are responsible for 15% of infections and 15,000 deaths annually, according to a study. [4] Due to the widespread usage of beta-lactam antibiotics to treat UTIs, resistant strains of the bacteria have in recent years emerged worldwide. The extended spectrum b-lactamase (ESBL) genes, which are primarily encoded by plasmids, mediate beta-lactam resistance. [5]

Even prior to the development of the first β-lactam antibiotic, penicillin, resistance to β-lactams started to emerge. Escherichia coli was discovered to possess the first β-lactamase before penicillin was made accessible for use in medicine. [6] The first reports of ESBLs in Klebsiella pneumoniae came from Germany in 1983. [7] Antibiotic resistance to beta lactam antibiotics most frequently occurs through the production of beta lactamase. These are produced by anaerobes as well as aerobic Gram-positive and Gram-negative bacteria [8]. These enzymes were named extended-spectrum β-lactamases (ESBLs) due to their extended range of activity, particularly against oxyimino cephalosporins. [9]

Although there are many other kinds of ESBLs, such as TEM, SHV, CTX, OXA, AmpC, and others, the majority of ESBLs are mutated variants of TEM or SHV enzymes and are well-characterized in the Enterobacteriaceae family, particularly Klebsiella pneumoniae [10]. Since Klebsiella species have quickly emerged as the most prevalent ESBL-producing bacteria, it is challenging to remove them from high-risk wards such intensive care units. [10] There are currently over 400 known ESBLs, most of which are derived from point mutations in the TEM, SHV, and CTX-M groups. [11]
Nowadays, preventing the spread of multidrug-resistant gram-negative bacteria across the community is a major problem. The study of antibiotic resistance in bacteria that cause UTIs is becoming more significant since ESBL producers have diverse resistance mechanisms. The distribution and pattern of antibiotic susceptibility of uropathogens vary geographically.

ESBLs have been implicated in several epidemic outbreaks and have become a major concern for hospitalised patients globally. By using molecular techniques to identify prevalent ESBL genes like TEM, SHV, and CTX-M in ESBL-producing bacteria and their pattern of antibiotic resistance, it is possible to gain insight into the epidemiology of ESBL and support targeted antimicrobial treatment. [12] The current study was undertaken since there is very little information on the molecular types of ESBL-positive Klebsiella species from this region of Central India. The present study aimed to determine the frequency of TEM, SHV, and CTX-M ß-lactamase genes using phenotypic and genotypic (RT-PCR) techniques, as well as the antibiotic susceptibility pattern of uropathogenic isolates of K. pneumoniae in the community and at the hospital Index Medical College.

Aims and objectives

This study was conducted to determine the prevalence of ESBL genes in *Klebsiella pneumoniae* isolated from patients visiting the outpatient departments and also who were admitted to various wards of the hospital as well to know the antibiogram profiles of the ESBLs producing *Klebsiella pneumoniae* isolates.

MATERIAL AND METHODS

Sample collection

Urine samples, both fresh mid-stream and catheterized, were collected at Index Medical College Hospital. The study included patients of various age groups and genders from both outpatient and inpatient departments of Index Medical College Hospital who were clinically suspected of having urinary tract infections. Samples were processed immediately after collection to prevent contamination.

These samples were inoculated on CLED agar and MacConkey's agar and incubated at 37^0 c for 18-24 hours. Significant bacteriuria was evaluated by measuring colony-forming units ($\geq 10^5$/mL) of pure growth of single isolates on CLED and MacConkey agar. [13] According to CLSI criteria, isolates were identified as Klebsiella pneumoniae using routine biochemical identification tests (CLSI, 2023). [14] The disc diffusion method was used to evaluate antimicrobial susceptibility testing in the presence of any possible growth in accordance with CLSI criteria. The antimicrobial which were tested included: Ampicillin (10 µg), Amikacin (30µg), Aztreonam (30µg), Ceftazidime (30µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Cefepime (30µg), Amoxicillin/clavulanic acid (20/10µg), Co-trimoxazole (25 µg), Gentamicin (10 µg), Imipenem (30 µg), Ciprofloxacin (5µg), Norfloxacin (10 µg), and Nitrofurantoin (300 µg). Mueller Hinton Agar and antibiotic discs were procured from Hi-Media India. All assays included ESBL positive control standard strain of *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 as negative control.

Screening for ESBL production using disc diffusion method

According to the CLSI recommendations, the standard disc diffusion technique was used to test the gram negative bacterial isolates for susceptibility to third generation cephalosporins (3GCs), that includes ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). The strain was deemed "suspicious for ESBL production" if a zone diameter of <22 mm for ceftazidime, <27 mm for cefotaxime, and <25 mm for ceftriaxone was observed. Strains that demonstrated resistance to any of the third-generation cephalosporins were designated for further ESBL confirmation using this approach. 207 of the 282 strains were suspected to produce ESBLs. These isolates were then subjected to phenotypic confirmation test.

Phenotypic confirmatory Disc Diffusion test (PCDDT) for ESBL production

Based on the CLSI guidelines, using the PCDDT method, cephalosporin/clavulanate combination discs were used to confirm the suspected ESBL strains. Briefly, the overnight growth in broth of Gram negative bacteria was adjusted to 0.5 McFarland Standard. According to CLSI guidelines, confirmation was performed using the confirmatory disk diffusion disc method. A lawn culture of test strains (0.5 Mc Farland inoculum type) on Mueller Hinton Agar was used to confirm the production of ESBLs. A disc of cefotaxime (30 µg) and ceftazidime (30 µg) was placed 20 mm apart from a disc of cefotaxime/clavullinic acid (30/10µg) and ceftazidime/clavullinic acid (30/10µg), respectively **(Figure.1)**. After overnight incubation at 37^0C the strain was considered ESBL positive if there was an increase in zone size of >5 mm in the zone size of Cephalosporin/Clavulanate combination disc when compared with cephalosporin alone. All the 207 strains were subjected to the combination disc method of which 159 strains were phenotypically confirmed as ESBL producers.

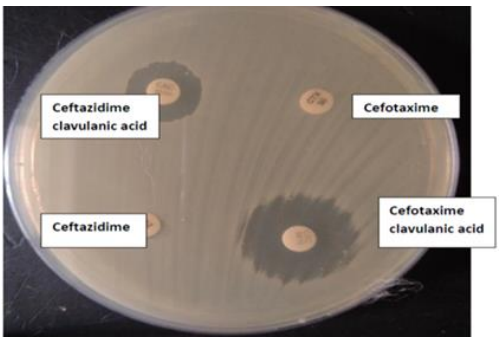

Figure 1: ESBL detection by Phenotypic Confirmatory Double-Disc Diffusion Test [PCDDT]

DNA extraction and characterization of bla genes

Of the 159 strains phenotypically confirmed as ESBL positive, 100 randomly picked non repetitive strains of K. pneumoniae isolates were then analyzed at their gene level. A single colony of test isolates was selected from a fresh culture plate, then transferred into 5 ml of luria bertania broth and incubated overnight at 37ºC. The genomic DNA from each isolate was extracted using the DNA Extraction Kit (TRUPCR® Bacterial DNA Extraction Kit), following the manufacturer's instruction. Bacterial lysate prepared from K. *pneumoniae* isolates were subjected to Real time qPCR for the detection of Beta-lactamase genes (CTX-M, TEM & SHV).

Amplification

A highly standardized in vitro nucleic acid amplification assay 'TRUPCR® ESBL Resistance Detection Kit (V1.0)' from 3B Blackbio Biotech India Ltd. was used for the detection & differentiation of antimicrobial resistance genes encoding resistance to antibiotics-Extended spectrum beta lactamase (ESBLs) genes i.e. $bla_{\text{CTX-M gr1}}$, bla_{TEM}, bla_{SHV} using Real time qPCR system. The reagents included in the kit are listed in **Table 1**.

Amplification of DNA was performed using CFX96 Real Time PCR system by Bio-Rad Laboratories, U.S.A. For PCR amplification for TEM, SHV and CTX-M genes the following reaction mixture was prepared: - 10 µl of template DNA + 10 µl of master mix containing 4.0 µl of qPCR Mix with UNG (Hot-start fast DNA polymerase Uracil-N-glycosylase (UNG) qPCR Buffer dNTPs) and 6.0 µl of Primer probe mix for ESBLs ($bla_{\text{CTX-M gr1}}$, bla_{TEM}, bla_{SHV}) and endogenous internal control (IC) detection **(Table 2)**.

Procedure of Real Time PCR

Master Mix prepared was added in a sterile cryovial & total volume obtained is 20 µl for one reaction. Mixing was done by vortex. Master Mix was dispensed in each PCR tubes with the help of Micropipette and 10.0 µl of isolated DNA templates were added to each master mix containing PCR tubes maintaining the sterile condition & each PCR tubes were capped and further placed in BioRad CFX96 Machine for Amplification. Initial denaturation step was done for 3 min at 95^0C followed by Denaturation step for 5 min at 95^0C & Annealing/Extension for 30 sec at 60^0C **(Table 3)**. The denaturation and annealing/extension step was repeated for 40 cycles and Acquisition on FAM, Texas Red, Cy5 & Hex was taken at annealing/extension step of each cycle. After finishing the complete cycle graph was read considering positive Cq value between 10-30 and accordingly the result was calculated.

Results and Discussion

According to this study, K. pneumoniae pattern of antibiotic resistance to various beta-lactam and non-beta-lactam antibiotics varied greatly. It was found that the majority of the K. pneumoniae isolates were multidrug resistant (MDR), implying they were resistant to three or more of the antibiotics used in the course of the study. 207 isolates (73.4%) of the 282 K. pneumoniae isolates found positive for ESBL production in the initial screening test, while 159 isolates (56.38%) tested positive for ESBL production in the phenotypic confirmation. According to the results of the PCR analysis of ESBL genotypes, all of the 100 K. pneumoniae isolates investigated in this study had at least one ESBL gene. Among the 100 isolates the number of ESBL producing K. pneumoniae with TEM, SHV and CTX-M were 68%, 35% and 41% respectively. As shown in table 4, several strains had one or more ESBL genes, and in a few of them, all three were found. An estimated 250 million instances of urinary tract infections (UTIs) occur worldwide each year, making it one of the most prevalent infections. [15] The large proportion of ESBL-producing Klebsiella sp. is a result of the extensive use of antibiotics. Extended spectrum beta-lactamase (ESBL) enzymes have become more prevalent in gram-negative bacteria in recent years, which has led to a widespread resistance to third-generation cephalosporins (3GCs).

The prevalence of K. pneumoniae urinary tract infections in the present research is in very similar with findings from earlier national studies. [16-18] India's reported prevalence rate of Klebsiella spp. that produce ESBLs ranges from 6 to 87%, which is in accordance with previous studies from nearby regions of the country. [19-24] The prevalence of ESBL in hospitals and communities across the globe is believed to vary from 3% to 100%. [25-28] According to this study, the most alarming aspect of the Klebsiella pneumoniae isolates that had passed the ESBL screening test is their widely variable antibiotic resistance rates against second- and third-generation cephalosporins, which range from 81.64% to 84.54% **(Table 4 and figure 2)**, which are comparable to those found in other authors' studies and establish these as MDR strains. [29-34] Imipenem (94.68%), amikacin (81.15%), and nitrofurantoin (80.19%) showed the highest sensitivity among the non-cephalosporin antimicrobials tested, suggesting that they are likely efficient drugs for treating UTIs caused on by K. pneumoniae that produces ESBLs. The rising use of cephalosporins in India might be linked to the increasing prevalence of multidrug resistance. [35,36]. It is well recognized that overuse of antibiotics leads to the development of drug resistant bacteria, which is one of the main causes of the clinically significant rise in the prevalence of bacteria that produce ESBL. [37]

Molecular techniques are costly, time-consuming, and need specialized equipment and knowledge, despite their apparent sensitivity. However, they are unquestionably helpful in understanding epidemiological studies and predisposition factors. [38] The phenotypic methods for ESBL detection seem to be limited to confirming the presence of an ESBL; they are unable to identify the specific subtype of ESBL. Additionally, certain ESBLs may not reach a level that disk diffusion tests can identify, which might result in treatment failure.

For molecular characterization 100 strains were randomly picked. 68 isolates were positive for *bla*TEM, 35 isolates were positive for *bla*SHV & 41 exhibited presence of *bla*CTX-M **(Table 5 and figure 3)**. 06 strains exhibited presence of both *bla*TEM and *bla*SHV, 16 isolates contained both *bla*CTX-M and *bla*TEM, 04 isolates showed presence of both *bla*CTX-M and *bla*SHV. Around 13 isolates

exhibited presence of all the three *bla* genes **(Table 6)**. Real-time detection and typing of genes encoding CTX-M1, TEM & SHV ESBLs in *K. pneumoniae* isolates using FAM (Blue), TEXAS RED (Red), Cy5 (Purple) & HEX (Green) channels is shown in **Fig 4 & 5.**

ESBL have gone from being an intriguing scientific finding to a reality of immense medical consequence. They were initially only found in hospital-acquired infections, but they have now been found in outpatient infections as well. Since there have been significant outbreaks recorded worldwide, they are emerging pathogens. The occurrence of ESBLs is still rather low in certain locations, such as Ujjain, Bijapur, and Kanchipuram (some Indian cities) [39–41]. However, ESBL production has gradually increased in India's major cities, such as New Delhi, rising from 80% in 2002 to 91% in 2005, 97% in 2007, and 100% in 2010 [20,42,43].Unintentional and widespread usage of third-generation cephalosporins is likely the cause of this. Another explanation for why patients opt for self-medication may be over-the-counter availability of these drugs [29].

Sharma et al. [44] observed a 60.0% positive rate for the SHV gene and a 56.0% frequency for TEM. 52.0% *bla*TEM, 45.0% *bla*SHV, and 37.0% *bla*CTX-M were found in K. pneumoniae isolates by Tripathi et al. [45] According to a research [46], the TEM detection rate was somewhat lower (47.33%), followed by the SHV (45.33%) and CTX-M (37.33%). Prevalence of TEM gene in the isolates was comparable to a research from Gujarat. [47] The prevalence of the TEM, SHV, and CTX-M genes is 67.0%, 55.0%, and 85.0%, respectively, according to Abraham et al. [48] Prevalence of TEM is comparable to this research. The more recent *bla*CTX-M type of ESBLs has replaced the older TEM and SHV types, which were formerly thought to be the cause of nosocomial infections.

K. pneumoniae is one of the most prevalent ESBL producing organisms, making challenging for the physicians to treat them specifically in the hospital settings. Since ESBL-producing strains are becoming more common in hospitals worldwide, it is important to determine the incidence of ESBL-positive strains in a hospital in order to develop an empirical treatment strategy for high-risk units where infections from resistant organisms are considerably more prevalent. [20] Further molecular studies of such types are undoubtedly required in other parts of India in order to identify the common ESBL enzymes present in those areas for epidemiological reasons.

Reagents	Description
qPCR Mix with UNG	Hot-start fast DNA polymerase Uracil-N-glycosylase (UNG) qPCR Buffer dNTPs (dATP, dCTP, dGTP, dUTP)
Primer Probe mix – 1 (for ESBLs)	Primer probe mix for ESBLs ($bla_{\text{CTX-M gr1}}$, bla_{TEM}, bla_{SHV}) and endogenous internal control (IC) detection
Positive Control (PC)	Positive control for all the antibiotic target genes and IC
Negative Control (NC)	Nuclease-free water

Table 1- Reagents required for PCR

Name of the Reagent	For 1 Reaction
	Tube-1
5x qPCR Mix with UNG	4.0 µl
Primer Probe mix – 1 (for ESBLs)	6.0 µl
Total reaction volume	**10.0 µl**

Table 2: Preparation of PCR mix

Steps	Temperature	Time	Dye Acquisition	Cycles
Initial Denaturation	95°C	3 min	-	1
Denaturation	95°C	5 min	-	40
Annealing/ Extension	60°C	30 sec	yes	

ISSN: 0975-3583, 0976-2833 VOL16, ISSUE 1, 2025

Table 3: PCR Amplification and Cycling Conditions

Antimicrobial Agents	Sensitive		Resistant	
	Number	Percentage (%)	Number	Percentage (%)
Ampicillin	0	0.00%	207	100%
Amikacin	168	81.15%	39	18.84%
Aztreonam	45	21.73%	162	78.26%
Cefotaxime	32	15.45%	175	84.54%
Ceftazidime	38	18.35%	169	81.64%
Ceftriaxone	34	16.42%	173	83.57%
Cefepime	75	36.23%	132	63.76%
Amoxicillin/Clavulanic acid	42	20.28%	165	79.71%
Ciprofloxacin	119	57.48%	88	42.51%
Co trimoxazole	86	41.54%	121	58.45%
Gentamicin	121	58.45%	86	41.54%
Imipenem	196	94.68%	11	5.31%
Norfloxacin	143	69.08%	64	30.92%
Nitrofurantoin	166	80.19%	41	19.80%

Table 4: Antibiotic susceptibility pattern of Klebsiella pneumoniae isolates (n=207)

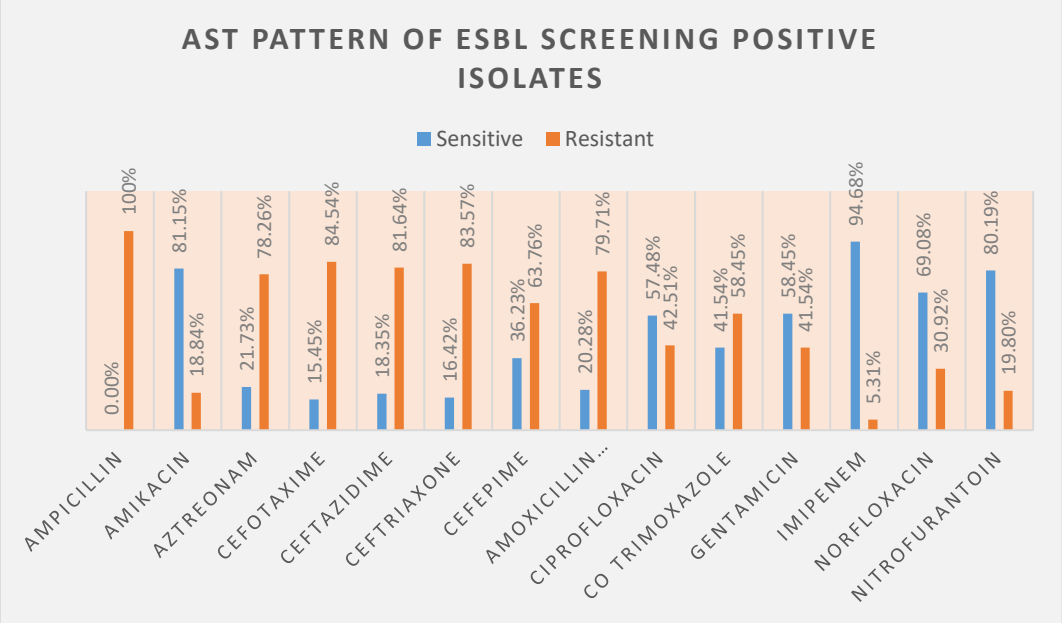

Fig. 2: Antibiotic susceptibility pattern of ESBL positive study isolates (n=207)

Amplified bla genes	Frequency (n=100)	Percentage (%)
bla_{TEM}	68	68%
bla_{SHV}	35	35%
bla_{CTX-M}	41	41%

Table 5. Prevalence of blaCTX-M, blaTEM and blaSHV genes among ESBL producing K. pneumoniae isolates.

ISSN: 0975-3583, 0976-2833 VOL16, ISSUE 1, 2025

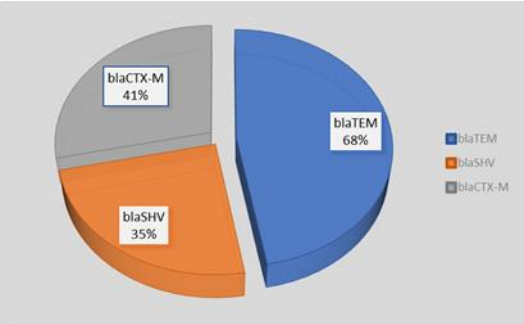

Fig 3. Prevalence of blaCTX-M, blaTEM and blaSHV genes among ESBL producing *K. Pneumoniae* isolates.

ESBL GENES (SINGLE/ IN COMBINATION)	**NUMBER OF STRAINS (n=100)**
***bla*$_{TEM}$ + *bla*$_{SHV}$**	**06**
***bla*$_{TEM}$ + *bla*$_{CTX-M}$**	**16**
***bla*$_{SHV}$ + *bla*$_{CTX-M}$**	**04**
***bla*$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{CTX-M}$**	**13**
ONLY *bla*$_{TEM}$	**33**
ONLY *bla*$_{SHV}$	**12**
ONLY *bla*$_{CTX-M}$	**08**
Strains with none of the 3 genes	**12**

Table 6. Distribution of TEM, SHV and CTX-M genes among ESBL producing *K. pneumoniae* isolates

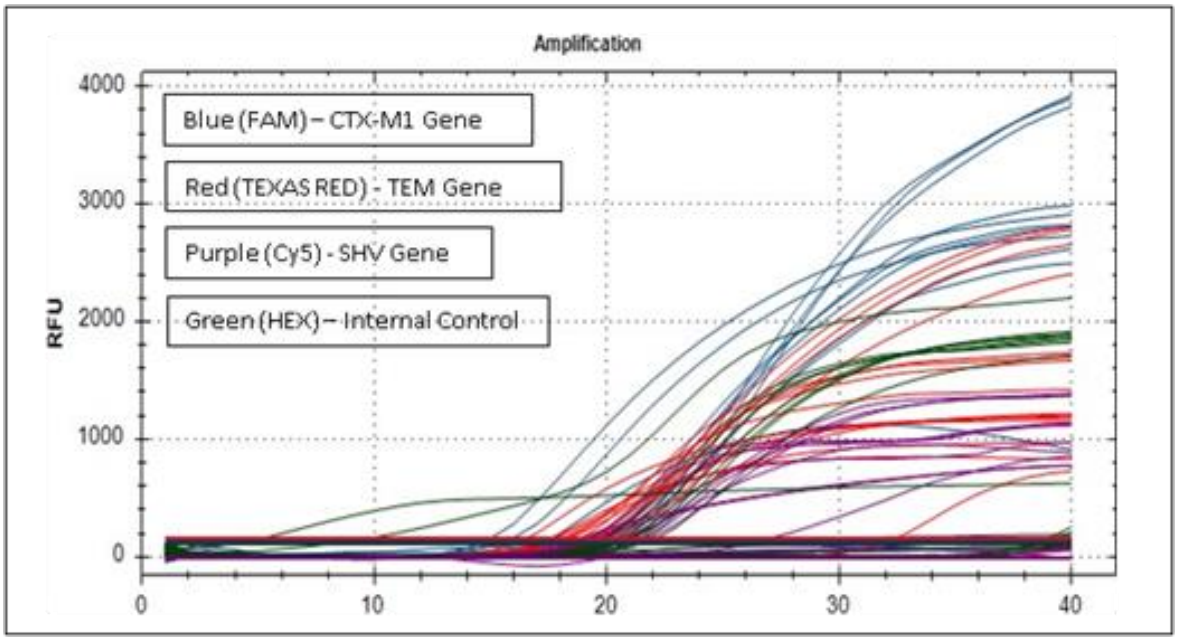

Fig 4. Real-time detection and typing of genes encoding CTX-M1, TEM & SHV ESBLs in K.P isolates using FAM (Blue), TEXAS RED (Red), Cy5 (Purple) & HEX (Green) channels of Bio-Rad CFX-96 RT-PCR.

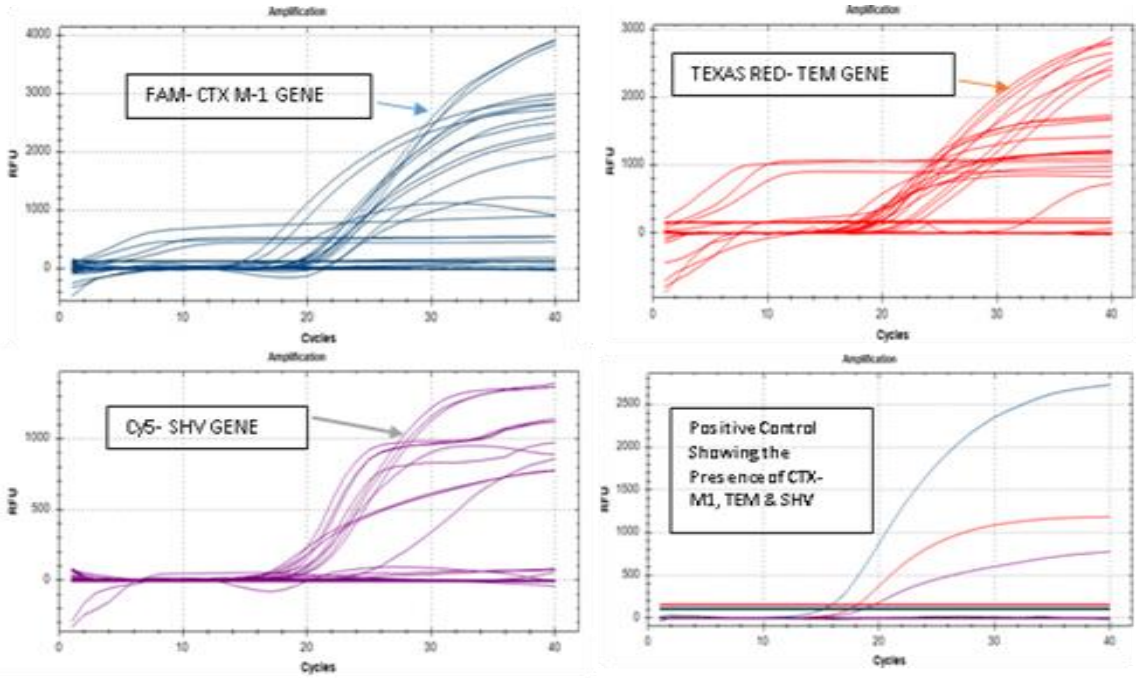

Fig 5. Graphical amplification curve for blaCTX-M1, blaTEM & blaSHV genes by RT-qPCR

Conclusion

When ESBL-producing isolates are reported from clinical samples, clinicians can utilize this information to treat ESBL-producing strains with suitable drugs and take preventative measures to keep these resistant organisms from spreading to other patients. A specialized phenotypic confirmatory test is essential for identifying ESBLs since the standard susceptibility tests used by clinical labs are unable to identify ESBL-positive bacteria and may mistakenly identify isolates that are sometimes responsive to any third-generation cephalosporin. PCDDT is an excellent phenotypic confirmatory test.

Using PCR amplification, rapid diagnosis can be accomplished without phenotypic characterization. The common genes (TEM, SHV, and CTX-M) that produce extended spectrum beta lactamase in Klebsiella pneumoniae isolated from various clinical samples may be quickly identified using real-time multiplex PCR. It will be useful for monitoring and identifying the best course of treatment for combating drug-resistant pathogens, saving significant time and money.

Based on these results, it can be said that an unusually high number of K. pneumoniae isolates which produce ESBLs are found in UTIs. All of our tertiary health facilities need to enhance the enforcement of infection control procedures, monitor and use extended spectrum cephalosporins sparingly, and conduct periodic surveillance of antibiotic resistance trends.

References

[1] Arjunan, M., Al-Salamah, A.A., Amuthan, M. 2010. Prevalence and antibiotics susceptibility of uropathogens in patients from a rural environment, Tamilnadu, *Am. J. Infect. Dis*., 6: 29– 33.

[2] Rahman, F., Chowdhury, S., Rahman, M.M., Ahmed, D., Hossain, A. 2009. Antimicrobial resistance pattern of Gram-negative bacteria causing urinary tract infection. *Stamford J. Pharm. Sci.*, 2: 44–55.

[3] Hryniewicz, K., Szczypa, K., Sulikowska, A., Jankowski, K., Betltjewska, K., Hryniewicz, W. 2001. Antibiotic susceptibility of bacterial strains isolated from urinary tract. *J. Antimicrob. Chemother.*, 47: 773–80.

[4] Klevens, R.M., Edward, J.R., *et al.* 2007. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Reports*, 122: 160-166.

[5] Topaloglu, R., Er, I., Dogan, B.G., Bilginer, Y., Ozaltin, F., Besbas, N., *et al.* 2010. Risk factors in community-acquired urinary tract infections caused by ESBL-producing bacteria in children. *Pediatr. Nephrol.*, 25(5): 919-25.

[6] Abraham EP, Chain E (1940) An enzyme from bacteria able to destroy penicillin. Nature 146-837.

[7] Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S (1983) Transferable resistance to cephotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection 11: 315- 318.

[8] Louis Rice CHEST 2001; 119: 391S-396S.

[9] Bradford P.A (2001) Extended- Spectrum Beta Lactamases in the 21st Century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14: 933-951.

[10] Lal P, Kapil A, Das BK, Sood S (2007) Occurrence of TEM and SHV gene in extended Spectrum β-Lactamases (ESBLS) producing *Klebsiella sp.* Isolated from a tertiary care hospital. Indian J Med Res 125: 173-178.

[11] Barguigua, A., El Otmani, F., Talmi, M., Bourjilat, F., Haouzane, F., Zerouali, K., *et al.* 2011. Characterization of extended-spectrum b-lactamase producing Escherichia coli and Klebsiella pneumoniae isolates from the community in Morocco. *J. Med. Microbiol.*, 60(9): 1344-52.

[12] Jain, A., Mondal, R. 2008. TEM and SHV genes in extended spectrum beta lactamase producing *Klebsiella* species and their antimicrobial resistance pattern. *Indian J. Med. Res.,* 128: 759-64.
[13] Trubiano A, Padiglione A. Nosocomial infections in the intensive care unit. Anaesth. Intensive Care. 2015; 16:598–602.
[14] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.
[15] Ronald, A.R., Nicolle, L.E., Stamm, E., Krieger, J., Warren, J. and Shaeffer, A. 2001. Urinary tract infection in adults: research priorities and strategies. *Int. J. Antimicrob Agents,* 17: 343-8.
[16] Vyas A, Karuna T, Kumar S, Gupta A, Sampath A, et al. (2023) Antimicrobial Resistance Trends in Urinary Tract Infection at Secondary Care Centres in Central India: Carbepenem Resistance Crossing 20% in Community. Infect Dis Diag Treat 7: 235. DOI: 10.29011/2577-1515.100235
[17] Mohapatra S, Panigrahy R, Tak V, J V S, K C S, Chaudhuri S, Pundir S, Kocher D, Gautam H, Sood S, Das BK, Kapil A, Hari P, Kumar A, Kumari R, Kalaivani M, R A, Salve HR, Malhotra S, Kant S. Prevalence and resistance pattern of uropathogens from community settings of different regions: an experience from India. Access Microbiol. 2022 Feb 9;4(2):000321. doi: 10.1099/acmi.0.000321. PMID: 35355869; PMCID: PMC8941965.
[18] Sarojamma V, Ramakrishna V. Prevalence of ESBL producing Klebsiella pneumoniae isolates in tertiary care hospital. ISRN Microbiology. 2011; 11:1-5.
[19] Hansotia, J., Agrawal, V., Pathak, A. and Saroji, A. 1997. Extended spectrum beta lactamases mediated resistance to third generation cephalosporins inKlebsiella pneumoniae in Nagpur, Central India. *Indian J. Med. Res.*, 105: 153-161.
[20] Mathur, P., Kapil, A., Das, B., Dhawan, B. 2005. Prevalence of extended spectrum beta-lactamase producing Gram-negative bacteria in a tertiary care hospital. *Indian J. Med. Res.,* 122: 305-8.
[21] Oberoi, L., Singh, N., Sharma, P., Aggarwal, A. 2012. ESBL, MBL and Ampc β lactamases producing super bugs-Havoc in the intensive care units of Punjab India. *J. Clin. Diagn. Res.,* 7: 70-3.
[22] Sharma, M., Pathak, S., Srivastava, P. 2013. Prevalence and antibiogram of extended spectrum β-lactamase (ESBL) producing gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and Klebsiella spp. *J. Clin. Diagn. Res.,* 7(10): 2173–2177.
[23] Sheemar, S., Chopra, S., Mahajan, G., Kaur, J., Chouhan, Y.S. 2016. Extended spectrum beta-lactamase and AmpC producing *Klebsiella pneumoniae*: A therapeutic challenge. *Trop. J. Med. Res.,* 19: 114-7.
[24] Jain, A. and Mondal, R. 2007. Prevalance and antimicrobial resistance pattern of extended spectrum beta lactamase producing Klebsiella spp. from cases of neonatal septicemia. Indian J. Med. Microbiol., 125: 89-94.
[25] Bean, D.C., Krahe, D., Wareham, D.W. 2008. Antimicrobial resistance in community and nosocomial Escherichia coli urinary tract isolates, London 2005-2006. *Ann. Clin.*
[26] *Microbiol. Antimicrob.,* 7: 13.Chanwit, T., Somporn, S., Wararat, C. 2007. "A correlation between phenotypes and genotypes of extended spectrum beta lactamase (ESBL) producing *Klebsiella pneumoniae* in a university hospital, Thailand." *J. Infect. Dis. Antimicrob. Agent,* 24: 117-23.
[27] Cristina, M.F., William, A.F., Nayane, C.O. *et al.* 2011. "Extended-spectrum beta- lactamase producing bacteria isolated from Haematologic patients in Manaus, State of Amazonas, Brazil." *Brazilian J. Microbiol.,* 42: 1076- 1084.
[28] Der, A.A., Angamuthu, K. 2005. Extended- spectrum betalactamases in urinary isolates of Escherichia coli, Klebsiella pneumoniae and other gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. *Saudi Med. J.,* 26: 956-9.
[29] Fouzia, B. and Damle, A.S. 2015. Int. J. Biomed. Adv. Res., 6(02): 91-97.
[30] Bora, A., Hazarika, N.K., Shukla, S.K., Prasad, K.N., Sarma, J.B., Ahmed, G. 2014. Prevalence of blaTEM, blaSHV and blaCTX-M genes in clinical isolates of Escherichia coli and Klebsiella pneumoniae from Northeast India. Indian J. Pathol. Microbiol., 57: 249-54.
[31] Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic characteristics of clinical isolates of *Klebsiella pneumoniae* and evaluation of available phenotypic techniques for detection of extended-spectrum β- lactamases. *Indian J Med Res* 2005; 122:330-337. http://www.ncbi.nlm.nih.gov/pubmed/16394326
[32] Kamatchi C, Magesh H, Sekhar U, Vaidyanathan R. Identification of clonal clusters of Klebsiella pneumoniae isolates from Chennai by extended-spectrum β-lactamase genotyping and antibiotic resistance phenotyping analysis. Am J Infect Dis 2009; 5(2):74-82. doi:10.3844/ajidsp.2009.74.82
[33] Shukla I, Tiwari R, Agrawa M. Prevalence of extended-spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care Hospital. Indian J Med Microbiol 2004; 22(2):87-91. http://www.ijmm.org/text.asp?2004/22/2/87/8078
[34] Aminzadeh Z, Kashi M, Shabani M. Bacteriuria by extended-spectrum β- lactamase producing Escherichia coli and Klebsiella pneumoniae isolates in a governmental hospital in south of Tehran, Iran. Iran J Kidney Dis 2008;2(4):197-200.
[35] Chaudhary, U., Aggarwal, R. 2004. Extended spectrum -lactamases (ESBL) - An emerging threat to clinical therapeutics. Indian J. Med. Microbiol., 22: 75-80.
[36] Thomson, K.S. 2010. Extended spectrum beta-lactamase, AmpC, and Carbepenamase issues. *J. Clin. Microbiol.,* 48(4): 1019-1025.
[37] Medeiros, A.A. 1997. Evolution and dissemination of beta-lactamases accelerated by generations of beta- lactam antibiotics. Clin. Infect. Dis., 24: S19-45.
[38] Nuesch-Inderbinen, M.T., Hachler, F.H.K.H. 1996. Detection of genes 15 coding for extended-spectrum SHV beta- lactamases in clinical isolates by a molecular genetic method, and comparison with the Etest. *Eur. J. Clin. Microbiol. Infect. Dis.,* 15: 398-402.

[39] Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R, et al. (2012) High prevalence of extended spectrum β-lactamases producing pathogens: results of a surveillance study in two hospitals in Ujjain India. Dove Press Journal: Infection and Drug Resistance 5: 65-73.

[40] Metri BC, Jyothi P, Peerapur BV (2011) The Prevalence of ESBL among *Enterobacteriaceae* in a tertiary care hospital of North Karnataka, India. J Clin Diag Res 5: 470-475.

[41] Kumar MS, Lakshmi V, Rajagopalan R (2006) Occurrence of extended spectrum β-lactamases among *Enterobacteriaceae* spp isolated at a tertiary care institute. Ind J Med Microbiol 24: 208-11.

[42] Wattal C, Chugh TD, Sharma A, Oberoi JK, Prasad KJ, et al. (2005) ESBL – An Emerging Threat to antimicrobial Therapy. Microbiology Newsletter Sir Ganga Ram Hospital 10: 8.

[43] Rastogi V, Nirwan PS, Jain S, Kapil A (2010) Nosocomial outbreak of septicaemia in neonatal intensive care unit due to extended spectrum β-lactamase producing *Klebsiella pneumoniae* showing multiple mechanisms of drug resistance. Ind J Med Microbiol 28: 380-4.

[44] Sharma J,Sharma M and Ray P. Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res 132, pp 332-336, 2010.

[45] Tripathi A, Dutta SK , Majumdar M , Dhara L, Banerjee D and Roy K. High Prevalence and Significant Association of ESβL and QNR Genes in Pathogenic Klebsiella pneumoniae Isolates of Patients from Kolkata, India. Indian J Microbiol 52(4):557–564, 2012.

[46] Chaudhary M and Payasi A. Molecular characterization and in vitro susceptibilities of β-lactamase producing Escherichia coli, Klebsiella species, Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus to CSE1034 and other β-lactams. Asian Pac J Trop Med ;7:s217,2014.

[47] Varun C and Parijath NG. Detection of TEM and SHV Genes in Extended Spectrum Beta Lactamase (ESβL) Producing E. Coli and Klebsiella pneumoniae Isolated From a Tertiary Care Cancer Hospital. Natl. J. Med. Res., 4(3): 201-204,2014.

[48] Abraham J, Varkey DR and Balaji V. Molecular characterization of extended spectrum beta lactamase producing strains from blood sample. Int J Pharm Pharm Sci, Vol 6, Issue 3, 276-278,2014.