DETERMINE THE MOLECULAR EPIDEMIOLOGY OF ESBL ISOLATES HOW OFTEN ESBL GENERATING KLEBSIELLA PNEUMONIAE WERE IN HOSPITAL SETTING

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Abstract

It is true that ESBL producers may lead to cardiomyopathy, UTIs, and respiratory infection. Recent observations in the area of public health indicate that gut colonisation, domestic member dissemination, foreign travel, and faecal carriage are also factors in the spread of ESBL-producing organisms. ESBLs are found in a variety of bacteria from the "Enterobacteriaceae family, including Klebsiella pneumoniae. The molecular epidemiology of ESBL isolates in this region of the nation is not well understood. The goal of this research was to determine how often ESBL generating Klebsiella pneumoniae were in both community and hospital settings. In method, randomly picked 100 isolates of "K. pneumoniae were taken for further genotypic studies. Phenotypically ESBL positive isolates were confirmed for the presence of bla genes by Uniplex PCR. Result depicted that 11 isolates was positive for all the three "blaTEM, blaSHV and blaCTX-M". Overall prevalence of TEM gene was 74.0%, SHV gene was 27.0% and CTX-M gene was 44.0% among the 100 isolates subjected to PCR. the TEM and SHV genes were more prevalent in the inpatients the CTX-M gene was more in the community samples. These ESBL-producing isolates are a therapeutic option in serious instances of resistant to multiple medications bacteria in this population, therefore this discovery is problematic for medical therapy.

Keywords: *ESBL isolates, genes, molecular, Klebsiella pneumoniae*

Introduction

Infectious illness therapy is increasingly threatened by antimicrobial resistance (Arora et al., 2023). The variety and extensive occurrence of β-lactamase enzymes have made it more difficult to control multidrug-resistant Gram-negative pathogens in particular (Naas et al., 2023). A possible method to tackle Gram-negative resistance is the use of novel β-lactamase inhibitors with increased action against such enzymes (Bush, 2023). This article discusses the categorization of β-lactamases, the evolution of β-lactamase medication, and all of the innovative β-lactamase inhibiting agents that have been at present authorised by the FDA along with the β-lactam/β-lactamase inhibitor products that can be bought for clinical use. It also covers their ways of action, ranges of limitation, roles as treatment, and testimony for use. (Kim et al., 2023).

These substances are used in conjunction with -lactam antibiotics to inhibit the activity of β – lactamases (Amir et al., 2023; Narendrakumar et al., 2023). They do this by permanently and irreversibly bind to the enzyme's active site. Inactive. Show extremely little antibacterial action and serve as "suicide substrates" when combined with penicillins (Fatima et al., 2023). The beta-lactamases are captured and rendered inactive by the stable intermediates they produce (Zeden et al.,

2023; Keller et al., 2023). Clavulanic acid, which was derived from Streptomyces clavuligaris, was the first ß-lactamase inhibitor to be utilized in clinical settings (Kim et al., 2023).

Its antibacterial action is minimal. However, when used in conjunction with amoxicillin, it considerably boosts the latter's antibacterial action. Sulbactam and tazobactam, two more ß-lactamase inhibitors, are taken with piperacillin and ampicillin, respectively (Choi et al., 2023; Darby et al., 2023). According to Kim et al., (2023), "class-A ß-lactamases such CTX-M, TEM, and SHV-ESBLs" are resistant to ß-lactamase inhibitors.

Klebsiella species are important pathogenic organisms that affect youth. They cause completely abscess, trauma and melt wound infections, septicemia and challenges of the neurological system, breathing problems tract, bladder, and abdomen cavity, and bacterial infection linked to medical services and communal environments (about 75% of circumstances are related to healthcare). (Darby et al., 2023). The infection's clinical manifestations are identical to those associated with various gram-negative bacillary infections". The study is aimed to understand the frequency of carriage and prevalence of "TEM, SHV and CTX-M type ESBLS-producing Klebsiella pneumoniae causing urinary tract infection among the hospitalized patients, healthy adults, old aged and children in the community.

Material and method

Maintainence of ESβL positive strains was an important aspect. All these isolates were preserved in nutrient agar stock culture tubes. These were then layered with 20% glycerol and stored at -20ºC. From this randomly picked 100 isolates of "K. pneumoniae were taken for further genotypic studies". Standard strains were procured from SSIMSRC, Davengere, Karnataka. The bacteriological media and antibiotic discs were obtained from Hi-media laboratories, Mumbai.

Extraction of plasmid DNA- Using GeNeiPureTM, that plasmid DNA was extracted from cell membranes of bacteria. A plasmid recovery kit using the producer's recommendations and the alkaline lysis concept. The eluted product was later used as template DNA and was stored at -200 c for amplification. The concentration of extracted DNA was assessed by spectrophotometer.

Phenotypically Uniplex PCR was used to validate the absence of bla dna in ESBL-positive cases. The apparatus used for pcr was "2720 Thermocycler Integrated Biosytems.". PCR Kit (PCR Master Mix Kit) for PCR was procured from GeneiTM Merck Laboratories. Preparation of 25µl PCR reaction mixture for a primer set was done as described later. The primers used were already worked upon by others in previously published studies. The primers used for TEM and SHV and for CTX-M they were used in this study. The expected amplicon size was as determined in the above-mentioned studies.

The statistical approaches were carried out using SPSS for Windows, & data was entered in MS-Excel (version 22.0). Statistics by using the Pearson's "Chi-square test and a p value of less than 0.05 was considered as statistically significant under categorical variables".

Result and Discussion

Occurrence of "BlaTEM, BlaSHV, BlaCTX-M gene among ESβL positive K". Pneumoniae (N=100) isolates by PCR

Of the uropathogenic K.pneumoniae ESβL producers, 100 were randomly selected for genetic characterization. Out of the 100 ESβL producers selected 74 strains possessed TEM genes with

amplicon sizes of 504 bp (Fig 1), 27 starins possessed SHV with amplicon sizes of 626 bp (Fig 2) and 44 strains possessed CTX- M with amplicon sizes of 544 bp genes (Fign3).

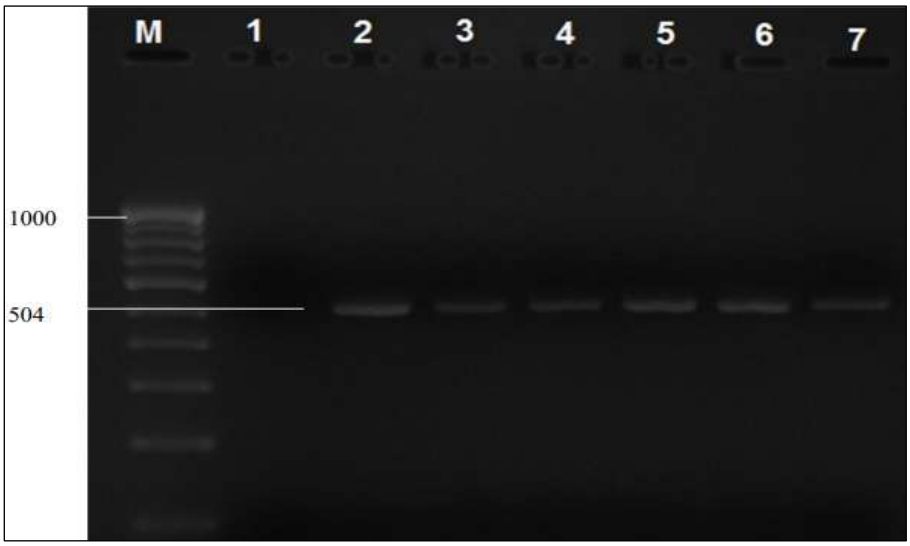

Figure 1: "Amplified 504 bp fragment bands of bla TEM genes detected by PCR (lane 1: negative control, 2-positive control & 3-7 = ESβL positive isolates, M = 100 bp DNA" ladder, Genei , Bangalore) 1000 504"

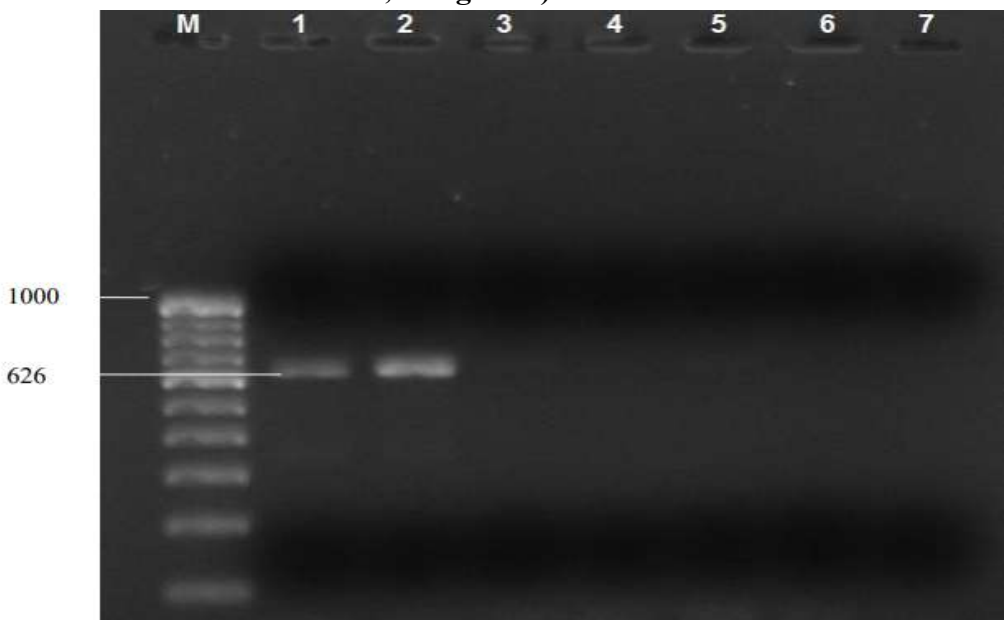

Figure 2: "Amplified 626 bp fragment bands of bla SHV genes detected by PCR (lane 1: positive control; 2: ESβL positive isolate & 3-6 ESβL negative isolates; 7: negative control; M = 100 bp DNA ladder, Genei , Bangalore)".

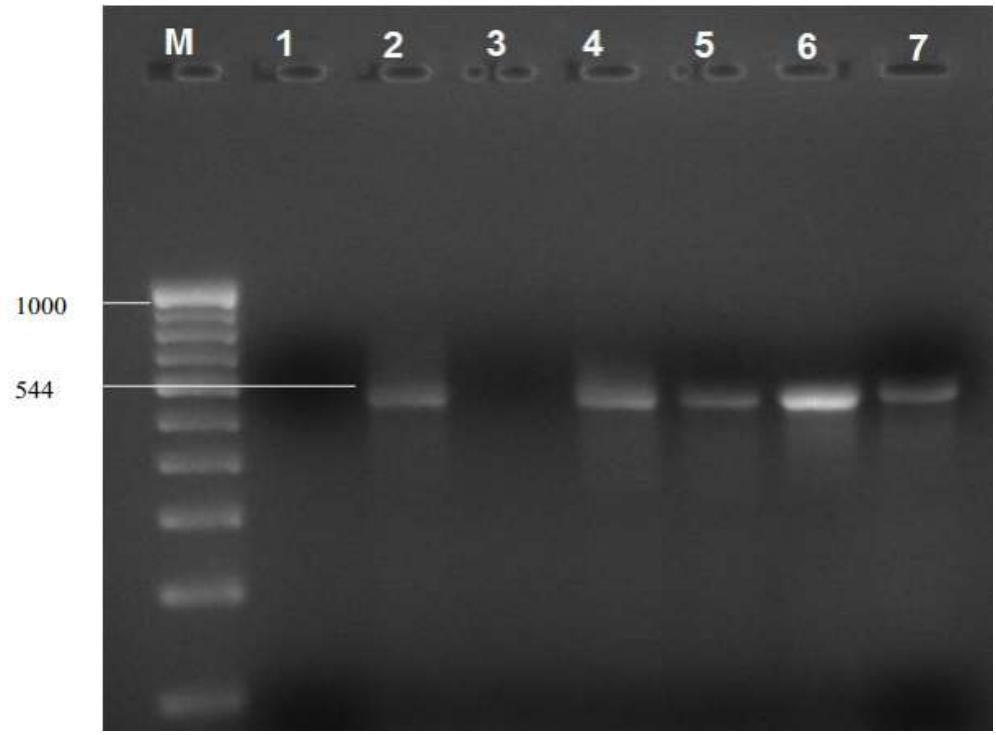

Figure 3: "Each bla CTX-M transcripts were found to have replicated 544 bp segment products by PCR (lane 1: the control group, lane 2: good control, lane 3: positive specimen, lane 4–7: ESβL positive isolated compounds, and column M: 100 bp DNA ladder), Genei , Bangalore)".

Table 1: Distribution of ESβL genes among the isolates (n= 100)

"ESβL GENES (SINGLE/ IN COMBINATION)"	**"NUMBER OF STRAINS (n=100)"**
"bla TEM+ bla SHV"	09
"bla TEM+ bla CTX-M"	25
"bla SHV+ bla CTX-M"	03
"bla TEM+ bla SHV+ bla CTX-M"	11
"ONLY bla TEM"	29
"ONLY bla SHV"	06
"ONLY bla CTX-M"	05
"Strains with none of the 3 genes"	14

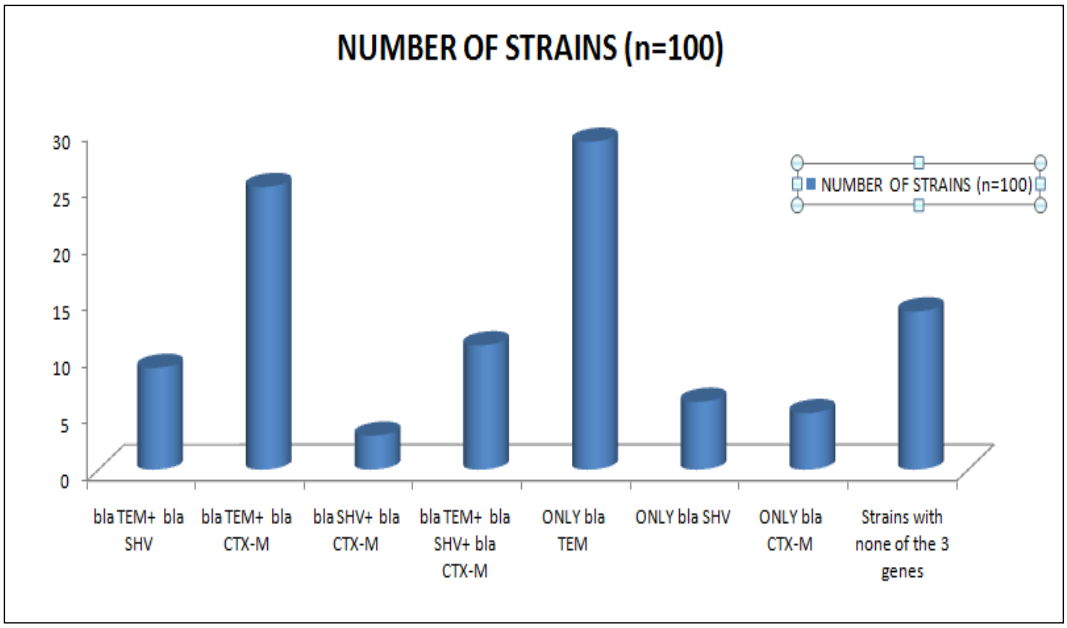

Figure 4: Distribution of ESβL genes among the isolates

Also more than one gene was detected viz., "Table 1 shows the results of the test for each of each of the three infections: 09 isolates tested good for blaTEM and blaSHV, 25 samples tested good for the two blaCTX-M and blaTEM, 03 infections tested positively overall both blaCTX-M but blaSHV, and 11 isolates tested good for every single one of them.

Overall prevalence of TEM gene was 74.0%, SHV gene was 27.0% and CTX-M gene was 44.0% among the 100 isolates subjected to PCR as depicted in Table 2.

Table 2: "Overall distribution of TEM.SHV and CTX-M genes (n= 100)"

bla TEM		74
bla SHV		27
bla CTX-M		44

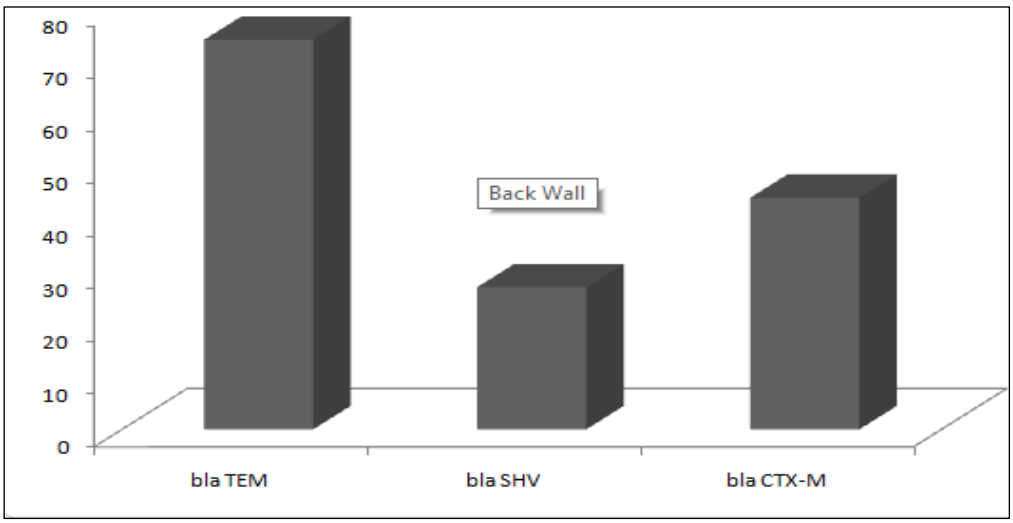

Figure 5: "Overall distribution of TEM.SHV and CTX-M genes"

Table 3: "Pattern of distribution TEM, SHV and CTX-M gene in hospital and community (n=100)"

Gene	Prevalence percentage in the hospital (n=69)	Prevalence percentage in the community (n=31)	P value
TEM	53(76.81%)	21(67.74%)	0.339
SHV	21(67.74%)	06(19.35%)	0.248
CTX-M	30(43.47%)	14(45.16%)	0.875

Out of the 100 randomly selected ESβL KP isolates 31 and 69 strains were from the OPD and IPD respectively. 67.74% of the OPD strains possessed the TEM gene whereas 76.81% of the IPD strain had TEM gene. 19.35% of the OPD strains possessed the SHV gene whereas 30.43% of the IPD strains had SHV gene. 45.16%of the "OPD strains possessed the CTX-M gene whereas 43.47 % of the IPD strains had CTX-M gene" (Table 3). Around 14 strains did not show presence of any of the genes that were investigated for. It can be inferred from the above results that while the TEM and SHV genes were more prevalent in the inpatients the CTX-M gene was more in the hospital samples. "Urinary tract infections (UTIs)" are among the most prevalent transmission, having a yearly rate of occurring throughout the world of around 250 million cases. This study provides prevalence data on ESβLs carrying Enterobacteriaceae at their gene level in the clinical setting of a Subharti Medical College Hospital Meerut, India.

Among people visiting the emergency room and primary care settings, urinary tract infections (UTI) are one of the most prevalent diseases. According to Jiang et al. (2020), pyelonephritis, sepsis, and silent bacteriuria are among the clinical symptoms of UTI. Typically, conventional antibiotics are used to treat UTI patients based on their individual needs. "Extended-spectrum beta-lactamase (ESBL)"-producing bacteria have, however, been cited as a cause of UTI on a more frequent basis in recent times.

Conclusion

In addition, the study enumerates distribution of ESβL producers and their bla genes. There is a need for such studies to be carried out on a regular basis. PCR technique was helpful in identifying and charaterising the genotypes. 74.0% TEM types followed by 44.0% CTX-M ones and 27.0% SHV harbouring ESβL- KP have been reported. Since urinary tract infections are among the most prevalent illnesses affecting individuals of all ages, it is important to keep an eye on the development and spread of resistant germs. The purpose of the research was well served the prevalence and incidence of TEM, and A SHV, and CTX-Mtype ESβLs-producing strains of Klebsiella pneumoniae, which caused urinary tract infections. Surveillance programs should be implemented at the regional level as well as national level as it is very important in reporting and understanding the spread of ESβL producers. DNA sequence studies can be carried out to ascertain the identity of the plasmids in future perspectives.

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