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Original Research Article

Detection of CTX Gene in Extended Spectrum Beta Lactamase Enzyme in *Klebsiella Pneumonaie* Isolates from the various Clinical Specimens of the Patients in a Tertiary Care Hospital.

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Abstract

Klebsiella pneumoniae, a Gram-negative bacterium of the Enterobacteriaceae family, possesses a rod-shaped morphology. Exhibiting opportunistic behavior, it thrives in individuals with compromised immune systems. This study aims to ascertain the presence of the CTX gene within Extended Spectrum Beta-Lactamase (ESBL) enzymes in *Klebsiella pneumoniae* isolates from clinical samples. Identification of these isolates was carried out using a conventional disc diffusion technique alongside the combined double disc method, while ESBLs were screened accordingly. Analysis of antimicrobial susceptibility patterns revealed notable resistance rates, with CAZ, CTX, exhibiting the highest resistance percentages. The majority of the ESBLs positive isolates were from Pus 100 (45.66%). The CTX genes were 61.8% recorded respectively.

Key words: Klebsiella pneumoniae, CTX, Resistance.

Introduction

All Penicillins, cephalosporins, and monobactams such as aztreonam exhibit resistance to extended-spectrum β-lactamases (ESBLs) [1]. ESBLs are predominantly associated with the opportunistic pathogen *Klebsiella pneumoniae*. The first documented case of ESBL-producing *Klebsiella pneumoniae* dates back to 1983 in Germany, and since then, resistance to cephalosporins has progressively increased on a global scale [2]. *Klebsiella pneumonia*e is known to cause bloodstream infections stemming from various sources including central venous line infections, urinary tract infections, intra-abdominal diseases, as well as community and ventilator-acquired pneumonia [3]. Numerous outbreaks of ESBL-producing microbe infections have been reported worldwide, with some hospitals experiencing an endemic presence of these strains, supplanting initial outbreak occurrences [4].

Isolates are typically classified as resistant to all penicillins, cephalosporins, and aztreonam following a significant increase in the minimum inhibitory concentrations (MICs) of ceftazidime or cefotaxime in the presence of clavulanic acid. CTX-M enzymes possess the ability to hydrolyze β-lactamases against cefotaxime, exhibiting a superior capability compared to benzylpenicillin hydrolysis, particularly in cephalothin hydrolysis. They also demonstrate a preference for hydrolyzing cefotaxime over ceftazidime. Although some ceftazidime MICs fall within the susceptible range, certain CTX-M β-lactamases display resistance to this drug [5].

Material & Methods

A Cross-sectional study was conducted at the Department of Microbiology, Mallareddy Narayana Multispeciality Hospital, Hyderabad, India, during over a period of one year April 2022 to April 2023. Ethical approval for this study was obtained from the Institutional Review Board (Approval No: MRMCWIEC/AP/84/2022). A total of 283 isolates of *Klebsiella pneumoniae* were collected from various infection sites within the hospital.

Bacterial isolation and identification were performed using diverse clinical specimens including urine, pus, blood, and sputum. These specimens were inoculated onto 5% sheep blood

agar, MacConkey agar, and Chocolate agar. Following incubation, cultures were assessed for colony identification and further characterized through gram staining and biochemical tests such as Indole, Triple Sugar Iron agar, citrate utilization, urease production, motility testing, and catalase and sugar fermentation assays [6].

The Antibiotic susceptibility patterns *of Klebsiella pneumoniae* isolates were determined using the Kirby–Bauer disc diffusion method [25], employing 20 different antibiotics commonly used in clinical practice: Cefazolin (CZ), Gentamicin (GEN), Tobramycin (TOB), Cefuroxime (CXM), Ceftriaxone (CTR), Ceftazidime (CAZ), Meropenem (MRP), Amoxicillin-Clavulanic Acid (AMC), Piperacillin-Tazobactam (PIT), Cefoperazone (CFS), Amikacin (AK), Ciprofloxacin (CIP), Cotrimoxazole (COT), Aztreonam (AT), Chloramphenicol (C), Tigecycline (TGC), Colistin (CL), Polymyxin B (PB), Norfloxacin (NX), and Cefotaxime (CTX). All inoculated plates were aerobically incubated at 37°C for 18-24 hours, and the results were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [22]. Confirmation of extended-spectrum β-lactamase (ESBL) production and screening were carried out using the Combined Double Disc Approximation method as described by CLSI [7].

Phenotypic Confirmatory Disc Diffusion Test (Combined Double Disc Method): For the phenotypic confirmation of extended-spectrum β-lactamases (ESBLs), a Ceftazidime (30 µg) disc was employed both alone and in conjunction with Clavulanic acid (30 µg/10µg). An increase in zone diameter of ≥ 5 mm for either of the Cephalosporin discs when paired with their respective Cephalosporin/Clavulanate disc was indicative of ESBL production. The antibiotics utilized were Ceftazidime (30 µg) and Ceftazidime-Clavulanic acid (30/10 µg), Cefotaxime (30 µg) and Cefotaxime-Clavulanic acid (30/10 µg) [7].

Interpretation: After incubation, if the isolate showed a zone diameter of 5mm or more with ceftazidime/clavulanic acid when compared to ceftazidime disc alone was considered as ESBL producer.

Genotyping Method: DNA extraction was conducted using the Hi media DNA Extraction Kit (HI-media HTB009) following the manufacturer's instructions, utilizing overnight bacterial cultures. The βHi-PCR® Extended Spectrum β-lactamases (ESBLs) Gene (Multiplex) Probe PCR Kit was employed for genotyping.

Statistical Analysis: Statistical analysis was carried out using M.S. Excel (2021) SPSS (Version 21). A significance level of $P < 0.05$ was considered statistically significant.

Results

In a tertiary care hospital in Hyderabad, India, a total of 283 samples of *Klebsiella pneumoniae* were identified. Among these samples, 104 (69.3%) were male, and 46 (30.7%) were female, resulting in a male-to-female ratio of 2.26:1. The majority of the ESBLs positive isolates were from Pus 100 (45.66%), followed by Sputum 51 (23.29%), Urine 43 (19.63%), and less ESBLs from Blood 25 (11.42%).

The ESBLs positive isolates were from the patients aged 41–60 years, 54 (24.66%), followed by >61 years, 48 (21.92%). 19 to 30 years 45 (20.55%), less from 31.40 years 18 (8.21%). Out of 219 ESBLs the majority were from males, 149 (68.04%), and females, 70 (31.96%).

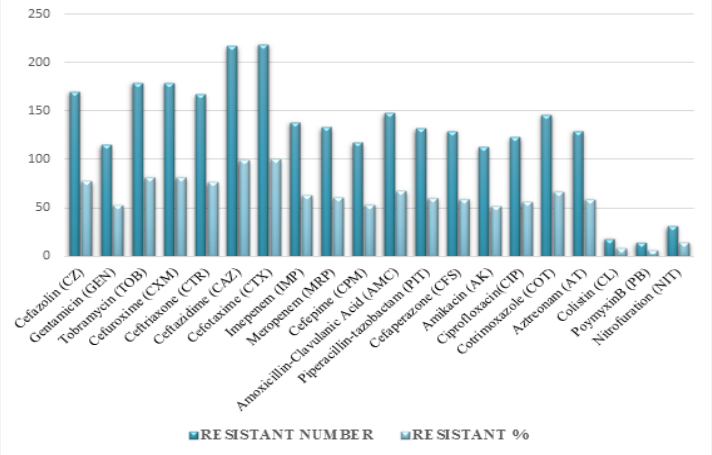

Figure 1: Antibiotic resistant patterninesbls (n = 219)

Out of 283 *Klebsiella pneumoniae,* 219 ESBLs, positive isolates, highest resistance was found to CTX 219 (100%) followed by CAZ 217 (99.09%), and polmyxin-B 14 (6.39%).

Out of 219 ESBL positive isolates identified, 217 ESBLs were positive for both Cefotaxime/Clavulanic Acid disc (99.8%) and Ceftazidime/Clavulanic Acid disc (99.8%).

Table 1: Comparison of various phenotypic methods with esbl genes

ESBLs Detected By RTPCR n=(219)			Phenotypic test	
			CAZ/Clavulanic acid	CTX/ Clavulanic acid
CTX (n=175)	Positive	175	174(99.4%)	173(98.8%)
	Negative	0	01(0.6%)	02(1.2%)

Phenotypic method such as combination disc method using ceftazidime and clavulanic acid disc tests 99.4% sensitivity, and 100% specificity respectively for CTX. Cefotaxime and clavulanic acid showed 100% specificity and sensitivity at 98.8%.

Multiplex PCR for Esbl Genes:

A multiplex real-time PCR, using the Hi-PCR®ESBL Gene (Multiplex) Probe PCR Kit, was conducted on all isolates to identify the presence of CTX, SHV, and TEM genes. A positive result was defined by a Ct value of <35 and a sigmoid curve-shaped amplification plot. In this study involving 283 *Klebsiella pneumoniae* isolates, 217 were found positive for ESBLs. Among them, 175 isolates (61.8%) were positive for the CTX gene.

Table 2: Esbl genes detected by rtpcr (n = 283)

	POSITIVE	NAGATIVE
CTX	175(61.8%)	108(38.2%)

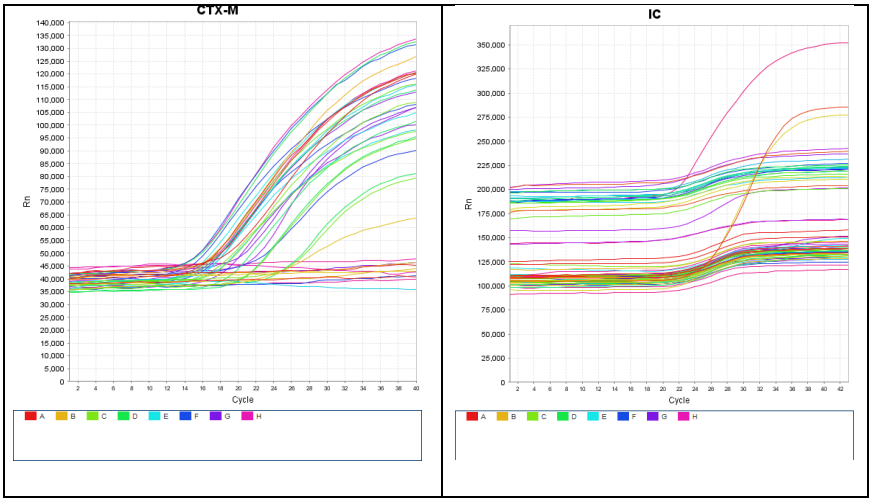

Figure 2: Isolates expressing CTX Gene and internal control by real-time PCR

Discussion

In this study, the majority of isolates were isolated from pus (35.3%), followed by sputum (18.2%), urine (15.1%), and blood (8.8%). Similar studies were conducted where 50% and 21% *Klebsiella pneumoniae* isolates were obtained from pus and urine samples, respectively In another study by [8] it was documented that 53% and 44% drug resistant *Klebsiella pneumoniae* strains were isolated from urine and pus samples respectively

The increased number of patients observed in the old age group could be because of their decreased immunity, which makes them more susceptible to infections. In a similar study conducted by [9]. similar age distribution findings were found. In this study, it was observed that the maximum number of patients belonged to the age group of 40–70 years compared to other age groups.

Regarding gender distribution in this study, a male predominance, accounting for 52.66% of the isolates obtained from patients, while females accounted for 24.73%. In a study conducted by [10] it was noted that approximately 71% of isolates were obtained from male patients, while roughly 29% were from female patients. In this current study, resistance rates of 77% for cefotaxime and 76% were observed for ceftazidime. These findings corroborated with a recent study conducted by [11] who reported resistance rates of 87% for cefotaxime and 85% for ceftazidime. Similarly, [12, 13].conducted a study in Ethiopia. In this study, β-lactamase enzyme production was evaluated at the genomic level by conducting PCR

amplification of encoding genes in 219 MDR isolates. A recent comprehensive multi-center study conducted in India's tertiary care hospitals revealed varying combinations of ESBL genes within single isolates, with these combinations differing across the study centers. This observation underscores the dynamic nature of ESBL gene distribution within our community, likely influenced by the global dissemination of this strain. Notably, the prevalence of CTX-M suggests that this gene has become commonplace in our community, reflecting a high degree of mobility in the encoding genes [14, 15]. The following five groups, made up of more than 130 different types of CTX-M enzymes, exist: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [16].

Conclusion

Timely and accurate diagnosis, coupled with effective treatment, is essential to battle multi-drug-resistant organisms. The latest research indicates a rising prevalence of multi-drug resistance in *Klebsiella pneumoniae*. Based on the findings of this study, it has been established that early detection of ESBL production can be effectively achieved through the combined disc method test, a phenotypic approach. The predominant gene isolated in our study was CTX.

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