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Original Article

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Phenotypic and Genotypic Detection of Extended Spectrum Beta Lactamase Enzyme in *Klebsiella Pneumoniae* Isolates from the Clinical Specimens of the Patients in a Tertiary Care Hospital

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ABSTRACT

Background: *Klebsiella pneumoniae* can cause a number of diseases, such as pneumonia, bloodstream infections, meningitis, and urinary tract infections. Depending on the infection site, the symptoms of *Klebsiell pneumoniae* infection vary. The purpose of this study is to determine the prevalence of the ESBL enzymes in *Klebsiella pneumoniae* isolates using phenotypic techniques from various hospital wards and the identification of ESBL resistance genes such as CTX, TEM and SHV.

Method: Different clinical samples from a tertiary care hospital were used to obtain *Klebsiella pneumoniae* isolates, which were then identified using a conventional disc diffusion technique and Combined double disc method, ESBLs were screened. The CTX, TEM, and SHV genes were genotypically detected using Multiplex PCR in accordance with the recommended procedure.

Result: The pattern of Antimicrobial susceptibility testing revealed that the most resistant antibiotics include, CAZ (76.67%), CTX(74.07%), CXM(72.67%), followed by TOB(72%), and PIT (67%). Out of 150 *Klebsiella pneumoniae* isolates, ESBL Combined double disc method positive are ceftazidime+clavulinicacid115 (74.7%) and, cefotaxime+clavulinic acid positive, 112 (74.7%). By using the Multiplex PCR technique, the CTX and SHV and TEM genes were 65.3% 51.3% and41.3% recorded respectively.

Conclusion: Most *Klebsiella pneumoniae* isolates that produced ESBLs have the CTX gene.

Key Words: β-*lactamase Enzyme, Klebsiella, pneumoniae, ESBL, CTX,TEM.*

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INTRODUCTION

All penicillins and cephalosporins, and monobactamslike aztreonam, are resistance to extended-spectrum -lactamases (ESBLs) [1]. Most frequently, ESBLs are found in the opportunistic pathogen *Klebsiella pneumoniae*, which is linked to severe infections in hospitalised patients, particularly immunocompromised hosts with serious underlying illnesses [2]. The first report of ESBL-producing *Klebsiella pneumoniae* came from Germany in 1983, and during the following decades, it is resistance to cephalosporins steadily increased globally [3].

Klebsiella pneumoniae cause bloodstream infections can result from infections of the central venous line, the urinary tract, intra-abdominal diseases, and community and ventilator-acquired pneumonia [4]. Worldwide, numerous outbreaks of infections caused by ESBL-producing microbes have been reported. In other hospitals, endemicity of the ESBL-producing strains has replaced the first outbreaks of infections [5].

Controlling the initial outbreak of ESBL-producing organisms in a hospital or specific area of a hospital is crucial because this could result in an increase in patient mortality [6]. Globally, 400 different ESBL types have been identified, with TEM and SHV being the most common. When the genes encoding TEM and SHV enzymes are mutated, ESBLs with wider substrate profiles are created [7]. The TEM and SHV forms of penicillinsare descended from a penicillinase with a number of single amino acid changes that enable them to hydrolyze extended spectrum cephalosporins [8].

In Gram-negative bacteria, TEM-1 is the most frequent -lactamase found. The synthesis of this enzyme causes up to 90% of E. coli ampicillin resistance [9]. Patients who spend a lot of time in the intensive care unit are the ones who are most likely to contract ESBL-producing Enterobacteriaceae (ICU). Serious repercussions, like treatment failure and mortality, may occur if ESBL-producing bacteria are not promptly identified [10].

According to estimates, 8% of all infections acquired in hospitals are brought on *by Klebsiella pneumoniae.* They make up between 3% and 7% of all nosocomial bacterial infections in the United States, ranking them eighth among hospital-associated pathogens and second only to E. coli as the leading cause of Gram-negative sepsis. [11]. Although 6 to 17% of all nosocomial urinary tract infections (UTI) are caused by *Klebsiella* species, the incidence is even higher in certain patient populations who are at risk, such as those with diabetes mellitus or neuropathic bladders [12]. Invasive equipment, such as catheters, and ventilator-associated pneumonia in hospitalised patients both significantly enhance the risk of *Klebsiella pneumoniae* in hospital-acquired infections [13].

Large, self-conjugating plasmids were a major factor in the development of antimicrobial resistance in *Klebsiella pneumoniae*. These additional chromosomal DNA molecules maintain a consistent copy number per bacterial cell, control their own replication rate, and encode elements that encourage the commencement of replication independently of the bacterial chromosome's replication [14]. It is a known property of *Klebsiella pneumoniae* to carry numerous -lactamase genes in the same strain, and this trait may help explain the pathogen's selective success [15].

Combinations of all gene types have been observed in this species; this may be caused by either carrying a plasmid encoding an array of antibiotic resistance genes or by acquiring transposons carrying several genes on the same plasmid [10]. There are numerous methods for determining whether an ESBL was created. Phenotypic tests for ESBL detection can only determine whether an ESBL was produced, they cannot determine the subtype of ESBL. While those ESBLs using a methodology comparable to standardised susceptibility testing are the most practical for the routine diagnostic laboratory, certain ESBLs may not reach a level to be detected by disc diffusion tests but nevertheless result in treatment failure in the infected patient [16]. These all rely on identifying a synergy between clavulanic acid and the indicator cephalosporin utilised in the initial screening [17].

The isolates are then reported as resistant to all penicillins, cephalosporins, and aztreonam after at least an eight-fold drop in the MICs of ceftazidime or cefotaxime in the presence of clavulanic acid [8].

DNA probes that were specific for the TEM and SHV genes were used to undertake early detection of -lactamase genes. However, utilising DNA probes might occasionally require a lot of labour. PCR with oligonucleotide primers that are specific for a -lactamase gene is the simplest and most popular molecular technique for determining whether a -lactamase from a family of enzymes is present [18]. Molecular approaches are expensive, time-consuming, and need specialised equipment and knowledge, but they also seem to be sensitive [19]. It is possible to learn more about the epidemiology and risk factors for these illnesses by using genetic approaches to identify the TEM and SHV genes in ESBL-producing bacteria and their pattern of antibiotic resistance [20]. The epidemiology and risk factors associated with these diseases can be learned from the pattern of antibiotic resistance and the detection of TEM and SHV genes in ESBL-producing bacteria [21].

METHODS

This was a Cross sectionel study was carried out at the Department of microbiology, Mallareddy narayana multispeciality hospital, Hyderabad, Indiaover a period of six months in 2022. This study was approved by ethical committe. In the hospital 150 isolates of *Klebsiella pneumoniae* were gathered from various infection sites. Bacterial isolation and identification. Different clinical specimens such as urine, pus, blood, bronchial wash, sputum considered in this research. The specimen inoculated on 5% sheep Blood agar, Mac Conkey agar, Chocolate agar. After incubation, cultures were observed for colony identification and further characterized by gram staining and biochemical (Indole, triple sugar iron agar, citrate, urease, and motility medium and catalase test, and sugar fermentation tests [22].

Antibiotic susceptibility testing

Antibiotic sensitivity pattern *of Klebsiella pneumoniae* isolates were examined using the Kirby– Bauer discs diffusion method [25] by using 20types of antibiotics in practice, Cefazolin(CZ), Gentamicin(GEN), Tobramycin (TOB), Cefuroxime(CXM), Ceftriaxone(CTR), Ceftazidime (CAZ), Meropenem (MRP),Amoxicillin-Clavulanic Acid (AMC), Piperacillin-tazobactam(PIT), Cefaperazone (CFS), Amikacin (AK), Ciprofloxacin(CIP), Cotrimoxazole (COT), Aztreonam (AT), Chloramphenicol (C), Tigecycline(TGC), Colistin (CL), PolymyxinB (PB), Norfloxacin (NX),and Fosfomycin (FOS), Cefotaxime (CTX),and all of the inoculated plates were aerobically incubated at 37C for 18-24 hours, and the results were interpreted based on the instruction provided by the CLSI .Confirmation of ESBL and screening done by Combined double disc approximation method as described by CLSI [22].

Phenotypic Confirmatory Disc Diffusion Test (Combined Double Disc Method)

A Ceftazidime (30 µg) disc was used alone and in combination with Clavulanic acid (30 µg/10µg) for phenotypic confirmation of the presence of ESBLs. A ≥ 5 mm increase in zone diameter for either of the Cephalosporin discs and their respective Cephalosporin/Clavulanate disc was interpreted as ESBL producer (The antibiotics used were; ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/ 10 µg), cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 / 10

Figure-1

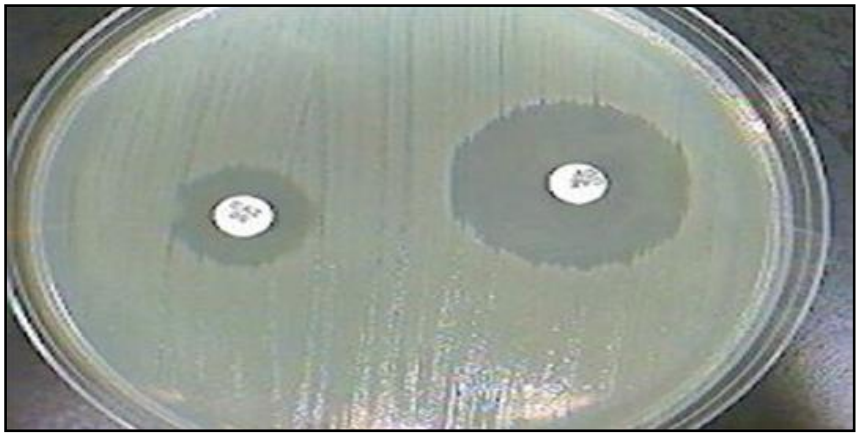

Figure 1: Combined Double disc test for ESBLs using ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 /10 µg).

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:

The overnight bacterial culture was used for DNA extraction using the hi media DNA Extraction Kit (HI-media HTB009) in accordance with the manufacturer's instructions.

β-lactamases (ESBLs) Gene (Multiplex) PCR

Hi-PCR® Extended Spectrum β-lactamases (ESBLs) Gene (Multiplex) Probe PCR Kit was used

Statistical Analysis

Statistical Analysis was performed using M.S. Excel (2020) and SPSS (Version 20). P-value<0.05 was considered significant.

RESULTS

In a tertiary care hospital, Hyderabad, in India .Out of 150 samples of *Klebsiella pneumoniae* identified. Of these 150 samples, 104 (69.3%) were male and 46 (30.7%) were female, making the male to female ratio 2.26:1.

Table 1: shows clinical specimens andsource of samples. Figure-2 represents the same.

Source	Frequency	Percent
pus/swab	73	48.7
blood	21	14.0
sputum	26	17.3
urine	30	20.0
Total	150	100.0

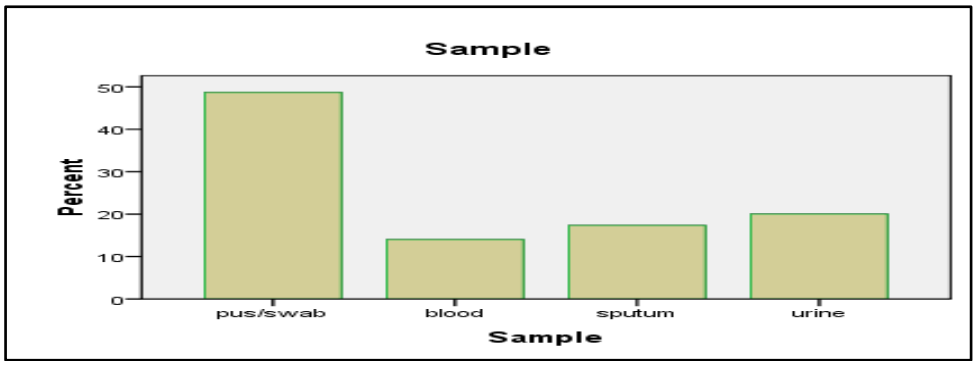

Figure-2

Klebsiella pneumoniae were isolated from pus/swab (48.7%), followed by urine (20%), sputum17.3% and 14% of blood.

Age distribution of the patients has been shown in Fig-3 represents the same.

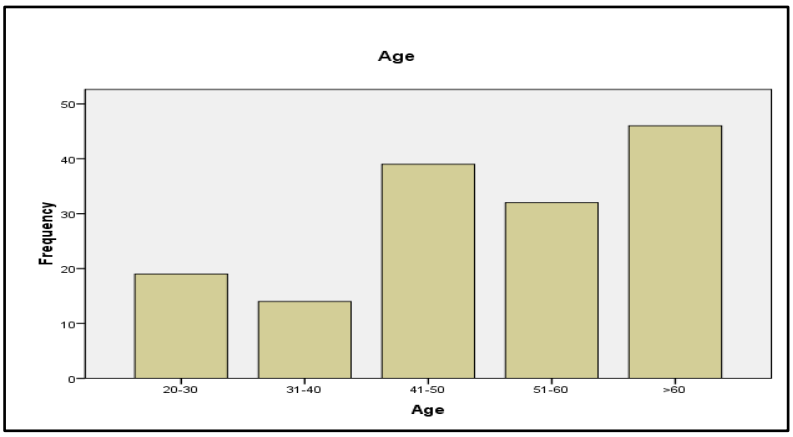

Figure-3

The samples for *the Klebsiella pneumoniae* were collected from in a tertiary care hospital for examining pattern of multidrug resistance. Most number of cases reported in the age group $60^{+}$and 41-50 followed by 51-60, 20-30 and 31-40. The age group at the highest risk was recorded for age group 60^{+}.

Antibiotic Resistance pattern *among the isolates of Klebsiella pneumoniae* is shown in Table-2 and Figure-4

Table-2

	Resistance	**Percentage**
Cefazolin(CZ),	73	48.67
Ceftriaxone (CTR)	77	51.3
Gentamicin(GEN)	80	53.33
Tobramycin (TOB),	108	72
Cefuroxime(CXM)	109	72.67
Ceftazidime (CAZ)	115	76.67
Meropenem (MRP)	66	44.0
Amoxicillin-Clavulanic Acid (AMC)	75	50
Piperacillin-tazobactam (PIT)	101	67.33
Cefaperazone (CFS)	86	57.33
Amikacin (AK)	77	51.33
Ciprofloxacin(CIP)	72	48
Cotrimoxazole (COT)	91	60.67
Aztreonam (AT)	76	50.67
Chloramphenicol (C)	56	37.33
Tigecycline(TGC)	6	4
PoymyxinB (PB)	29	19.33
Norfloxacin (NX)	100	66.67
Cefotaxime(CTX)	112	74.07
Cefepime (CPM)	83	55.3

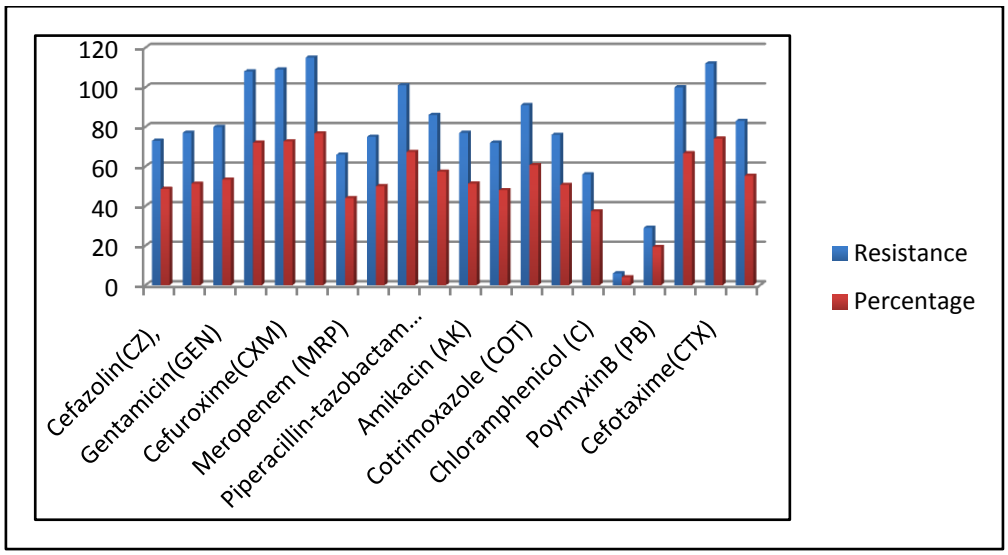

Figure-4

Itcanbe seen that highest resistance was observed for the antibiotic Ceftazidime (76.67%) Cefotaxime (74.07%), and Tobramycin (72%) and the least resistance Tigecycline (4%), Poymyxin-B (19.33%). The Tigecycline and Poymyxin-B will be more effective for the microbe.

Combined double disc method test for ESBLs

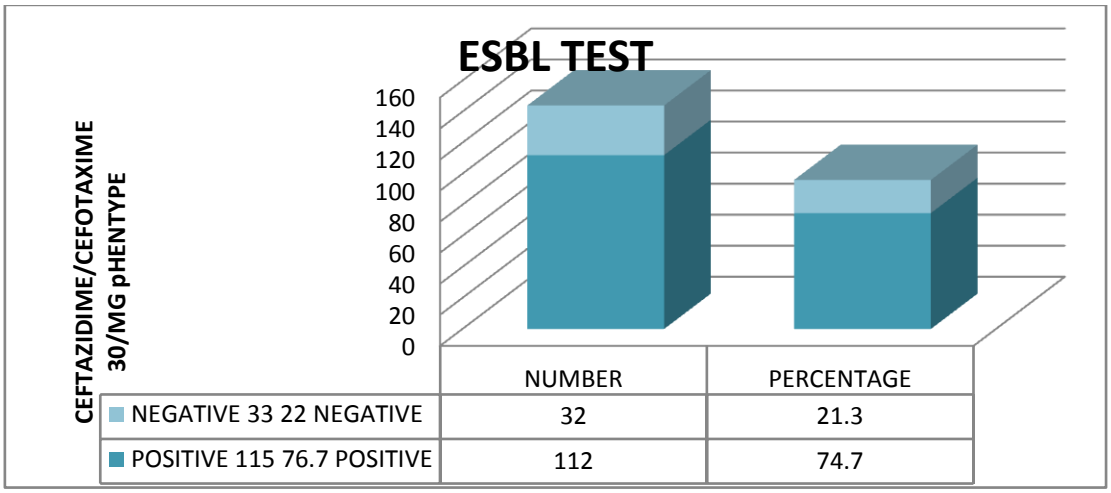

Figure-5

Combined double disc test

Using the combined double disc approximation technique, all of the isolates were simultaneously screened for the production of ESBL. Out of 150 samples combined double disc ceftazdime/clavulnate(30/10mg) positive 115(76.7%) andcefotaxime/clavulnate(30/10mg) 112(74.7%).

Detection of ESBLs Encoding Genes

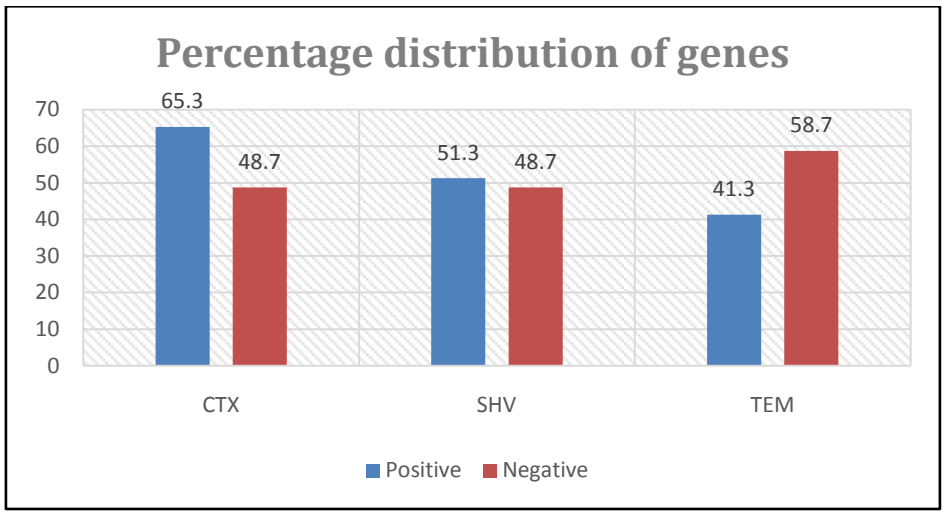

Figure-6

In this study highest positivity CTX (65.3%) was observed followed by SHV (51.33%) positive and TEM (41.3%).

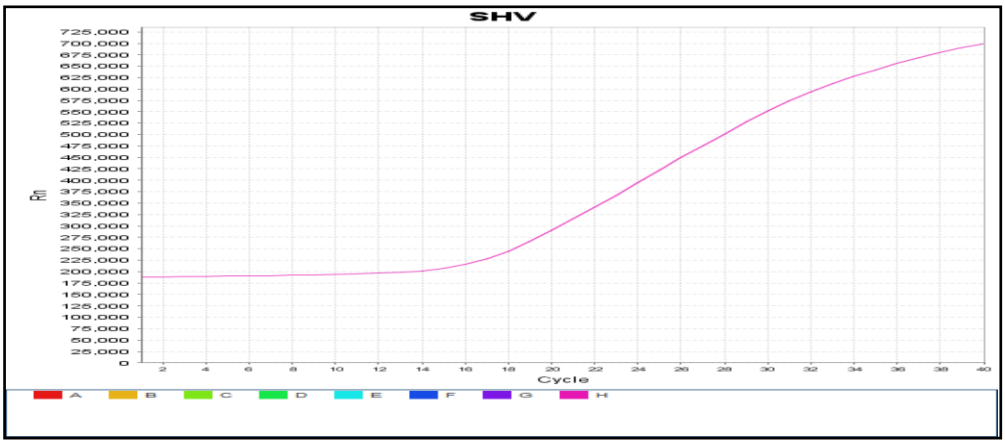

Figure 7: Multiplex PCR real time amplication of SHV gene after 35cycles

DISCUSSION

One of the most significant multi-drug resistant pathogenic bacteria is *Klebsiella pneumoniae*, which is responsible for a number of disorders including infections of the urinary and respiratory tracts, burn infections, and pneumonia, blood infections, and wounds.

All *Klebsiella pneumoniae* isolates used in this investigation were obtained from a tertiary-care hospital in different wards, where various samples from various clinical conditions were evaluated and collected. *Klebsiella pneumoniae* were isolated from pus/swab (48%), followed by urine (20%), sputum17.3% and 14% of blood.

In this study higher percentage of pus sample 48.7%, this indicate that pus (wound) infection is the most common infection in hospitals. The likelihood of *Klebsiella pneumoniae* hospital-acquired infections is greatly increased by the presence of invasive devices, such as sepsis and fever associated pneumonia in hospitalized patients. Second highest percent sample is urine (20%).

ESBLs are a small group of enzymes that are mediated by plasmids. In Frankfurt, Germany, an oxyamino beta-lactamase, the first ESBL, was reported in 1983. resistance to pencillins, monobactams, and cephalosporins [23, 24].

In this study ESBL genes SHV, CTX and TEM were 65.3%, 51.3%, 41.3% that means CTX is more frequently found. TEM gene recorded less frequent. Which is quiet smillarthan the record found in [25, 26].

With growing use of higher generation cephalosporins, the incidence of beta-lactamases in general and the ESBL in particular is increasing, posing a significant issue. [25]. beta-lactamases for SHV (class A) SHV-1 and TEM-1 have a similar overall structure and share 68% of the same amino acids. *Klebsiella pneumoniae* is home to the SHV-1 beta-lactamase, which accounts for up to 20% of the ampicillin resistance in this species that is plasmid-mediated.

Additionally, the active site of this family of ESBLs has altered amino acids, most frequently at positions 238 or 238 and 240. There are reported to be about 60 SHV variants. Among the most prevalent are SHV-5 and SHV-12 [26].

In this study the high resistance pattern was seen for the antibiotics Ceftazidime (CAZ) (76.67%), Cefotaxime (CTX) (74.07%) followed by Tobramycin (72%) whereas least resistive pattern recorded for the Tigecycline (TGC) (4%), PoymyxinB (PB) (19.33%) and Chloramphenicol (C) which is less frequent than [27, 28].

Tests on antibiotic profiles against all identified species revealed 76.67 % and 76% Chlormphenicol and ampicillin resistant, in Erbil, Iraq, and Egypt [27, 28].

Combined double disc method was used for the detection of the ESBLs. The CTX, TEM, and SHV genes detected. highest CTX positive and least TEM positive.

The constitutive expression of a chromosomally encoded B-lactamase (blaSHV-1) that gives resistance to ampicillin, amoxicillin, carbenicillin, and ticarcillin, however, explains this high incidence of ampicillin resistance [29]. The resistance rate for another medication, amoxicillin-clavulanic acid, is equally high (70%) was found in Nigeria, Iran, and Saudi Arabia [30, 31].

There was a small difference between the results and those from a study conducted in Erbil-Sulaimani-Duhok/Iraq by Khalid et al. [32].

The most often studied kinds of these are TEM, SHV, OXA, and CTX-M, with CTX-M being most frequently linked to antibiotic resistance [33, 34 & 35]. 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 [36].

The CTX-M-15 kind is the one that is most frequently encountered in hospitals and the general population [37]. There are currently more than 140 different forms of TEM type beta-lactamases, with TEM-1 being the most common variety seen in *Klebsiella pneumoniae* and *E. coli.* Additionally, there are about 100 distinct kinds of SHV, which are primarily found in *Pseudomonas aeruginosa and Acinetobacter* species [38].

CONCLUSION

The prevalence and spread of *Klebsiella pneumoniae* strains that produce ESBLs are concerning, and these strains are frequently isolated from various clinical samples from various conditions. Both ESBL genes were found in these isolates, CTX, SHV and TEM were 65.3%, 51.3%, 41.3% that means CTX is more frequently found. The most resistance recorded for the Ceftazidime (CAZ), Cefotaxime (CTX), and Tobramycin (TOB).

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