Distribution of TEM and SHV Genes in ESBL-Producing *Klebsiella pneumoniae* Strains Isolated From Various Clinical Samples in Al-Basrah Province, Iraq

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KEYWORDS
Klebsiella pneumoniae, ESBLs, TEM and SHV

ABSTRACT
Klebsiella pneumonia is an opportunistic pathogen causes several diseases including sepsis, pneumonia, and wound infections. Antimicrobial resistance (AMR) in MDR K.pneumonia carrying genes for extended-spectrum β-lactams (ESBLs) has been considered a risk for health problems around the world, which has greatly limited treatment options. The current study relied on n=26 Klebsiella pneumoniae isolates that were previously isolated by the studies of master's student "Intithar Mahdi Mozan" between October 2022 and December 2022,the samples were collected from three hospitals in Al-Basrah province,Iraq. K. pneumoniae isolates reactivated and re-identified by cultivating isolates on MacConkey agar and Klebsiella pneumoniae chromogenic media and then re-identified K. pneumoniae isolates through diagnostic gene 16S rDNA by PCR technique, the results revealed that all n= 26 isolates of K. pneumoniae had a molecular weight of approximately 130 bp at a 100%. Double disk approximation test (DAM) was used to detected produced extended-spectrum β-lactams (ESBLs), the result of current study was showed positive results for the ESBLs. Extended-spectrum β-lactamses (ESBLs) genes was detected by using three specific primers: blaTEM, blaSHV and, blaCTX-M. Out of n=26 K. pneumoniae isolates results was shown the 23 (88.46%) K. pneumoniae isolates had gave positive results for the blaTEM gene. While 3 (11.538%) K. pneumoniae isolates revealed negative results for blaTEM gene. On other hand results were shown that only 5 (19.23%) K. pneumoniae isolates had positive results for blaSHV gene and 21 (80.769%) K. pneumoniae isolates had negative results for the blaSHV gene. Also the results shown the 5(19.23%)K. pneumoniae isolates had positive results for blaTEM and blaSHV genes.

1. Introduction

Klebsiella pnemouniae is involved in one-third of community-acquired and nosocomial Gram-negative infections worldwide. *K. pneumoniae*, a nosocomial opportunistic infection, causes pneumonia, urinary tract infections, and bacteremia. Due to severity, resistance, and treatment challenges, researchers worldwide are focusing on its treatment [1,2]. *K. pnemouniae* is bacterium that causes urinary tract, respiratory, and wound infections [2,3]. It is present in the environment and can carry multiple β-lactamase genes in the same strain [4]. The widespread use of extended-spectrum β-lactamases and the overuse of antibiotics have contributed to the establishment of multidrug-resistant *K. pneumoniae* [5]. Combinations of all types of *bla* genes have been reported in this species, possibly due to the carriage of an antibiotic-resistant plasmid or acquisition of transposons containing different *bla* genes [6]. Antibiotic resistance is a major health concern that prolongs the need for medical care and increases its cost, resulting in hundreds of deaths annually. [7,8]. The World Health Organization reports state that antibiotic-resistant *K. pneumoniae* is considered a serious health threat and have designated *K. pneumoniae* as a multidrug-resistant (MDR) pathogen that presents an immediate risk to human health [8, 9]. The misuse of traditional antimicrobial drugs has been responsible for the rise in multidrug-resistant (MDR) *K. pneumoniae* strains [10]. Numerous resistance mechanisms have been evolved by *K. pneumoniae* against various antimicrobials [5]. The ability of efflux pump systems and biofilm formation to occur is one of the most crucial factors for the development of the MDR [11]. Various harmful compounds can be forced out of cells via efflux pumps, which are protein-based structures [12]. *K. pneumoniae* can evade antibiotics and the human immune response by forming biofilms [13]. Since their discovery in the 1980s, ESBL-producing bacteria have been found all over the world and are often separated from nosocomial and community-acquired viral infections [11,12]. Clavulanic acid inhibits these bacterial

enzymes, which can lead to resistance against a variety of β-lactam antibiotics, such as monobactams and third-generation cephalosporins [13]. There are currently over 400 known ESBL enzymes, β-lactamases, including TEM-1, TEM-2, and SHV-1, with over 150 members, and the majority of which have developed as a result of changes to the active centre of traditional plasmid β-lactamases [14]. Therapeutic limits have been applied more and more to infections caused by ESBL-producing bacteria, raising the risk of treatment failure, extended hospital stays, high healthcare expenses, and high mortality. *Klebsiella* species are now considered to be one of the six drug-resistant bacteria needing urgent new therapeutic compounds [14]. The two main types were *TEM* and sulphydryl variable *SHV*, but *CTX-M* type is more prevalent in some nations. Gramme negative bacteria produce these rapidly evolving bacteria, which can hydrolyze all cephalosporins, aztreonam, and related oxyimino-beta lactams in addition to older penicillins, though clavulanic acid can inhibit this [3]. Around the world, many cases of infection outbreaks caused by ESBL-producing organisms have been reported. The widespread of strains that produce ESBL has succeeded initial infection outbreaks in several hospitals [11]. Because of the potential for higher patient mortality, it is crucial to contain the first epidemic of ESBL-producing pathogens in a hospital [12]. The identification of *TEM* and *SHV* genes in ESBL-producing bacteria by molecular approaches, together with their antibiotic resistance pattern, might provide useful knowledge into the illnesses' epidemiology and related risk factors [15].Although being expensive, lengthy, and requiring specialised equipment and skills, molecular approaches appear sensitive [16,17].The objective of the current investigation was to detect bla_{SHV} and bla_{TEM} genes in isolates of ESBL *K. pneumoniae* obtained from a variety of clinical samples obtained from hospitals in the Al-Basrah province, Iraq.

Materials and Methods

Collection of samples

The investigation was conducted using (n = 26) *K. pneumoniae* isolates that were previously isolated during a master's study by “Intithar Mahdi Mozan" The samples had been taken between October 2022 and December 2022 from the AL-Sadr teaching hospital, AL-Fayhaa teaching hospital, and Basrah teaching hospital in Basrah province, Iraq. The samples were taken from urine samples (from patients with UTIs and inpatients with urinary catheters), sputum samples (from outpatients with chest infections), and wound swab samples.

Reactivation and re-identification of Klebsiella Pneumonia isolates

Klebsiella pneumoniae isolates (n=26) were reactivated and re-identified according to [18,19] and kept in refrigerated until used in other tests.

Detection of Extended Spectrum β-Lactamase (ESBLs)

Double Disk Approximation Method (DAM)

The Mueller-Hinton agar plates were used to cultivate the *K. pneumoniae* isolates to determine if they produced extended spectrum β-Lactamase (ESBLs) enzymes. Around discs containing 20µg amoxicillin / 10µg clavulonic acid the 30µg cefotaxime disc, 30µg ceftriaxone disc, 30µg ceftazidime disc, and 30µg aztreonam disc were placed in a circular pattern about 20–30 mm around the amoxicillin-clavulonic acid disc. After an overnight incubation period, the results detected according to standers [20].

DNA extraction

Genomic DNA extracted from *K. pneumoniae* isolates according to(Presto^{TM} Mining DNA Bacteria, Geneaid, USA) kit protocol.

Detection of 16S rDNA

K. pneumoniae isolate DNA extraction was amplified by PCR for 16S rDNA amplification with a particular primer that was approximately (130 bp) in length [21, 22]. standard molecular DNA ladder (2000 bp) was used compare the PCR results.

Detection of ESBLs Genes

Two different primers that were utilized for amplified *bla*$_{SHV}$ and *bla*$_{TEM}$ genes by PCR with a particular primer that was approximately (713 bp) and (800 bp) in length respectively according to [23].

Results

In the current study, the results of cultivated *K. pneumoniae* isolates on MacConkey agar and *Klebsiella pneumoniae* chromogenic media showed that the colonies of all n=26 *K. pneumoniae* isolates displayed mucoid pink, white, and purple colors on the used media, respectively. Also, the results of molecular diagnostics employing the PCR technique, which relies on the diagnostic gene 16S rDNA, revealed that all n= 26 isolates of *K. pneumoniae* had a molecular weight of approximately 130 bp at a 100% finger (1).

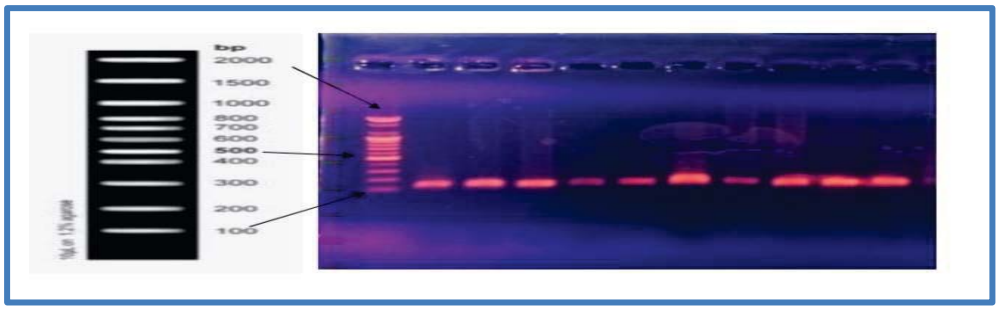
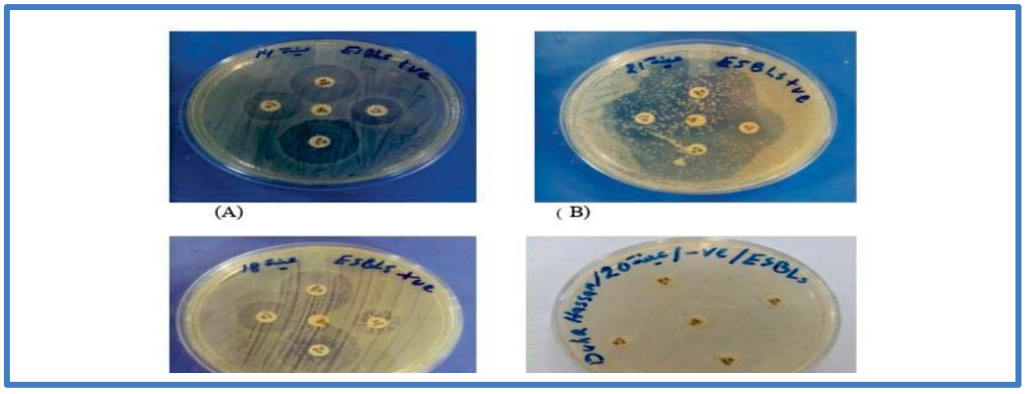


Figure (1) PCR Amplified Products of 16S rDNA. Lane 1:(2000 bp DNA ladder), Lane:(no. 2-11) 16S rDNA band of *K. pneumoniae* isolates . using 1.5% agarose gel, 70V, 45min.

Furthermore, positive results were shown in 22 (84.6%) out of the n=26 *K. pneumoniae* isolates in the current study produced extended-spectrum β-lactamases (ESBLs), by using the double-disc approximation method (DAM).While 4(15.4%) isolates revealed negative results for produced ESBLs, as in Figure (2). **Double-disc approximation (DAM) test used to detect ESBLs produced by *K. pneumonia* isolates the (A, B, and C) positive results, while (D) negative results.**



PCR was used to amplify the *bla*$_{TEM}$ and *bla*$_{SHV}$ genes. The amplified genes' bands were characterized approximately at (800 bp) , compared to the stander molecular DNA ladder at (2000 bp), as shown in figure (3). out of n=26 *K. pneumoniae* isolates results was shown the 23 (88.46%) *K. pneumoniae* isolates had gave positive results for the *bla*$_{TEM}$ gene. On the other hand, the 3 (11.538%) *K. pneumoniae* isolates revealed negative results for *bla*$_{TEM}$ gene. While *bla*$_{SHV}$ gene band were characterized approximately at (713 bp), compared to the stander molecular DNA ladder (2000 bp), figure (4). the current study's results were shown that only 5 (19.23%) *K. pneumoniae* isolates had positive results for *bla*$_{SHV}$ gene and 21 (80.769%) *K. pneumoniae* isolates had negative results for the *bla*$_{SHV}$ gene. Also, the results shown the 5(19.23%), *K. pneumoniae* isolates had positive results for *bla*$_{TEM}$ and *bla*$_{SHV}$ genes.

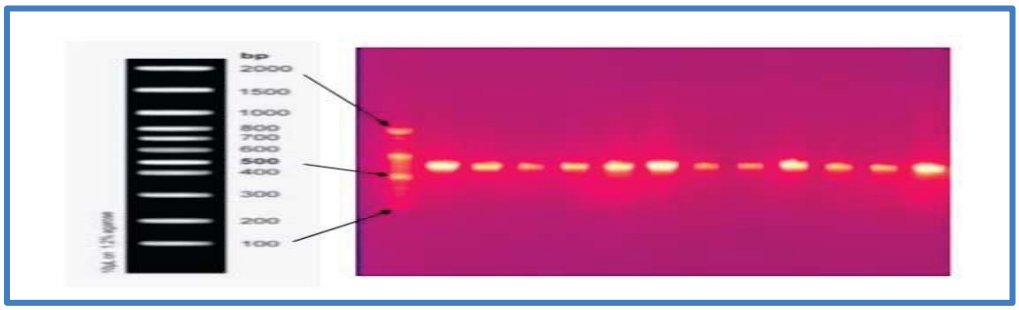
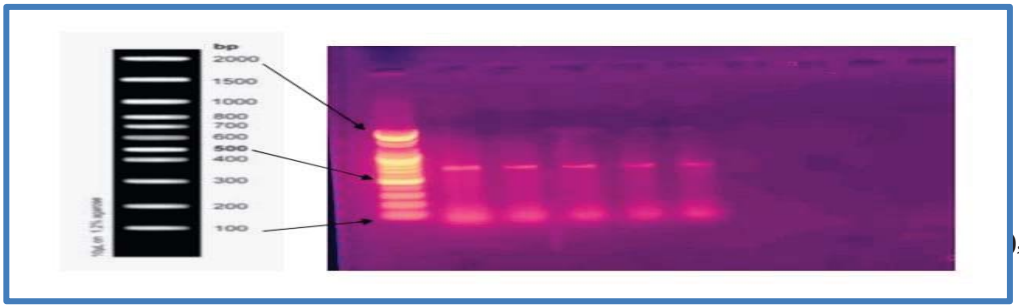


Figure (3) PCR Amplified Products of *bla*$_{TEM}$ gene. Lane 1:(2000 bp DNA ladder), Lane:(no. 2-13) *bla*$_{TEM}$ gene . using 1.5% agarose gel, 70V, 45min.



The current study reactivated n=26 *K. pneumoniae* isolates from previously isolated during a master's study by "Intithar Mahdi Mozan." The samples had been taken between October 2022 and December 2022 in brain-heart infusion broth and confirmed their identification based on morphological characteristics on MacConkey agar and Hi-chrome agar [19,20]. *K. pneumoniae* colonies appeared large and mucous on MacConkey agar due to lactose fermentation, while purple on Hi-Chrome agar [21]. MacConkey agar's distinguishing qualities make it useful for therapeutic and scientific purposes. Lactic acid production during lactose fermentation lowers the pH of the agar, resulting in pink colonies of lactose-fermenting Gram-negative bacteria [22].cIn current study was used the16S rRNA for reconfirm detection of *K. pneumoniae* isolates, because the 16S rRNA gene is a useful target for molecular analyses, contributing significantly to the detection and identification of bacteria in clinical samples[23,24]. It is the most popular technique in standard clinical microbiological laboratories for suspected bacterial infections. 16S rDNA sequencing is used in clinical microbiology labs to identify bacteria, classify taxa and species, identify culture-negative bacteria, and classify unculturable ones [25,26].cThe double disk approximation test is a useful method for detecting bacteria's ability to produce ESBLs. Current study, ESBL phenotypes were found in 22 (84.6%) *K. pneumoniae* isolates that gave positive results for produced extended-spectrum β-lactamases (ESBLs). The similar results were reported in Jordan [27], Sudan [28], Iran [29], India [30], Iraq [31,32 ,33,34 & 35]. On other hand, other studies in China, Nigeria and India reported high percentages of ESBL-producing isolates [36,37].cThe results showed the out of n=26 *K. pneumoniae* isolates, 23 (88.461%) isolates was positive for *bla*$_{TEM}$, while only 5 (19.23%) isolates were positive for *bla*$_{SHV}$ table (1) ,This result agrees with the study of [38] ,that reported gave *bla*$_{TEM}$ (60%). And the study of [39] which reported detection of the genotype of β-lactam genes for 112 *Klebsiella pneumoniae* samples in Najaf Hospital. 63(56.25%) isolates gave positive results for *bla*$_{TEM}$ and 49(43. 75%) isolates for the *bla*$_{SHV}$ gene. Current study results were showed agreement with the study of [40] in Wasit_Iraq, .In the study of [41] in Diwaniyah Governorate, reported for gave positive results for both *bla*$_{SHV}$ (66.7%) and *bla*$_{TEM}$ (55.6%) genes.The results of the current study demonstrated the potential of the inclusion of the two genes in one isolate, 5 individual isolates were found to carry two β-lactamase genes (*bla*$_{TEM}$ and *bla*$_{SHV}$), and this in turn leads to the expansion of

multidrug resistance and the dissemination of virulent strains, which enhances the worldwide spread of β-lactam genes in other regions of the world.

Conclusions

Extended-spectrum β-lactam (ESBL) enzymes bla_{TEM} gene were found in majority of isolates of *K. pneumoniae*. The 5 isolates included both the bla_{TEM} and bla_{SHV} genes, The threat posed by this bacteria's resistance to several medications and the potential for horizontal gene transfer—the transmission of these resistance genes by mobile elements.

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