ORIGINAL ARTICLE

Molecular detection and sequencing of SHV gene encoding for extended-spectrum β-lactamases produced by multidrug resistance some of the Gram-negative bacteria

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Abstract

Background and Objective: The increase in extended-spectrum β-lactamases (ESBLs) producing microbes in recent years has led to the great challenge for the clinician in the treatment. The study aims to investigate the molecular basis of ESBL encoding-resistant gene, SHV to modern β-lactams. Also, for gene sequencing and comparing their genetic relatedness utilizing phylogenetic analysis. **Patients and Methods:** A total of 100 clinical isolates are submitting after identification to phenotypic confirmatory double disk synergy technique. Polymerase chain reaction was performed for the determination of SHV gene, and sequencing analysis for the amplified gene is also achieved. **Results:** A total of 27 isolates, 3 (11.1%), 15 (55.55%), and 9 (33.0%) of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were positive for a phenotypic and confirmatory test for ESBLs, respectively. SHV gene was detected only in 5 (31.25%) isolates of *P. aeruginosa*, 2 (12.5 %) *E. coli,* and 9 (56.25%) in *K. pneumoniae*. **Conclusions:** SHV gene plays an essential role in the resistance of ESBL producer isolates to new β-lactams. The sequencing of this gene revealed 98–99% compatibility range with the global standard gene in National Center of Biotechnology Information.

Key words: Extended-spectrum beta-lactamases, gram-negative bacteria, SHV gene

INTRODUCTION

It is well documented that the resistance of pathogenic organisms has become a serious problem with failures in the treatment of infectious diseases.[1] The clinically most important β-lactam resistance mechanism in Gram-negative pathogens is enzymatic inactivation of the antibiotics by β-lactamases.[2] Extended-spectrum β-lactamase (ESBL) is a class of beta-lactamase enzymes gives widespread resistance to β-lactam antibiotics including penicillin, cephalosporins, and monobactam, so it compromises the efficacy of all β-lactams but not to carbapenem. ESBL group of enzymes is found widely and causes a severe infection on human health leading to various diseases.[3] These enzymes stimulate hydrolysis of the β-lactam ring and thereby inhibit these antibiotics.[4] Among ESBLs, the most widespread and clinically relevant are the class ESBLs of transmission electron microscopy (TEM), SHV, and CTX-M types. TEM and SHV type ESBLs are derived from penicillinases TEM-1, TEM-2, and SHV-1 and are characterized by several single amino acid substitutions.[5] ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited.[6] Beta-lactamase-producing bacteria can play an important role in polymicrobial infections. They can have a direct pathogenic impact in causing the infection as well as

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an indirect effect through their ability to produce the enzyme beta-lactamase.[7] Such as urinary tract infections, otitis media, *Klebsiella pneumoniae*, abdominal infections, and meningitis are among the common and serious diseases caused by these pathogens.[8] Express detection of these resistant organisms offers one of the best solutions to improve patient investigation and to monitor hospital-acquired infection as well as reduce the misuse of antibiotics.[9] The present study aimed to characterize an ESBL-producing isolated from a Gram-negative bacteria using primary screening test for investigation on ESBLs, phenotypic confirmatory analysis for the determination of potentiality of above bacteria to produce ESBL and to determine the existence of SHV gene encoding ESBL by detection of gene expression by polymerase chain reaction (PCR) and to detect the SHV gene sequences of *K. pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* and to compare their genetic relatedness utilizing phylogenetic analysis

PATIENTS AND METHODS

The total number of 100 from different clinical sites including burns, wounds, otitis media, and urinary tract infection (UTI) was used for this study and taken information about patients (sex, age, and duration of stay in the hospital, disease, and kind of therapy). These samples are obtaining from AL-Ramadi Teaching Hospital and AL-Karkh Hospital in Baghdad, during a period extending from November 2017 to February 2018. The study proposal was submitted successfully into Ethical Approval Committee in the University of Anbar and obtained the Committee Authentication. The study isolates were bacteriologically diagnosed depending on the conventional, macroscopical, and microscopical in addition to biochemical and confirmatory tests. Identified isolates were stored in a medium containing brain heart infusion broth 20% glycerol. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as international standard isolates.

Detection of ESBLs

Primary ESBL screening

Antibiotic susceptibility tests of all ESBL isolates were performed using a disk diffusion method on Muller-Hinton agar with antibiotic disk of ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), cefoxitin (30 µg), and aztreonam (10 µg). The inoculum to be used in this test was prepared by adding growth from five isolated colonies grown on MacConkey agar plates to 5 ml of nutrient broth, this culture was then incubated for 3 h to produce a bacterial suspension of moderate turbidity and taken from the growth 10 µl by micropipette and added to 10 ml of normal saline that compared with turbidity of ready-made 0.5 McFarland tube standard. A sterile swab was used to obtain an inoculum from the standardized culture; this inoculum was then swabbed on Muller-Hinton agar plate. The antibiotic discs were placed on the surface of the medium evenly with sterile forceps and then incubated at 37°C for a full 18 h; then, the inhibition zones were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI).[10]

Confirmatory test for ESBLs

In the confirmatory test for ESBLs, all isolates which show resistance to third-generation cephalosporins (ceftazidime and ceftriaxone) were submitted to double disc synergy test. In this test, amoxiclav (amoxicillin+ clavulanic acid, 30/10 µg) disc was placed in the center of the inoculated plate and ceftazidime (30 µg) and cefotaxime (30 µg) disks alone are set at 10 mm distance from the amoxiclav disc, the plates were overnight incubated at 37°C for 16–18 h. The isolates interpreted as ESBL if there clear synergy of the edge of the inhibition zone of the antibiotic disc toward the amoxiclav disc.[10]

DNA extraction

SaMag bacterial DNA extraction kit is used with SaMag-12 automatic nucleic acid extraction system for the extraction of genomic DNA from study isolates (Samaga, Cepheid, Italy). The extraction process consisted of steps of lysis, binding, washing, and elution. First, a bacterial suspension was done by taking five colonies from bacteria growth on the MacConkey agar and added to 2 ml brain

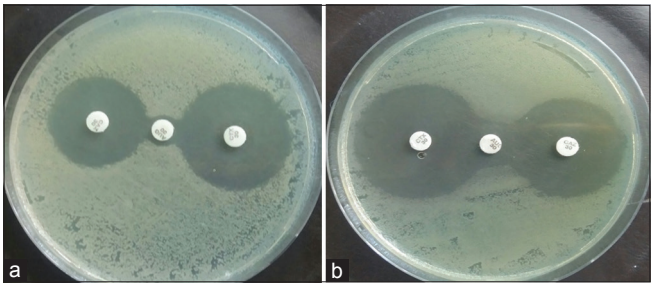

Figure 1: (a and b) The positive result for double disk synergy test between the AUG and CTX, CAZ among clinical study isolates. AUG - (amoxicillin + clavulanic acid, 30/10 µg), CTX - Cefotaxime (30 µg), CAZ - Ceftazidime (30 µg)

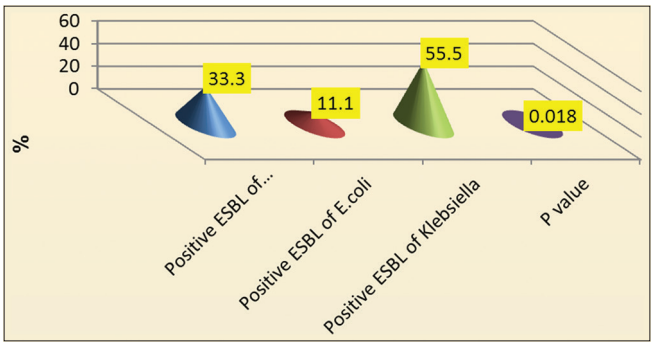

Figure 2: Phenotypic confirmatory test for the production of extended-spectrum β-lactamases among study isolates

heart infusion broth and incubated for 24 h at 37°C, 1 ml from bacterial suspension was transferred to 1.5 ml microcentrifuge tube at ×5000 g for 5 min, then discard supernatant and added 220 µl buffer BL2 to pellet and mix by vortexing for 5–10 s. After that, 200 µl suspensions had been taken to sample tube (supplied in the kit) and added 10 µl from control positive to all tubes of the device, finally insert these samples tube to automated extraction DNA device (Samag-12).

Molecular method for the detection of ESBLs

The genomic DNA was used as templates in the specific PCR amplification for the detection of the blaSHV, blaSHV-F (5'-ATTCAGTTCCGTTTCCCAGCGG -3') (GC 54.55%), (TM 68°C), and blaSHV-R (5'-AAGATCCACTAT CGCCAGCAG-3') (GC 52.38 %), (TM 64°C) having product size 231 bp. These forward and reverse primers were purchased from α-Canada Co., Italy. PCR is performing according to the Maxime PCR Pre-Mix kit, i-Taq DNA Polymerase 5U/µl, dNTPs 2.5 mM, reaction buffer (×10), and gel loading buffer ×10. About 5 µl from Taq PCR PreMix, 10 picomols/µl (1 µl) from forwarding primer, 10 picomols/µl(1 µl) from the reverse primer, 1.5 µl DNA, 16.5 µl distill water to reach final volume 25 µl then mixed well by vortex. All tubes transferred into the thermal cycler.

The optimum condition for the detection SHV gene as follows: Initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s, amplification at 72°C for 45 s, and the final extension at 72°C for 7 min. The amplified products were separated in 1% agarose gel and the electrophoresis was performed according to standard procedure.

Sequencing of DNA

Sequencing of PCR product was achieved by NICEM Company (South Korea) at which the PCR DNA products had been sending with their specific primers in a freezer bag. The sequencing part of this research was designed between the sequence of standard gene BLAST program which is already present at online National Center of Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov and using BioEdit program. The evolutionary analysis was conducted using Molecular Evolutionary Genetics Analysis software version 6 software.[11]

Statistical analysis

Statistical significance is considering with the threshold $P < 0.05$. The significant differences were detected using Chi-square; Chi-square test was used to study the association (dependence) between antibiotic susceptibility (sensitivity, resistance, and intermediate), production of ESBL, and prevalence of these types of the genes blaSHV among bacterial isolates.

RESULTS

A total of 100 clinical samples were collected during this study, out of these specimens, 65 (65%) were positive for culture distributed as follows: 28 (43.07%) *P. aeruginosa* isolates clinical specimens distributed as follows: 2 (7.1%) isolates from wounds, 9 (32.1%) from ear, and 17 (60.7%) from burn, and 25 (38.4%) isolates of *Klebsiella* distributed as follows: 19 (76%) isolates from burn, 5 (20%) isolates from UTI, and 1 (4%) isolates from ear, and 12 (18.4%) isolates from *E. coli* distributed as follows: 5 (41.6%) isolates from burn and 7 (58.3%) isolates from UTI, and others isolates from UTI including 12 isolates were Gram-positive bacteria, and 23 isolates were without growth (culture negative). Negative growth is due to the fact that patients are antimicrobials chemotherapy during culture time. These isolates are screening using the Kirby–Bauer disk diffusion method by measuring the diameter of inhibition zones around antibiotic discs showed that *K. pneumoniae* were resistance to ceftriaxone 22 (88%), ceftazidime 21 (84%), aztreonam 22 (88%), cefepime 12 (92.3), and more sensitive to meropenem and imipenem. *E. coli* were resistance to ceftriaxone 12 (100%), ceftazidime 10 (83.3%), aztreonam 12 (100%), and cefoxitin 10 (83.3%) and show resistance to meropenem 2 (16.7%) and 3 (25%) for imipenem. Pseudomonas showed resistance to ceftriaxone 20 (71.4%), ceftazidime 19 (67.9%), aztreonam 6 (21.4%), cefepime 13 (68.4%), and cefoxitin (100%) and one isolate shows resistance to imipenem 1 (3.6%). In the confirmatory test, 27 of 60 (45%) are identified as ESBL positive; 9 (33.3%) of *P. aeruginosa* 15 (55.5%) of *Klebsiella* and 3 (11.1%) of *E. coli*. Statistical analysis shows a statistically significant ($P > 0.05$) (0.018).

In the genetic part, PCR assay has been used to detect the presence of extended-β-lactamases gene blaSHV in *K. pneumoniae*, *E. coli,* and *P. aeruginosa* isolate from clinical environment. In the present study, 16 of 27 isolates from *K. pneumoniae*, *E. coli*, and *Pseudomonas* have carried SHV gene as follows: 9 (56.25%) in *K. pneumoniae*, 5 (31.25%) in *P. aeruginosa,* and 2 (12.5%) in *E. coli* as represented in Figures 3 and 4.

Results of sequencing and phylogenetic analysis of SHV gene

The study result revealed of six isolates no 1, 13, 23, 9, 3, and 10 of amplified SHV gene, DNA which was sent for sequencing and phylogenetic. Alignment study of SHV gene which encoded for *K. pneumoniae* isolates (No. 1, 13, and 23) revealed that there is closely related genotype to the novel ones which isolated from Senegal, Spain: Madrid, Australia, China, Iran, France, Brazil, India, Thailand: Chonburi, USA: Chicago, IL, Germany: Hamburg, and Vietnam: Hanoi deposit in the Gene Bank with accession numbers documented in the following

Table 1. Further, the sequencing of these genes appeared 98% compatibility range in comparison with the global standard gene in a gene bank in NCBI. On the other hand,

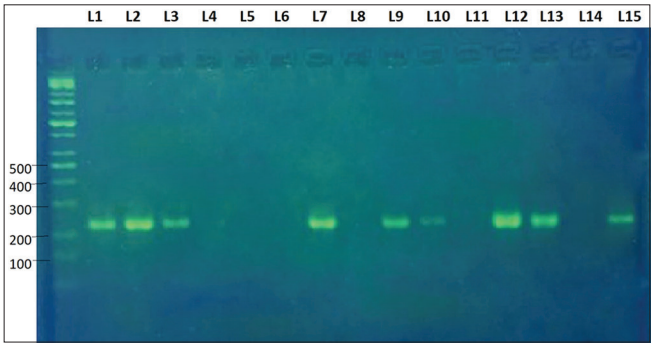

Figure 3: Distribution of SHV gene in study isolates. Agarose gel electrophoresis (2%) with Novel Juice dye agarose at 5 volt/cm^2. ×1 tris/borate/ethylenediaminetetraacetic acid buffer for 1:30 h. Bands with SHV gene (231 bp) obtained from *K. pneumoniae* isolates, which revealed positive results are represented by L1, L2, L3, L7, L9, L10, L12, L13, and L15 while L5, L6, L8, L11, and L14 were negative results. DNA ladder with 100–2000 bp on the left and right used as a molecular weight marker for DNA

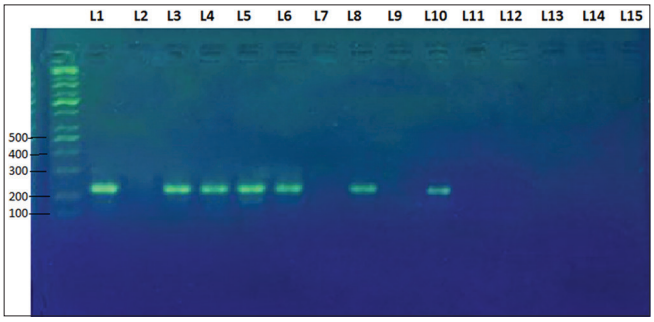

Figure 4: Distribution of SHV gene in study isolates. Agarose gel electrophoresis (2%) with Novel Juice dye agarose at 5 volt/cm^2. ×1 tris/borate/ethylenediaminetetraacetic acid buffer for 1:30 h. Bands with SHV gene (231 bp) obtained from *P. aeruginosa* and *E. coli*. Isolates, which revealed that positive results are represented by L1, L3-L6, L8, and L10 while L5, L6, L8, L11, and L14 were negative results. DNA ladder with 100–2000 bp on the left and right used as a DNA molecular weight marker

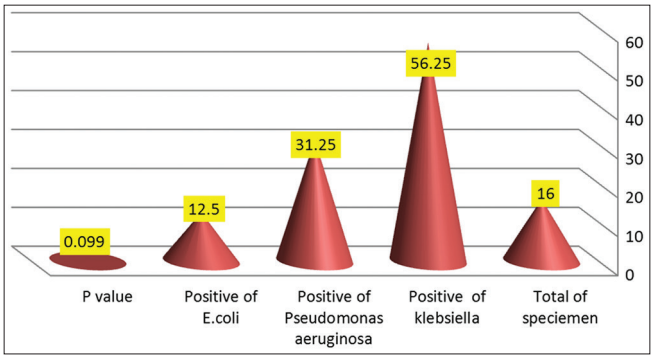

Figure 5: Distribution of the positive cases of blaSHV among study isolates

SHV gene which encoded for *P. aeruginosa* isolated No. 9 and 3 revealed that there are similar with those countries with compatibility range (99%) in Brazil, Egypt, United Arab Emirates, India, Japan, Tunisia, France, and Switzerland, followed by Greece (98%), the USA (74%), Brazil: Belo Horizonte (74%), and Colombia (71%) with accession numbers documented in the following Table 2. Furthermore, SHV gene which encoded for *E. coli* isolates No. 10 revealed that there is closely related genotype to the novel ones which isolated from India, Iran, China, France,

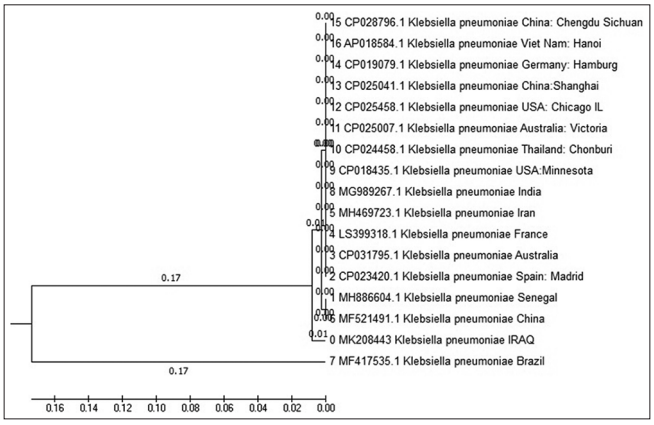

Figure 6: Phylogenetic analysis tree of Iraqi SHV genes which encoded for *Klebsiella pneumoniae* genetic distance with other global

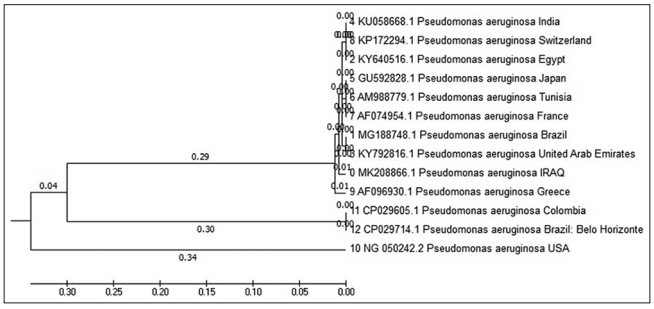

Figure 7: Phylogenetic analysis tree of Iraqi SHV genes which encoded for *Pseudomonas aeruginosa* genetic distance with other global

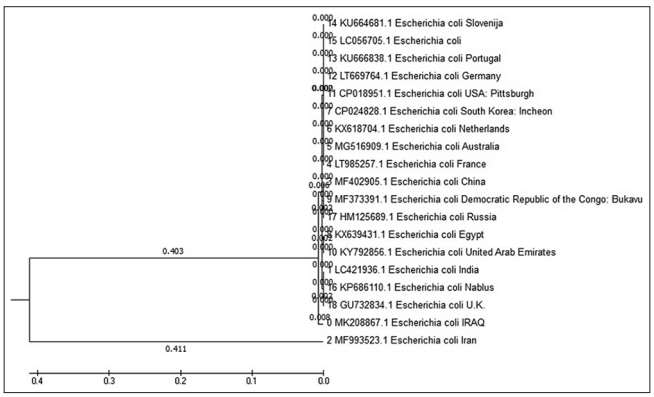

Figure 8: Phylogenetic analysis tree of Iraqi SHV genes which encoded for *Escherichia coli* genetic distance with other global

Table 1: The BLAST results of SHV gene *K. pneumoniae* in the gene bank, and compatibility of DNA sequences obtained from NCBI

S. No	Accession	Gene	Country	Source	Compatibility (%)
1	ID: MH886604.1	(blaSHV) gene	Senegal	*K. pneumoniae*	98
2	ID: CP023420.1	(blaSHV) gene	Spain: Madrid	*K. pneumoniae*	98
3	ID: CP031795.1	(blaSHV) gene	Australia	*K. pneumoniae*	98
4	ID: LS399318.1	(blaSHV) gene	France	*K. pneumoniae*	98
5	ID: MH469723.1	(blaSHV) gene	Iran	*K. pneumoniae*	98
6	ID: MF521491.1	(blaSHV) gene	China	*K. pneumoniae*	98
7	ID: MG653189.1	(blaSHV) gene	Brazil	*K. pneumoniae*	98
8	ID: MG989267.1	(blaSHV) gene	India	*K. pneumoniae*	98
9	ID: CP018435.1	(blaSHV) gene	USA: Minnesota	*K. pneumoniae*	98
10	ID: CP024458.1	(blaSHV) gene	Thailand: Chonburi	*K. pneumoniae*	98
11	ID: CP025007.1	(blaSHV) gene	Australia: Victoria	*K. pneumoniae*	98
12	ID: CP025041.1	(blaSHV) gene	USA: Chicago, IL	*K. pneumoniae*	98
13	ID: CP025458.1	(blaSHV) gene	China: Shanghai	*K. pneumoniae*	98
14	ID: CP019079.1	(blaSHV) gene	Germany: Hamburg	*K. pneumoniae*	98
15	ID: CP028796.1	(blaSHV) gene	China: Chengdu, Sichuan	*K. pneumoniae*	98
16	ID: AP018584.1	(blaSHV) gene	Vietnam: Hanoi	*K. pneumoniae*	98

NCBI: National Center Biotechnology Information, *K. pneumoniae*: *Klebsiella pneumoniae*

Table 2: The BLAST results of SHV gene which encoded for *P. aeruginosa* in the gene bank, and compatibility of DNA sequences obtained from NCBI

S. No	Accession	Gene	Country	Source	Compatibility (%)
17	ID: MG188748.1	(blaSHV) gene	Brazil	*P. aeruginosa*	99
18	ID: KY640516.1	(blaSHV) gene	Egypt	*P. aeruginosa*	99
19	ID: KY792816.1	(blaSHV) gene	United Arab Emirates	*P. aeruginosa*	99
20	ID: KU058668.1	(blaSHV) gene	India	*P. aeruginosa*	99
21	ID: GU592828.1	(blaSHV) gene	Japan	*P. aeruginosa*	99
22	ID: AM988779.1	(blaSHV) gene	Tunisia	*P. aeruginosa*	99
23	ID: AF074954.1	(blaSHV) gene	France	*P. aeruginosa*	99
24	ID: KP172294.1	(blaSHV) gene	Switzerland	*P. aeruginosa*	99
25	ID: AF096930.1	(blaSHV) gene	Greece	*P. aeruginosa*	98
26	ID: NG_050242.2	(blaSHV) gene	USA	*P. aeruginosa*	74
27	ID: CP029605.1	(blaSHV) gene	Colombia	*P. aeruginosa*	71
28	ID: CP029714.1	(blaSHV) gene	Brazil: Belo Horizonte	*P. aeruginosa*	74

NCBI: National Center Biotechnology Information, *P. aeruginosa*: *Pseudomonas aeruginosa*

Australia, the Netherlands, and South Korea: Incheon. Egypt, Democratic Republic of the Congo: Bukavu, United Arab Emirates, USA: Pittsburgh, Germany, Portugal, Slovenija, Japan, Nablus, Russia, and the U.K. deposit in the Gene Bank with accession numbers documented in the following Table 3. The sequencing of these genes appeared 99% compatibility range in comparison with the global standard gene in a gene bank in NCBI. Furthermore, the sequencing and BLAST analysis of partial SHV gene and types of polymorphism for the gene are reflecting in Table 4.

DISCUSSION

It is well known that Gram-negative bacilli are the most commonly isolated living organisms from clinical specimens, and due to the high production of ESBL in this group, this requires laboratory testing methods to correctly detect these enzymes for good management.[12] In our study, GNBs distribution was as follows: *E. coli* were 12 (18.4%) and *K. pneumoniae* was 25 (38.4%). In our research, *P. aeruginosa* accounted for the most ESBL-producing organisms in

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Table 3: The BLAST results of SHV gene which encoded for *E. coli* in the gene bank, and compatibility of DNA sequences obtained from NCBI

S. No	Accession	Gene	Country	Source	Compatibility (%)
29	ID: LC421936.1	(blaSHV) gene	India	*E. coli*	99
30	ID: MF993523.1	(blaSHV) gene	Iran	*E. coli*	99
31	ID: MF402905.1	(blaSHV) gene	China	*E. coli*	99
32	ID: LT985257.1	(blaSHV) gene	France	*E. coli*	99
33	ID: MG516909.1	(blaSHV) gene	Australia	*E. coli*	99
34	ID: KX618704.1	(blaSHV) gene	Netherlands	*E. coli*	99
35	ID: CP024828.1	(blaSHV) gene	South Korea: Incheon	*E. coli*	99
36	ID: KX639431.1	(blaSHV) gene	Egypt	*E. coli*	99
37	ID: MF373391.1	(blaSHV) gene	Democratic Republic of the Congo: Bukavu	*E. coli*	99
38	ID: KY792856.1	(blaSHV) gene	United Arab Emirates	*E. coli*	99
39	ID: CP018951.1	(blaSHV) gene	USA: Pittsburgh	*E. coli*	99
40	ID: LT669764.1	(blaSHV) gene	Germany	*E. coli*	99
41	ID: KU666838.1	(blaSHV) gene	Portugal	*E. coli*	99
42	ID: KU664681.1	(blaSHV) gene	Slovenija	*E. coli*	99
43	ID: LC056705.1	(blaSHV) gene	Japan	*E. coli*	99
44	ID: KP686110.1	(blaSHV) gene	Nablus	*E. coli*	99
45	ID: HM125689.1	(blaSHV) gene	Russia	*E. coli*	99
46	ID: GU732834.1	(blaSHV) gene	U.K.	*E. coli*	99

NCBI: National Center Biotechnology Information, *E. coli*: *Escherichia coli*

Table 4: Type of polymorphism of SHV genes which encoded for three study isolates of *K. pneumoniae*, two isolates of *P. aeruginosa*, and one isolate of *E. coli*

Number and source of isolates	Type of substitution	Location	Identities (%)	Nucleotide	Sequence ID
1 (*K. pneumoniae*)	Transition	272	99	C>T	ID: MH469723.1
	Transversion	440	-	T>A	
13 (*K. pneumoniae*)	Transition	272	98	C>T	ID: MH469723.1
	Transition	294	-	G>A	
	Transversion	440	-	T>A	
23 (*K. pneumoniae*)	Transition	272	99	C>T	ID: MH469723.1
9 (*P. aeruginosa*)	Transversion	335	99	T>A	ID: MG188748.1
	Transition	354	-	A>G	
3 (*P. aeruginosa*)	Transversion	334	99	C>G	ID: MG188748.1
10 (*E. coli*)	Transition	539	99	A>G	ID: MF993523.1
	Transversion	505	-	T>A	
	Transversion	465	-	A>T	

Klebsiella pneumoniae: *K. pneumonia, Pseudomonas aeruginosa*: *P. aeruginosa*

all clinical samples 28 (43.07%). After screening all the GNBs, according to CLSI recommended screening criteria, 60 (92.3%) were found to be potential ESBL producers. However, ESBL production was confirmed in only 27 (45%) by the phenotypic confirmatory method. Thus, in our study, the confirmatory method could not detect ESBL production in 33 isolates (55%). This results may be due to additional resistance mechanisms may be due to the production of other types of enzymes such as ambler Class C β-lactamases as concluded in a study by Al-Ouqaili *et al*.,[13] and MBL enzymes in a study laid down by Al-Ouqaili *et al*.[14] or by another mechanism for resistance such as biofilm in a survey by Al-Ouqaili *et al*.[15]

Nine (33.3%) of *P. aeruginosa* isolates were ESBL positive, which is in agreement with those observed by Hakemi *et al*.,[16] which showed that ESBL positive were 31.1%. However, it is in contrast with two other studies in Iran that their results

were lower (18%) and higher (42.8%) than our result this discrepancy may be related to more usage of beta-lactam drugs and the time of the study.[17,18] *P. aeruginosa* isolated in this study was resistance to ceftazidime (67.9%), cefepime (68.4%), is similar with a study Hakemi *et al*.,[16] (66%) for ceftazidime and (67%) cefepime, and low resistance was observed to imipenem (3.6%). Nithyalakshmi *et al*.[19] agreed with our study in finding low resistance to imipenem (3.4%). The resistance tool of problematic infections affiliated from *P. aeruginosa* to imipenem and meropenem may be due to diminished expression of an OprD-like protein which works as a carbapenem-specific channel and expressed by the OprD gene. This interpretation is reflected clearly in a study laid down by Al-Ouqaili *et al*.[20]

K. pneumoniae isolates were resistant to ceftriaxone 22 (88%) and ceftazidime 21 (84%), these results were a similarity with Natoubi *et al*.[21] showed that the resistance of *K. pneumoniae* to ceftriaxone and ceftazidime was 81.08% and 81.08%, respectively. In this study, *K. pneumoniae* was the most frequent ESBL producer, *Klebsiella* ESBL producer was 55.5% that similarity with Al-gamy *et al*.[22] showed that ESBL production was 55% isolates of *K. pneumoniae* isolated from hospital-acquired infections in Riyadh. However, these contrast with Saha *et al*.,[23] ESBL producers were observed among *Klebsiella* spp. (75%) and with AL-Subol and Youssef,[24] they were detected (67.5%) of *K. pneumoniae*, different rates for the prevalence of ESBL-producing *K. pneumoniae* are detecting in the variation difficult to explain but may be due to differences in antibiotic exhaustion and a variety of specimens handling time. Further, *E. coli* isolates, were resistant to ceftriaxone 12 (100%), ceftazidime 10 (83.3%), aztreonam 12 (100%), cefoxitin 10 (83.3%), meropenem 2 (16.7%) this agree with Hassan *et al*.,[25] showed the resistance rates to ceftazidime, ceftriaxone, and aztreonam among *E. coli* isolates were 97.8%, 98.6%, and 98.5% respectively, and in another study by Devrim *et al*.,[26] show the rate of meropenem and imipenem resistance was 7.4%.

In the present study, 11.1% isolates of *E. coli* were ESBL producers agree with the investigation by Batchoun *et al*.[27] observed that ESBL production of *E. coli* was 10.8%. In contrast to our study, the study by Devrim *et al*.,[26] showed that ESBL present in 27 (72.9%), study of Chourasia *et al*.,[27] which showed prevalence ESBL in *E. coli* was (60.4%), and Balan[29] observed that 19.6% isolates of *E. coli* were ESBL producers.

In the genetic part of this study, 16 (59.25%) of 27 isolates are carrying carried SHV gene and 11 (40.7%) isolates expressed a negative result to the genes used in this study due to other genes encoding ESBLs not used in this study. The results of the blaSHV gene among *Klebsiella* isolates were 56, 25% this agree with Haji *et al*.[30] and 55.5% of *K. pneumoniae* isolates. These results were different from another study by Hassan *et al*.[25] showed that SHV gene was 86% of *K. pneumoniae* isolates. The rate of SHV in this study was a little higher than that reported in a Turkish hospital Dagi *et al*.[31] showed that SHV in *K. pneumoniae* isolates were 24.4%. SHV gene has appeared in *E. coli* with the low ratio (12.5%). This ratio was in agreement with those observed by study in Iran Moosavian and Deiham[32] SHV was 9%. These results were different from other studies Hassan *et al*.,[25] detect the prevalence of SHV in *E. coli* was 22%, and study by Mirzaee *et al*.[33] showed that the prevalence of SHV was 31.6%.

The study revealed that the prevalence rate of blaSHV in *Pseudomonas* (31, 25%) this similar to Toupkanlou *et al*.[34] (36%). In contrast to our study, a study by Ghaima and Abdulhassan[35] detection the prevalence of ESBL-SHV gene in *P. aeruginosa* was 52.3%, Bahmani and Ramazanzadeh[36] detected that the prevalence of ESBL-SHV gene in *P. aeruginosa* was 10.57% and in a study by Bokaeian *et al*.,[37] blaSHV was 6.6%, and Chika and Agbakoba[38] was 43.5%.

The phylogenetic tree revealed that the SHV encoded for *K. pneumoniae* revealed that the compatibility range (98%), and SHV encoded for *E. coli* revealed that the compatibility range (99%) while SHV gene which encoded for *P. aeruginosa* isolated showed a variation in the compatibility range with countries (99%) in Brazil, Egypt, United Arab Emirates, India, Japan, Tunisia, France, and Switzerland, followed by Greece (98%), the USA (74%), Brazil: Belo Horizonte (74%), and Colombia (71%).

CONCLUSIONS

SHV encoding resistance is detecting in high prevalence, particularly among *K. pneumoniae* isolates. Furthermore, SHV plays an essential role in the resistance of ESBL producer isolates to new β-lactams. Further, the sequencing of these genes revealed 98–99% compatibility range with the global standard gene in gene bank documented in NCBI. The phylogenetic tree of SHV gene which encoded for *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. Revealed that the compatibility range (98%), (71–99%) and (99%) of bacterial isolates respectively with the global standard gene in gene bank documented in NCBI.

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