Molecular Study of SHV-11 and SHV-12 Genes among Klebsiella Pneumoniae Isolated from UTI Patients in Erbil City

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ABSTRACT

Among 350 urine samples collected from urinary tract infection patients in Erbil city, (50) isolates were identified as Klebsiella pneumoniae using cultural, morphological and biochemical characteristics. Their identity confirmed by Vitek² system. Extended-spectrum β-lactamase (ESBL) production determined by phenotypic and molecular methods. Phenotypic detection methods include an initial screening by using (ATM30, CTX5 and CT30), by which (52%) were resistant. Phenotypic ESBL production was confirmed by DDST and CDM. Eight per cent of the isolates were ESBL producer by DDST and (42%) were ESBL producer by CDM. Fifty per cent of the isolates were ESBL producer by both methods. Polymerase Chain Reaction (PCR) amplification used for detection of SHV-11 (a non-ESBL) gene in the genomic DNA and SHV-12 (an ESBL) gene in the plasmid DNA. SHV-11 found in the genomic DNA of 96% of ESBL producer isolates, while 88% of phenotypic ESBL negative isolates contained SHV-11 in their genomic DNA. Seventy two per cent of the phenotypic ESBL producer isolates had SHV-12 in their plasmid DNA, whereas 44% of the phenotypic ESBL negative isolates contained SHV-12 in their plasmid DNA. Out of 25 phenotypic ESBL producer isolates, 72% carried SHV-11 and SHV-12, whilst only 44% of phenotypic ESBL negative isolates contained both genes.

Keywords:
Extended-spectrum β-lactamase; PCR; SHV-12; SHV-11

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1. INTRODUCTION

Klebsiella pneumoniae (K. pneumoniae), is a Gram-negative bacterium belongs to the Enterobacteriaceae family. It is an important opportunistic pathogen and a frequent cause of urinary tract infections (UTIs) and pneumonia (Podschun and Ullmann, 1998). It is the second Gram negative causative agent of UTI (Schembri et al., 2005). UTIs represent one of the most common diseases encountered in medical practice. There is a growing concern regarding antimicrobial resistance worldwide, particularly in K. pneumoniae and other causative agents of UTIs (Rashed et al., 2008).

The predominant mechanism for resistance to β-lactam antibiotics in gram-negative bacteria is the production of β-lactamase. In addition, production of extended-spectrum β-lactamases (ESBLs) is another important mechanism which is responsible for resistance to the third-generation cephalosporins (Paterson et al., 2003).

ESBLs are plasmid mediated enzymes that are able to hydrolyse a wide variety of penicillins and cephalosporins (Turner, 2005). ESBLs are more prevalent in K. pneumoniae than in any other Enterobacterial species. K. pneumoniae isolates usually carry a chromosomal β-lactamase, most commonly SHV (Hæggman et al., 2004) and the plasmid variants have probably derived from the chromosomal SHV genes (Chaves et al., 2001). Plasmid-mediated SHV-type ESBLs are wide spread in clinical isolates of K. pneumoniae (Ryoo et al., 2005).
The SHV-β-lactamases comprise one of the most clinically significant classes of β-lactamase family. PCR and hybridisation studies revealed that \textit{bla} \textit{SHV} genes are native to \textit{K. pneumoniae} chromosome (Ford and Avison, 2004). It has also been found on plasmids of \textit{K. pneumoniae} and some other members of Enterobacteriaceae family such as \textit{E. coli}, \textit{K. oxytoca} and \textit{Proteus mirabilis} (Hirakata et al., 2005). \textit{SHV-1} is encoded both chromosomally and on plasmids in \textit{K. pneumoniae}. SHV-β-lactamases belong to group 2be β-lactamases. Since \textit{SHV-β} lactamase gene is a normal part of the \textit{K. pneumoniae} chromosome, evidences have shown that plasmid-borne \textit{bla} \textit{SHV} genes are originated from chromosomal \textit{bla} \textit{SHV} genes as a result of translocation via transposons and insertion sequences such as insertion sequence 26 (IS26). The plasmid borne \textit{bla} \textit{SHV} carries IS26 insertions (820 bp IS) either upstream of the promotor or in it, which in turn reinforces the promotor strength.

The aim of the present study was PCR amplification of \textit{SHV-11} gene genomic DNA and \textit{SHV-12} gene in plasmid DNA in of the phenotypic ESBL producer and phenotypic non-ESBL producer isolates of \textit{K. pneumoniae} in order to know whether presence of these genes related to antibiotic resistance or not.

2. MATERIALS AND METHODS

2.1 BACTERIAL SAMPLES

Three-hundred and fifty urine samples were collected during the period of 2nd of June to 5th of October 2012 from patients with urinary tract infection admitted to Hawler Teaching, Rizgary, Raparin and West Emergency hospitals in Erbil city.

Urine samples were cultured on MacConKey agar by streaking method and incubated at 37°C for 18-24 hours. The suspected colonies were sub-cultured on nutrient agar for obtaining pure culture.

The morphological characteristics of the colonies were studied. The \textit{K. pneumoniae} isolates grow well on ordinary media at 37°C for 18-24 hours. On MacConkey agar the colonies typically appear large, pink and mucoid (Arora and Arora, 2008). The biochemical tests performed for suspected bacterial isolates include catalase, oxidase, urea hydrolysis, citrate utilization, sugar fermentation and motility tests (Atlas et al., 1995). Moreover, VITEK 2 was used for confirming the identity of the isolates.

2.2 DETECTION METHODS FOR ESBLs

2.2.1 PHENOTYPIC DETECTION METHODS

With rising in number of ESBL producing clinical isolates of bacteria there is also requirement for their detection in the laboratory (Al-Jasser, 2006). According to the Clinical and Laboratory Standards Institute (CLSI, 2011) guidelines for detection of ESBL-production, an initial screening method and phenotypic confirmatory tests based on synergy between an indicator cephalosporin and clavulanic acid was used.

Although these three antibiotic discs (CTX, CI and ATM) were used as indicator of ESBL production as the antibiotic sensitivity testing was done, phenotypic confirmatory tests was done for all of the isolates.

According to the CLSI, (2011) recommendations, a disc of ceftazidime (30μg) alone and in combination with clavulanic acid (30/10μg) discs were used. The discs were placed on a plate streaked with the isolate to be tested within fifteen minutes. An increase of 5 mm or more in the zone diameter of ceftazidime tested in combination with clavulanic acid versus its zone when tested alone, confirm the presence of ESBL phenotype.

2.2.1.1 DOUBLE DISC SYNERGY TEST (DDST)

In the same way as the antibiotic sensitivity testing was done, a disc of amoxicillin/clavulanic acid (20/10μg) placed
16 mm apart (centre to centre) from a disc containing a third generation cephalosporin (ceftaxime, ceftriaxone and aztreonam) on a plate of Muller-Hinton inoculated and streaked with the tested bacterium. Extension of the edge of one of the cephalosporins or aztreonam zone on the side exposed to the disc containing clavulanic acid indicate the presence of ESBL and the isolate should be considered as ESBL producer (Freitas et al., 2003).

2.2.1.2. COMBINATION DISC METHOD (CDM)

According to the CLSI, (2011) recommendations, a disc of ceftazidime (30μg) alone and in combination with clavulanic acid (30/10μg) discs were used. The discs were placed on a plate streaked with the isolate to be tested within fifteen minutes. An increase of 5 mm or more in the zone diameter of ceftazidime tested in combination with clavulanic acid versus its zone when tested alone, confirm the presence of ESBL phenotype.

2.2.2 MOLECULAR DETECTION METHOD

Since the phenotypic ESBL detection methods do not provide an accurate identification of an ESBL producer, detection of the β-lactamase gene is a more precise approach. In this study, specific primers were designed for amplification of SHV-11 which is a non-ESBL β-lactamase gene and SHV-12 which is an ESBL β-lactamase gene. PCR was done for genomic DNA extracts of all fifty isolates by using SHV-11 primers and plasmid DNA extracts by using SHV-12 primers.

2.3 GENOMIC DNA EXTRACTION

The G-spin™ genomic DNA extraction kit was used for purification of genomic DNA of the isolates. It is designed for rapid isolation of genomic DNA from a variety of sample sources.

2.4. Plasmid DNA extraction

Plasmid DNA was extracted from 5 millilitre (ml) of an overnight culture of the isolates of K. pneumoniae grown in LB broth medium containing an appropriate antibiotic by using plasmid DNA purification kit (DNA-spin Plasmid DNA Purification Kit/ iNtRON/ Korea). The extracted plasmid DNA was stored at -20°C until using.

2.5 PCR AMPLIFICATION OF BLASHV-11 AND BLASHV-12 GENES

Specific oligonucleotide primers were designed for SHV-11 and SHV-12 genes according to blasSHV-11 and blasSHV-12 nucleotide sequences by using Genius software.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHV-11 F</td>
<td>CCGGCCGATTTTGTGATTTCG</td>
<td>463</td>
</tr>
<tr>
<td>SHV-11 R</td>
<td>CCGCCATTACCATGAGCGAT</td>
<td></td>
</tr>
<tr>
<td>SHV-12 F</td>
<td>GGATCTGGTGAGACTACTGCG</td>
<td>461</td>
</tr>
<tr>
<td>SHV-12 R</td>
<td>TGGTATACGGCGCAAGGCC</td>
<td></td>
</tr>
</tbody>
</table>

PCR amplification was done for genomic DNA extracts using SHV-11 primers and PCR program was 94°C for 2 min. (initial denaturation), 94°C for 1min. (denaturation), 64°C for 1min. (annealing), 72°C for 1min. (extension) and 72°C for 10 min. (post extension) and 35 cycles. The reaction mixture is shown in the table (2).

Table (2): The reaction mixture of (25µl) reaction volume for PCR used in the present study

<table>
<thead>
<tr>
<th>Component</th>
<th>Final Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>GoTaq Green Master Mix 2x</td>
<td>1X</td>
<td>12.5 µl</td>
</tr>
<tr>
<td>Forward primer 10 µl</td>
<td>0.31 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td>Reverse primer 10 µl</td>
<td>0.31 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td>Template DNA 10 μg-1 µg</td>
<td></td>
<td>1 µl</td>
</tr>
<tr>
<td>Nuclease-Free water</td>
<td>9.5 µl</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td></td>
<td>25 µl</td>
</tr>
</tbody>
</table>

PCR amplification was done for SHV-12 gene by using plasmid DNA extracts of all isolates as template and SHV-12 primers. The PCR program was 94°C for 2 min. (initial
denaturation), 94°C for 1min. (denaturation), 54°C for 1min. (annealing), 72°C for 1min. (extension) and 72°C for 10min. (post extension) and 35 cycles. The PCR reaction tube components were the same as for SHV-11.

For running PCR products of both SHV-11 and SHV-12, 1.2 gm agarose powder was weighed and added to 100 ml of TBE buffer and bring to boil. After cooling, the gel casted and after solidification, the PCR products were loaded into the wells in the gel, 5µl/well. Five µl of 1500bpDNA ladder (Gene Direx) loaded into one of the wells in the gel to determine the size of the amplified products. The gels were run at 45V/15 min., then at 135V/30-45min. The gel stained with ethidium bromide, visualised by UV-trans illuminator and photographed.

The method described by Sambrook and Russell, (2001) was used for gel electrophoresis. The extracted plasmids were electrophoresed in 0.7% agarose gel with Tris-Borate Ethylene-Diamine Tetra-Acetic acid (TBE), stained with ethidium bromide and visualized under UV-light.

3.RESULTS AND DISCUSSION

Among 350 urine samples collected from patients admitted to Hawler Teaching, Rizgary, Raparin and West Emergency hospitals in Erbil city during the period of 2nd of June to 5th of October 2012, fifty isolates were identified as K. pneumoniae.

The isolates of K. pneumoniae identified. On MacConkey agar, the colonies of K. pneumoniae are typically appear as large, mucoid and pink (figure1) with red pigment usually diffusing into the surrounding medium indicating fermentation of lactose and acid production (Winn et al., 2006).

Figure (1): K. pneumoniae colonies on MacConkey agar

All of the isolates were positive for citrate, catalase and urease tests, whereas, oxidase negative. They all ferment glucose and lactose and gave yellow slant and yellow butt (acidic/acidic) reaction, while H2S negative. Furthermore, they were non-motile. The identity of the isolates was confirmed by using Vitek2 system. The sheet of the results of identification by Vitek2 system is shown in the appendix. It shows that the isolates were identified as K. pneumoniae subsp. pneumoniae with probability of 99% which is considered as an excellent level of identification.

ESBL screening by disc diffusion method was done for all fifty K. pneumoniae isolates by using three antibiotic discs as screening agent which were CTX, CI and ATM according to CLSI, (2011) recommendations. Out of these fifty, thirty-four isolates (68%) were resistant to at least one of the three ESBL screening agents. Twenty-six (52%) of the isolates were resistant to all the three tested antibiotics, one isolate was resistant for CI and ATM, one isolate resistant for CTX and ATM, one isolate resistant for ATM alone. The present results agree with that of Shukla et al., (2004) who reported that 72% of the isolates were resistant to the 3GCs tested. However, they are incompatible with the results of Subha and Ananthan, (2002) who found that 87% of their isolates showed resistance to all the three 3GCs.

Extended-spectrum β-lactamases have been found in a wide range of Gram-negative bacteria and has emerged as an important mechanism of resistance in these bacteria. However, the major strains expressing these enzymes belong to the family Enterobacteriaceae (Ramazanzadeh, 2010), K. pneumoniae remains as the major ESBL-
producing organism isolated worldwide (Iroha et al., 2011). In addition, over reliance on 3GCs for treatment of Gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics. As ESBLs are frequently encoded by genes located on different transferrable genetic elements, a variety of epidemiological situations have been identified (Widmer, 2008).

Among fifty isolates tested to know whether they are ESBL producer or not by DDST, only four (8%) of them gave positive result. As it is shown in the figure (2), the synergy between a cephalosporin and clavulanic acid indicate a positive result. This result agrees with that of Subha and Ananthan, (2002) who reported that 6.66% of K. pneumoniae isolates are ESBL positive by DDST. Although DDST is considered as a reliable method for ESBL detection, it has been known to suffer from the non-standardization of the distance of disc placement (Tofteland et al., 2007). Another reason may be AmpC enzymes which are induced by clavulinate (which inhibit them poorly) and may then attack the cephalosporins, masking synergy arising from inhibition of the ESBL (Pfaller and Segreti, 2006). This can be a cause of disagreement of the current study with the previously mentioned ones, in addition to geographical differences.

Out of fifty isolates tested for detection of ESBL production by CDM, twenty-one (42%) of them were confirmed as ESBL producer. An increase of 5 mm or more in the inhibition zone diameter for ceftazidime tested in combination with clavulanic acid versus its zone when tested alone, confirm the presence of ESBL phenotype (figure 3).

The present result is in agreement with that of Nasehi et al., (2010) who showed that 38.5% of their K. pneumoniae isolates had ESBL phenotype by this method. Among 52% of the isolates which were resistant to all three ESBL screening agents, twenty-five (50%) were confirmed as ESBL producer by both methods together, CDM identified (42%) alone and (8%) identified by DDST. It has been reported that 57.1% of K. pneumoniae were phenotypic ESBL producer based on phenotypic confirmatory tests, DDST and CDM which is in agreement with the current result (Taşli and Bahar, 2005). The present result is in agreement with the study that was carried out by Fazly Bazzaz et al., (2009) who showed that the prevalence of ESBL producing K. pneumoniae was 61% by the phenotypic disc confirmatory tests. The overall high prevalence of ESBL-positive K. pneumoniae may be due to the lack of chromosomally encoded AmpC β-lactamases in the genus Klebsiella (Tofteland et al., 2007).

Out of 25 phenotypic ESBL producer isolates in the present study, 17 (68%) resist all three tested screening agents, while only 9
(36%) of phenotypic ESBL negative isolates were resistant during ESBL screening test. The current results indicate that ESBL screening can be used for presumptive identification of potential ESBL producers among urinary isolates of *K. pneumoniae*.

The use of phenotypic tests for detection of ESBL producing microorganisms remains an issue, causing a great deal of discussion and controversy. Some factors such as production of different β-lactamases by the same microorganism could lead to erroneous conclusions (Sanguinetti, 2003). Tests based on the detection of ESBLs by PCR are more conclusive in defining ESBL production (Steward, 2001). Recently, molecular methods have been shown to be absolutely necessary to detect specific ESBL type (Taşli and Bahar, 2005).

In this study *SHV-11* which is a non-ESBL β-lactamase gene and *SHV-12* which is an ESBL β-lactamase gene were amplified by using specific primers designed for them. PCR was done for genomic DNA extracts of all fifty isolates using *SHV-11* primers and for their plasmid DNA extracts using *SHV-12* primers.

After extraction of genomic DNA from all *K. pneumoniae* isolates by PCR and gel electrophoresis were carried out to screen and determine the presence of *blaSHV-11* in the DNA of *K. pneumoniae*. PCR was performed on template DNA of each bacterial isolate separately for amplification of *blaSHV-11* gene via the use of specified oligonucleotide primers that flanked DNA sequence to be amplified. After amplification of *blaSHV-11* gene, PCR products of all bacterial samples were ran in agarose gel and visualised by UV-transilluminator to detect the presence of the gene.

The present results show that out of fifty isolates of *K. pneumoniae*, forty-six (92%) contain *blaSHV-11* gene in their genomic DNA. Although, all isolates in the current study were resistant for ampicillin, only 92% possess *blaSHV-11* in the genomic DNA. This suggests that ampicillin resistance in those isolates which lack *blaSHV-11* in their genomic DNA may be due to *blaSHV-1* or plasmid encoded *blaSHV-11*, because *blaSHV-1* which is the ancestor of *blaSHV-11* is responsible for about 20% of plasmid encoded ampicillin resistance in *K. pneumoniae* (Tzouvelekis and Bonomo, 1999). Moreover, this result is compatible with that of Younes, (2010) who stated that only 90% (54/60) of tested *K. pneumoniae* in his study were positive by PCR for *SHV* genes, despite expectation that *K. pneumoniae* isolates present an intrinsic resistance to ampicillin (Heritage et al., 1999). Similar resistance findings reported that 95% (178/187) of *K. pneumoniae* isolates collected from Portugal showed reduced susceptibility to ampicillin had *blaSHV* (Mendonça, 2009). Ozdemir et al., (2013) showed that the gene which was responsible for resistance to AMP reside in a 5000 bp mobile DNA segment which had the ability to translocate itself.

The figure (4) shows the results of PCR amplification of *blaSHV-11* of the phenotypic ESBL positive *K. pneumoniae* UTI isolates. After running of PCR products in agarose gel, the isolates which gave positive result in PCR, produced a band of 463 bp, which indicated the presence of *blaSHV-11* gene.

![Figure (4): Agarose gel electrophoresis (1.2% agarose for 60-90 minutes) of amplified PCR products of *blaSHV-11* gene (Genomic DNA) of phenotypic ESBL positive isolates](image-url)

**Lane L:** 1500 bp DNA ladder
Lane 1-41: Amplified PCR product of blaSHV-11 of phenotypic ESBL positive isolates

Among phenotypic ESBL producer isolates, blaSHV-11 found in the genomic DNA of twenty-four (96%) of the isolates. The present result is similar to that of Ghafourian et al., (2011) who reported that among 67 phenotypic ESBL producer isolates of K. pneumoniae, PCR amplification of blaSHV gene in genomic DNA was positive in 94% of the isolates. It has also been shown that 100% of phenotypic ESBL producer isolates of K. pneumoniae had blaSHV gene in their genomic DNA by PCR amplification (Tribuddharat et al., 2007).

Among phenotypic ESBL negative isolates of K. pneumoniae, twenty-two (88%) contained blaSHV-11 in their genomic DNA as shown in the figure (5).

![Agarose gel electrophoresis](image)

Figure (5): Agarose gel electrophoresis (1.2% agarose for 60-90 minutes) of amplified PCR products of blaSHV-11 gene (Genomic DNA) of phenotypic ESBL negative isolates

Lane L: 1500 bp DNA ladder

Lane 7-46: Amplified blaSHV-11 of ESBL negative isolates

Consistent with previous results by Younes, (2010) who demonstrated that blaSHV-11 was the most prevalent gene described in his study, blaSHV-11 was highly prevalent in the current investigation and among 25 phenotypic ESBL negative isolates 23 isolates possess blaSHV-11. Moreover, out of 21 isolates included in the study of Lee et al., (2006) seventeen isolates contain blaSHV-11 alone and associated with blaSHV-12 in 4 isolates. The blaSHV-11 has been described most often in K. pneumoniae (Nuesch-Inderbinen et al., 1997) and may be the ancestor of blaSHV-2a and blaSHV-12 (Ford and Avison, 2004). The non-ESBL phenotype conferred by SHV-11 shows that the Leu35Gln substitution between SHV-11 and SHV-1 has little or no significance with respect to hydrolysis of expanded-spectrum cephalosporins, therefore, its appearance is likely to be due to drift rather than antibiotic selection (Howard, et al. 2002).

In addition, the result of the present study is in concordance with that of Oliveira et al., (2010) who performed PCR for genomic DNA of all isolates in their study and found that SHV genotype was present in 78.1% of the K. pneumoniae isolates.

PCR amplification of blaSHV-12 gene was performed for plasmid DNA extracts of all of the isolates by using specific oligonucleotide primers. Among fifty isolates, twenty-nine (58%) had blaSHV-12 gene in their plasmids. Among blaSHV-carrying ESBL producer K. pneumoniae isolates, 51.4% of the genes encoded non-ESBL SHV-11, while the rest produced SHV-type ESBLs, including SHV-12 (34.6%) (Kiratisin et al., 2008). This is disagree with the results of the present study which may be due to that PCR amplification was done only for ESBL positive isolates in their study.

Among phenotypic ESBL producer isolates, 72% had blaSHV-12 in their plasmid DNA and gave a band of 461 bp, whereas 24% (6 isolates) lack the gene (figure 6). This absence of blaSHV-12 and presence of ESBL phenotype could be attributed to hyper production of non-ESBL β-lactamases, SHV-1 or SHV-11, due to high gene copy number or a single base pair change in promoter sequence, or modifications in outer membrane proteins co-existing with TEM-1, SHV-1 and SHV-11. These result in conferring an ESBL similar phenotype causing false positive results (Wu et al., 2001). The presence of other types of
ESBL genes could be counted as another reason for ESBL phenotype in these isolates.

The present findings are consistent with the study of Paterson et al., (2003) who demonstrated that SHV-type ESBLs were the most common ESBLs, occurring in 67.1% (49 of 73) of isolates of K. pneumoniae with phenotypic evidence of ESBL production. It is also agree with that of (Taşli and Bahar, 2005). They detected SHV-type ESBLs in 74.3% of the isolates and among 18 sequenced SHV amplicons, 5 were SHV-12 (27.7%).

Lin et al., (2010) and Severin et al., (2010) found that 100% and 65.3% of their phenotypic ESBL positive K. pneumoniae isolates had SHV type ESBL in their plasmid DNA. The latter showed that (27.6%) of SHV type ESBLs were of SHV-12 type.

Of the phenotypic ESBL negative isolates of K. pneumoniae included in the present study, eleven (44%) isolates contained blaSHV-12 in plasmid DNA, while the rest lack blaSHV-12 in their plasmid DNA (figure 7).
phenotype. This result agree with Oliveira et al., (2010) who reported that fifteen isolates possess the SHV family but gave negative ESBL phenotype.

Twelve (48%) of the phenotypic ESBL negative isolates contain blaSHV-11 in genomic DNA, while lack blaSHV-12 in plasmid DNA. This indicates that presence of blaSHV-12 in plasmid DNA is essential for giving a positive ESBL phenotype.

Two (8%) of the phenotypic ESBL negative isolates lack both genes in genomic and plasmid DNA, therefore, their negative phenotype may be due to absence of these genes. This is agree with that of Bali et al., (2010), in which one of the positive isolates of K. pneumoniae with CDM had no ESBL with PCR. These isolates may have other types of ESBL. It has been shown that the majority (29/32) of K. pneumoniae isolates harbour transferable blaSHV whereas, a strain carry blaSHV-11 and contain the large SHV transposon was failed to conjugate with the recipient strain (Younes, 2010). This indicated chromosomal location which agrees with other findings that the large SHV transposon is chromosomal location (Turner et al., 2009). Furthermore, a strain of K. pneumoniae possessed blaSHV-12 could not be transferred by conjugation indicating a non-conjugative plasmid location (Younes, 2010).

Considering their plasmid profile, 40% of phenotypic ESBL positive isolates contained one plasmid, 56% possess two plasmids and one phenotypic ESBL positive isolate had four plasmids.

Conclusions

Phenotypic ESBL screening can be used for presumptive identification of potential ESBL producer isolates. High prevalence of phenotypic ESBL producers among Klebsiella pneumoniae urinary isolates. Both SHV-11 and SHV-12 genes are more prevalent in phenotypic ESBL producer isolates. Finally, presence of SHV-11 in genome and SHV-12 in plasmid DNA is necessary for phenotypic ESBL production.

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