DOI: 10.24996/ijs.2018.59.1C.1

ISSN: 0067-2904

Antibiotic Resistant Gene Exchanged Between *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Escherichia coli infections are becoming difficult treated because of extensive resistance to antibiotic among these organisms and manufacturing extended-spectrum beta lactamases enzymes (ESBLs) make them resistant to beta-lactam antibiotics. This study aims to offer a summary of the main horizontal transmission apparatuses between *E. coli* as well as *Staphylococcus aureus* and emergence resistance to antibiotics. Fifty of the *E. coli* and 50 of *S. aureus* isolates were examined to obtain minimum inhibitory concentration (MIC) results. These isolates were then tested by conventional polymerase chain-reaction for the existence or absence the sulfhydryl variable *SHV* beta-lactamase genes. About (48%) of isolated *E. coli*, while (32%) of *S. aureus* were revealed *SHV* gene.

Keywords: *Extended spectrum beta lactamase (bla_{SHV}) gene, Antibiotic Resistant Genes Exchanged, Horizontal gene transfer.*

تبادل الجين المقاوم للمضادات الحيوية بين بكتريا الاشريكية القولونية وبكتريا المكورات العنقودية الذهبية.

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الخلاصة

إصابات الإشريكية القولونية أصبحت صعبة المعالجة بسبب المقاومة الواسعة للمضادات الحيوية بين هذه الكائنات وتصنيعها انزيمات البيتا لاكتام واسعة الطيف مما يجعلها مقاومة للمضادات الحيوية . تهدف هذه الدراسة إلى تقديم ملخص عن انتقال الجينات افقيا بين الإشريكية القولونية و المكورات العنقودية الذهبية ومقاومتها للمضادات الحيوية. تم فحص 50 عزلة من الإشريكية القولونية و 50 من عزلات المكورات العنقودية الذهبية للحصول على الحد الأدنى من التركيز المثبط. وتم اختبار هذه العزلات بعد ذلك بواسطة تفاعل البوليميراز المتسلسل التقليدي للكشف عن وجود أو عدم وجود جينات (*SHV*) . حيث وجدت هذه الدراسة ان حوالي (٪ 48) من الإشريكية القالونية تحوي على هذا الجين ، في حين وجد هذا الجين بنسبة (32٪) في المكورات العنقودية الذهبية .

Introduction

Bacteria are unicellular with a relatively minor- genomic size [1]. Difference detected are remarkable in cellular manners, properties of metabolic and phenotypic behaviors [2]. Several

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mechanisms may be explaining the massive genetic range that occurs in the creation of bacteria , which include inner modifications of genetic evidence, ordinarily happen due to amassing of mutation and recombination of inter- genomically homologous as well as acquisition of exact genes from other species by the route of horizontal genes transfer (HGT) [3,4]. The drug-resistant strains of bacteria as *Escherichia coli* and *Staphylococcus aureus* were believed mediated through (HGT), HGT might arise across huge phylogenetic range, like bacterium to eukaryote, bacterium to archaea and animal to bacterium and so on [4]. The genetic material transfer across species occurs successfully by: (i) Transfer of the DNA- sequence from the donor or giver into the receiver cell [5]. (ii) Integration of the picked up DNA- sequence into the genome of the receiver or (into an independent replicating component for instance a plasmid) [6]. (iii) Gaining gene (s) expression in the novel atmosphere [7]. The third stage count on the compatibility among moved genes with the transcription and translation apparatus of the host microorganism [8]. Mechanisms may exploit by bacteria for transfer of genetic elements are transformation, transduction and conjugation [9, 10, 11]. *S. aureus* colonizes the skin, nose, throat, and gastrointestinal (GI) tract of humans [12]. Bacteria in gastrointestinal tract of human assist as a pool for antibiotic resistant genes, which could be moved to other transient and resident intestinal bacteria or bacteria that move across, but then do not settle in large intestines for a considerable long time [13].The process of plasmids conjugation that coding antibiotic- resistant apparatus had cause global distribution of these genes , which conjugative plasmids transfer themselves between bacteria, predominantly in *Enterobacteriaceae* [14].Conjugative plasmids able to encode resistant to extended spectrum beta-lactamases (*SHV*) gene [15, 16].

Aim of study: To investigate the transmission of (*SHV*) gene between *Escherichia coli* and *Staphylococcus aureus* bacteria.

MATERIALS AND METHODS:

Bacterial strain collection and antibiotic identification:

Fifty *E. coli* isolates obtained from infant diarrheal patients , while fifty isolates of *S. aureus* were recovered from a nose of healthy adult persons. To detect antibiotics resistance , minimum inhibitory concentration (MIC) tests for *E. coli* and *S. aureus* were detected by using VITEK® 2 Compact (test) system.

Extraction of DNA from Bacteria:

Extraction of DNA from Bacteria was done according to Promega Corporation, 1 ml of overnight Brain heart infusion (BHI) culture was centrifuged for 2 min at 13,000 ×g and the supernatant then discarded. In *S. aureus*, growth was suspended in 480 µl of 50 mM EDTA. Lysis enzyme was added 120 µl of (Lysozyme) and incubated at 37 °C for 30 min, subsequently the admixture was centrifuged for 2 min, the supernatant after that detached and adding 600 µl of nuclei lytic solution then incubated for 5 min at 80 °C, which cooled to temperature of room, later 3 µl was added of RNase solution, mixed, incubated at 37 °C for 15 min and then cooled, while in *E. coli* was lysed according to manufactures, which Precipitation of protein done by adding 200 µl of precipitated solution and vortexes which then placed in ice for 5 min and finally centrifuged for 3 min . After that supernatant was mixed with 600 µl of isopropanol at room temperature and centrifuged for 3 min. Subsequently supernatant was poured and 600 µl of 70 % ethanol was added and centrifuged for 2 min , which the pellet was air-dried for 15 min and finally, mixed with 100 µl of rehydrated solution for overnight at 4 °C.

Oligonucleotide Sequence:

Primers used to investigate the *SHV* gene are: *SHV*-F (5'–3' CGCCTGTGTATTATCTCCCT) and *SHV*-R (5'–3' CGAGTAGTCCACCAGATCCT), which have size (293) bp [17].

PCR amplification: Cycle of PCR includes steps for template denaturation, primer annealing and primer extension Table-1. After 20–40 cycles, the amplified product e analyzed for size by gel electrophoresis.

Table 1-Show PCR amplification

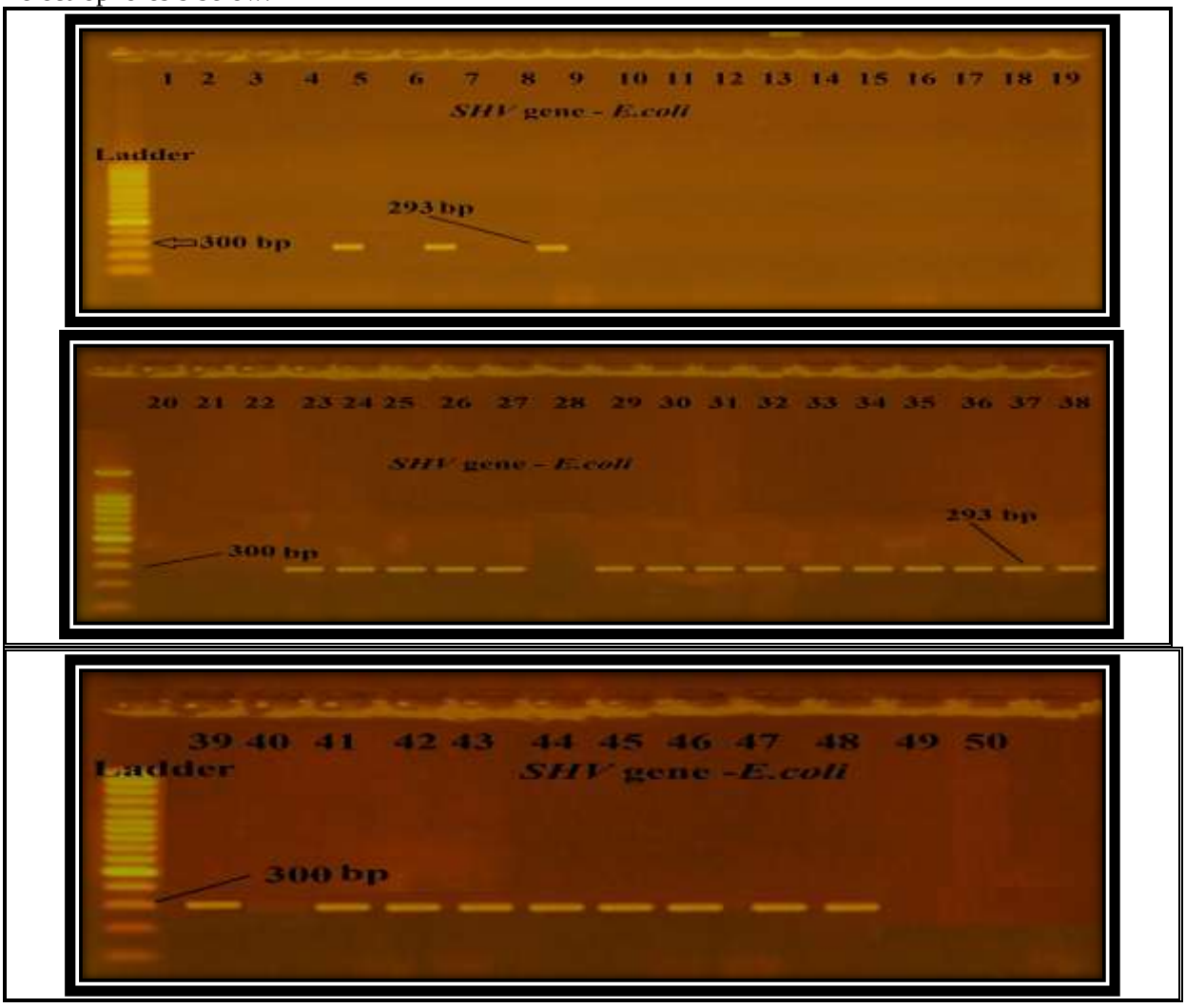
Step	Temperature, °C	Time
Initial denaturation	95	5 min
Annealing	60	30 s
Extension	72	1 min
Final Extension	72	10 min

Gel electrophoresis: PCR amplicon were investigated by electrophoresis on (1%) agarose-gel having Ethidium-bromide (0.5 mg / ml) in tris-acetate-EDTA buffer (TAE buffer) and then photographed under (UV) illumination.

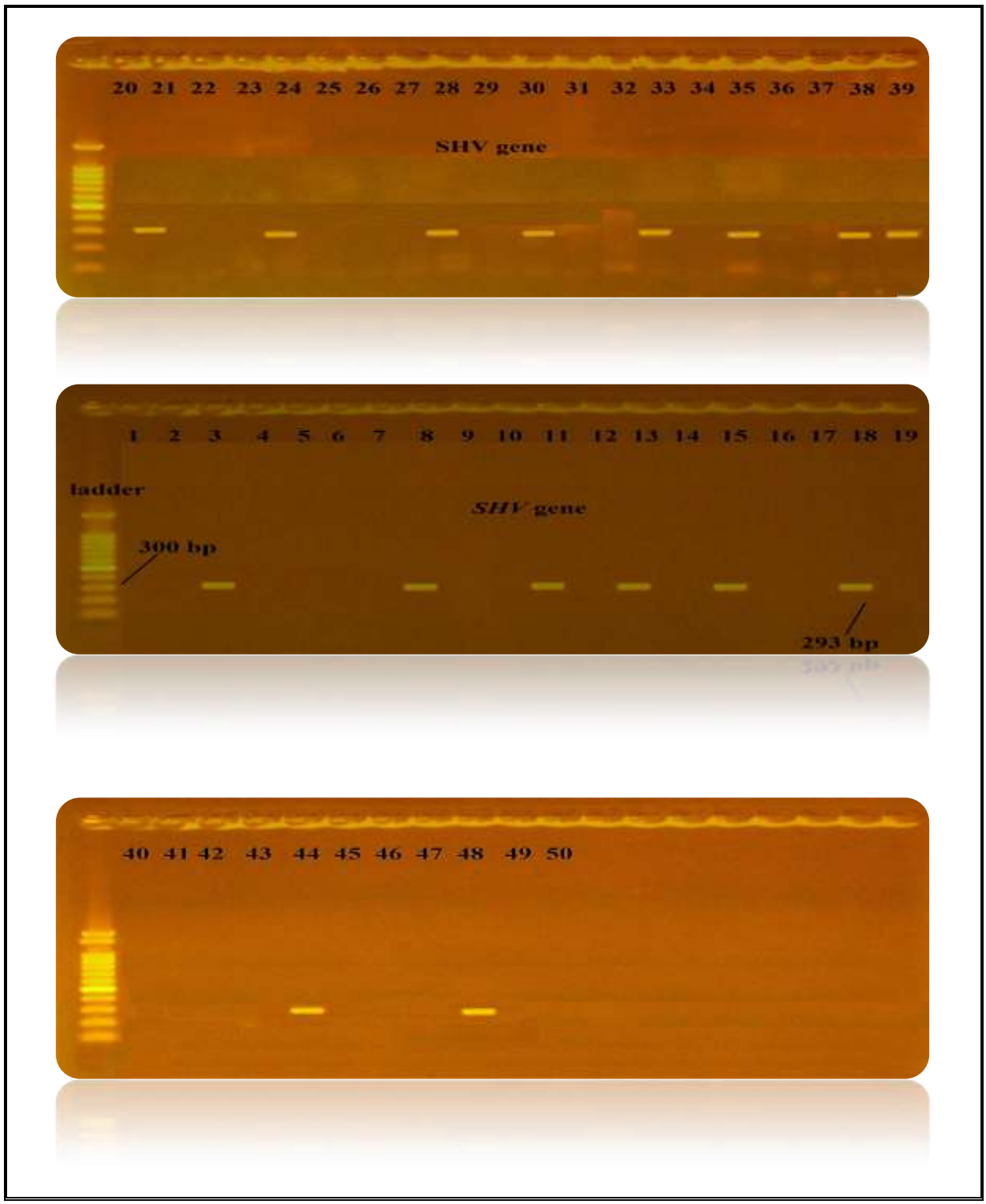
Results and Discussion:

In this study, 100 bacterial isolates including 50 *S. aureus* and 50 *E. coli* were studied for antibiotics resistant and also investigated for beta-lactamase *SHV* (bla_{SHV}) genes. *E. coli* expressing resistance to amoxicillin - clavulanate (≤ 8/4 µg / ml) , ampicillin (≤ 8µg / ml) , gentamicin (≤ 4 µg / ml) and trimethoprim -sulfamethoxazole (≤2 / 38µg/ml) , while *S. aureus* expressing resistance to benzyl penicillin (≤ 2 µg / ml) , oxacillin (≤ 2 µg / ml) , gentamicin (≤ 4 µg / ml) and trimethoprim - sulfamethoxazole (≤ 2 / 38 µg / ml) .

Genotypically, beta-lactamase *SHV* (bla_{SHV}) genes was studied. The bla_{SHV} was detected among (24) isolated of *E. coli* (48%), while in *S. aureus* among (16) (32%) as showed in Figures -(1, 2, 3). The development of drug-resistance organisms due to the opportunity of horizontal-genes transmission as well as misused of antibiotics in human treatment [18, 19] . Microflora can exchange genes and may transmission it to other commensal microorganisms or pathogens in the intestines, in food or on mucosal surfaces [20 , 21]. Unfortunately, the resistant organisms can transfer from adult to infant by several ways carrying the antibiotic resistant genes , which in this study was noticed (bla_{SHV}) genes that possess size of (293 bp) , which is originally present in gram negative bacteria such as (*E.coli*) as shown in the picture (1) , could be transfers to other bacteria , which the same size of that gene (293 bp) recognized in isolates of *S. aureus* as shown in the picture (2) of gel electrophoresis below.



Picture 1- electrophoresis of *SHV* – genes in *E. coli.*



Picture 2- electrophoresis of *SHV* – genes in *S. aureus.*

The human gastrointestinal tract provides environment for antibiotic resistant genes to develop and occurrence through populations of bacteria because of density of cells and capability of gene transmission through a range of several apparatuses [22, 23]. Transfer of genes by conjugation, transformation and transduction can occur due to physical or chemical exposure such as antibiotics, which lead to activation of certain genes, called SOS genes, in order to repair the damaged DNA [24, 25].Radman in (1975) that discovered SOS genes or response in *E. coli*, which is a stimulating DNA- repair system [26, 27].

During (SOS) response, genetic-exchange and mutation inside cells occurred to repair the damaged DNA [28]. According to (SOS) situation, *SHV*-gene may be transferred or exchanged between *E.coli* and *S. aureus* when exposure to antibiotics. The result of our study is similar to that reported by other study, which reported that the (SOS) responses are encouraged by antibiotic such as ampicillin and oxacillin ,which lead to gene exchanged between gram positive (*Streptococcus uberis*) and gram negative (*Pseudomonas aeruginosa*) bacteria ,which result in acquisition of antibiotic resistant genes [29, 30].

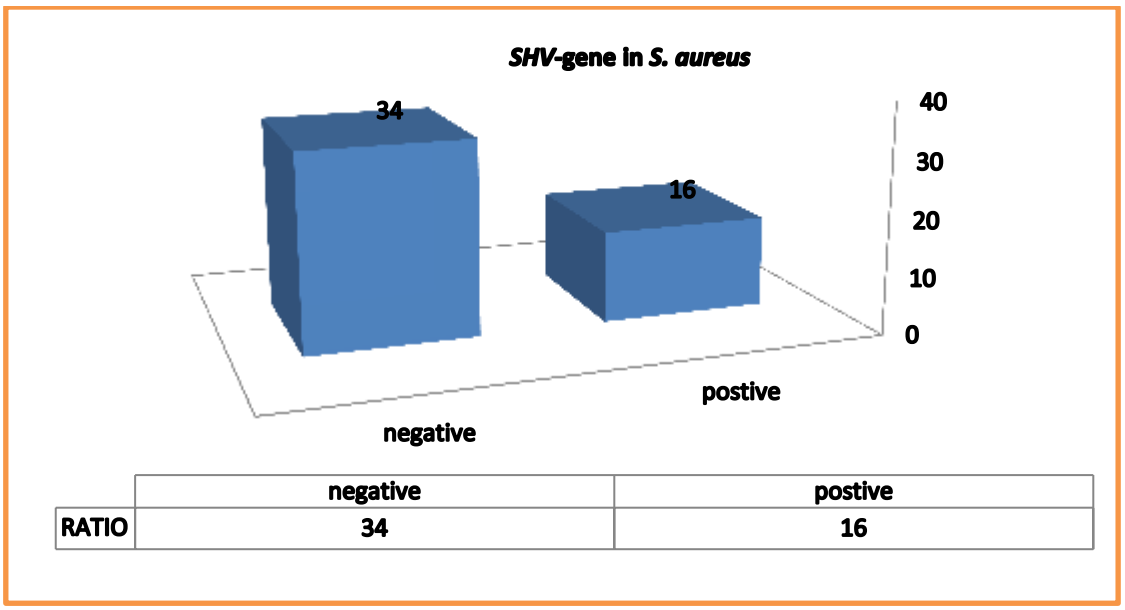


Figure 1-show SHV gene in S. aureus.

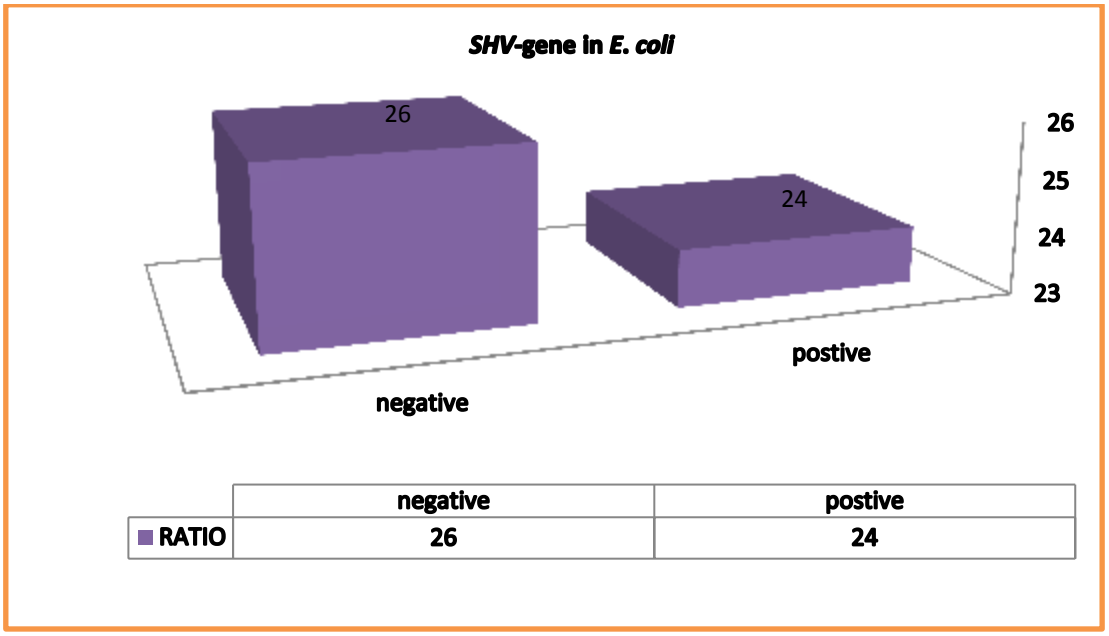


Figure 2- show SHV gene in E. coli.

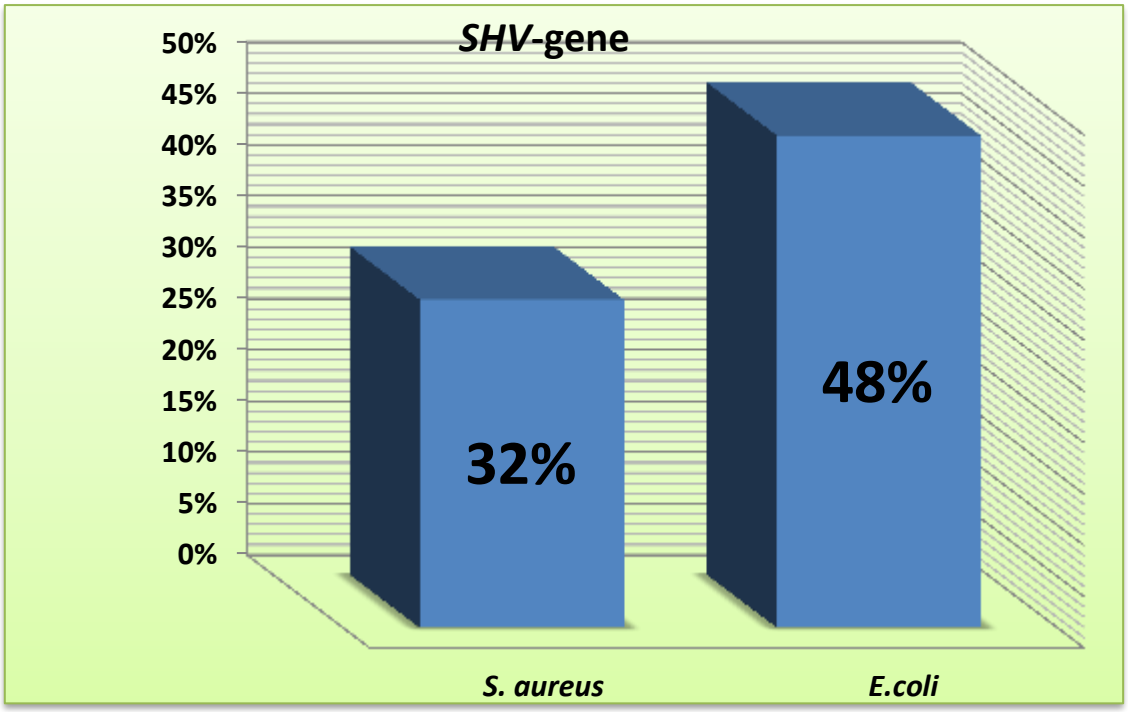


Figure 3- show SHV gene in S. aureus and E. coli.

Conclusion

Genes of antibiotic resistant can be developed by transmission through horizontal way from transient bacteria or by re-arrangement of genetic material, which transient organisms are a possible causes of antibiotic resistance bacteria. The transferred genes may have the same or different sequences if mutated.

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