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Detection of Extended Spectrum β-Lactamase (ESBL)amongGram–negative bacteria isolates from workers in a restaurant in Wasit province, Iraq.

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Abstract

Aims: The aim of this study was to identify ESBL-producing bacteria among the gram negative *E. coli*, *Pseudomonas*, *Salmonella* and *Proteus* isolated from restaurant workers.

Methods: A set of six multiplex PCR assays was performed following the use of predesigned specific group primers and optimised thermal cycle conditions. The primersused were: TEM, SHV, OXA-1-like, DHA, GES, PER-1 and PER-2, VIM, KPC, IMP and VEB. Strains were analysed by PCR and tested for antibiotic resistance.

Results:All *Salmonella*(n=3), *Proteus* (n=6), and *Pseudomonas*(n=15) stains were negative for ESBL genes. The rate of ESBL gene in*E. coli*isolates(n=15) varied; SHV(60%), OXA (46%), CTM-X(60%), and PER1(46%). All other genes werefound low in all investigated bacteria: TEM (26%), VIM (6.6%), OXA-48 like (6.6%), and LAT (6.6%).*E. coli* isolates were highly resistant to CTX, CRO, CAZ, FEB, ATM, SXT and AMC. *Proteus* strains were highly resistant to CRO and TE. *Pseudomonas* isolates were highly resistant to CTX, CRO, CAZ, SXT and AMC. Majority of *Salmonella* strains were sensitive to all tested B-lactam antibiotics.

Conclusion: The study revealed the presence of ESBL genes in *E. coli* strains isolated from restaurants workers in Wasit and high resistance rate of certain commonly antibiotics. Further studies that involve bigger sample size are required to deeply investigate the molecular characteristics of ESBL producing bacteria. Sequencing and further analysis of amplicons are also essentialto obtain clear identification of ESBL producer and to determine antibiotics resistance.

Keywords: Extended Spectrum β-Lactamase , PCR , Antibiotic sensitivity test, Gram negative bacterium .

INTRODUCTION

B-lactamase production by Gram negative bacteria is a common mechanism for antibiotic resistance.Thus,it has become acritical issue in medical practice. The increasing incidence of bacteria releasing extended-spectrum β-lactamases (ESBLs) has not only been reported in hospitalized and healthy individuals but also in restaurants. It has contributed to increased mortality and morbidity in healthcare and public expenditures. ESBLs are plasmid mediated thus they are often subjected to continuous mutations. ESBLs are predominantly observed in *Escherichia coli*, *Klebsiellaspp.*, *Pseudomonas spp.*, *Acinetobacter baumannii* and othergenera of the *Enterobacteriacea*[1].

A number of studies reported that there is an increase in antibiotic resistance in ESBL strains toampicillin, trimethoprim/sulfamethoxazole and ciprofloxacin in clinical[2–4].Alsoit was reportedthat the presence of TEM and SHV are prevalent in community isolated*Enterobacteriaceae*, *Klebsiella*and*E. coli*[5, 6].Less prevalent ESBLs were also reported such as GES-type, PER type and VEB type β-lactamase.Also, some GES variants have been found carbapenems resistant[7].Moreover, some strains have been found predominantly produced a plasmid mediated AmpC β-lactamase which belongs to the CMY-2 group. Isolates' resistance to carbapenems were found to produce KPC carbapenemases, and also isolates from *Enterobacteriaceae* were found to be β-lactamase OXA-48producers[8].

In the last decade, it was reported that *Salmonella* genomic island 1 (SGI1) isolated from human and cattle is multidrug resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline. Later, five antibiotic-resistance genes were detected in this strain including aadA2, sul1, floR (cmlA-like), tet(G) and blaP1 (also named blaPSE-1 or blaCARB-2) that confer resistance to streptomycin and spectinomycin, sulphonamides, chloramphenicol and florfenicol, tetracyclines, and β-lactam antibioticsrespectively[9].

Upregulation of β-lactamase production genes limit the therapeutic potential of antibiotics and thus a sensitivity test is still important in identifying the effective treatment against ESBL pathogens. Because antibiotic resistance originated from the presence of more than one β-lactamase, and due to the high diversity of these enzymes, multiplex PCR assays with optimised thermal cycling conditions and designed group of primers are suggested forrapid detection of β-lactamases genes, allowing direct sequencing from amplicons. The aim of this study is to identify clinical isolates *of E. coli, Salmonella, Proteus and pseudomonas* from restaurant workers to determine which genes are involved in ESBLs and to identify the susceptibility patterns to a number of antibiotics in order to characterise genes involved in antibiotics resistance.

MATERIALS AND METHODS

Bacterial isolates

Thirty nine isolates were obtained from restaurant workers in Wasit, Iraq. Biochemical identification for species was performed by cultural characteristics and standard biochemical procedures confirmed by the API 20E (bioMerieux SA, Marcy IEtoile, France).Identified pathogens were *E. coli* (n=15), *Salmonella* (n=3), *Proteus* (n=6) and *Pseudomonas* (n=15).

Antibiotic susceptibility testing

Bacterial isolates were grown on nutrient agar for 24 hrs at 37°C. For sensitivity test,isolates were suspended with a

sterile PBS and compared with 0.5 McFarland turbidity standards. The antibiotic susceptibility test was performed usingthe disc diffusion method on Muller Hinton agar(Oxoid). Bacteria were cultured with antibiotics discs for 24 hrs at 37°C. Following incubation, the diameters (mm)of each antibiotic inhibition was measured in (mm). The following antibiotic discs used were imipenem (IPM;10µg), meropenem(MEM;), cefotaxime (CTX;30µg) ,cefotetan(CN;15µg), amikacin (AK;30µg), tetracycline (TE), ceftazidime(CAZ;), cefepime (FEP;), aztreonam(ATM;), ciprofloxacin(CIP;), amoxicillin /clavulanic acid (AMC) (20µg/10µg), trimethoprim/ sulphamethoxazole (SXT;1.25µg/23.75µg), cefoxitin (FOX;), all were obtained from BIOANALYSE

Table 1: Detection of ESBL genes using a seyt of 6multiplex PCR assays.

Type of bacteria	**No of multiplex**																				
E.coli	**Multiplex 1 (size of band bp)**				**Multiplex 2 (bp)**				**Multiplex 3 (bp)**						**Multiplex 4 (bp)**		**Multiplex 5 (bp)**			**Multiplex 6 (bp)**	
	800	**713**	**564**	**688**	**404**	**561**	**326**	**346**	**162**	**895**	**997**	**538**	**683**	**399**	**520**	**648**	**399**	**281**	**139**	**390**	**538**
1	+	–	+	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–
2	–	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–
3	–	+	+	+	–	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–
4	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
5	–	+	+	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–
6	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
7	+	+	+	+	–	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–
8	–	–	+	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–
9	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–
10	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–
12	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Salmonella	**Multiplex 1**				**Multiplex 2**				**Multiplex 3**						**Multiplex 4**		**Multiplex 5**			**Multiplex 6**	
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Proteus	**Multiplex 1**				**Multiplex 2**				**Multiplex 3**						**Multiplex 4**		**Multiplex 5**			**Multiplex 6**	
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
6	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas	**Multiplex 1**				**Multiplex 2**				**Multiplex 3**						**Multiplex 4**		**Multiplex 5**			**Multiplex 6**	
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Detection of β-lactamase genes

β-lactamase geneswere detected by a multiplex PCR technique. DNA was prepared rapidly from a single bacteria colony in a volume of 100µl of sterile distal water heated at 95°C for 10min). A cell suspension was centrifuged at 7000rpm for 5min. For each PCR multiplex reaction, the total DNA used was 2µl for a final volume reaction of 50µl composed of PCR buffer (1X), MgCl2(1.5mM), dNTP (200mM), a variable concentration of specific-group primers (Table 1) and 1U of Taq polymerase(Bioneer ,Korea).The thermal cycles conditions applied for all β-lactamase genes were as fellows; initial denaturation at 94°C for 10min, followed by 30 cycles of 94°C for 40 sec , 60°C for 40 sec and 72 °C for 1min and a final extension step at 72°C for 7min.

The presence of β-lactamase genes was detected by specific primers (Table 1). Amplicons were loaded on 2% agarose gel containing ethidium bromide and visualised after running 100V for 1hr. A DNA ladder of 100bp was the marker size used for all experiments (Biobasic, Canada and Promega USA)respectively.

Sequencing analysis of multiplex PCR products

The amplified β-lactamase genes in all PCR assays were detected by a DNA sequence analyser. PCR amplicons were purified using the EXOsap purification kit (Clermont *et. al* 2013) and bidirectional sequencing. Each sequence was individually compared with well-known β- lactamase gene sequences using the BLAST.

Table 2: Antibiotic susceptibility pattern of Gram negative bacteria including E coli, Salmonella, Proteus and Pseudomonas

Bacteria	**Antibiotics**													
E. coli	**IPM**	**MEM**	**CTX**	**CN**	**AK**	**CRO**	**TE**	**CAZ**	**FEP**	**ATM**	**CIP**	**SXT**	**AMC**	**FOX**
1	S	S	R	S	S	R	S	R	R	R	S	R	R	S
2	S	S	R	S	S	R	R	R	R	I	R	R	R	S
3	S	S	R	I	S	R	R	R	R	R	R	R	R	R
4	S	S	R	S	S	R	I	R	R	R	S	R	R	S
5	S	S	R	S	S	R	I	R	R	R	S	R	R	S
6	S	S	S	R	S	R	S	S	S	S	S	S	R	S
7	S	S	R	R	S	R	I	R	R	R	S	R	R	S
8	S	S	R	S	S	R	I	R	R	R	R	S	R	R
9	S	S	R	R	S	R	R	R	S	R	S	R	R	R
10	S	S	R	R	S	R	R	R	R	I	I	R	R	R
11	S	S	I	I	R	I	R	I	R	S	S	S	R	R
12	S	S	R	S	S	I	S	R	R	R	S	R	R	R
13	S	S	S	I	I	I	I	I	S	S	S	S	I	S
14	S	S	S	S	S	S	S	I	S	S	S	S	R	I
15	S	S	S	I	I	S	S	S	S	S	S	S	I	S
Proteus	**IPM**	**MEM**	**CTX**	**CN**	**AK**	**CRO**	**TE**	**CAZ**	**FEB**	**ATM**	**CIP**	**SXT**	**AMC**	**FOX**
1	S	S	R	R	R	R	R	R	R	S	I	R	S	S
2	S	S	R	S	S	R	R	R	R	R	I	R	S	S
3	I	S	R	I	S	R	R	R	R	S	S	S	R	S
4	S	S	S	S	S	R	R	R	R	S	S	I	S	S
5	I	S	S	I	S	R	R	R	S	S	R	S	R	S
6	R	S	S	R	I	S	R	I	S	S	R	R	R	R
Pseudomonas	**IPM**	**MEM**	**CTX**	**CN**	**AK**	**CRO**	**TE**	**CAZ**	**FEP**	**ATM**	**CIP**	**SXT**	**AMC**	**FOX**
1	S	S	R	R	I	R	I	R	I	I	I	S	S	R
2	R	R	R	S	I	R	S	S	R	S	I	R	R	R
3	S	S	R	S	I	S	R	S	S	S	R	S	R	S
4	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	R	S	S	R	S	R	R	R	S	S	I	S
6	S	S	R	S	S	R	S	R	S	R	S	R	R	R
7	S	S	S	S	S	S	S	S	S	S	S	S	R	S
8	S	S	I	S	S	S	S	S	S	R	S	S	I	S
9	S	S	S	S	S	S	S	R	S	R	S	I	S	I
10	S	S	R	I	I	R	S	R	R	R	R	R	R	I
11	S	S	R	S	S	R	S	I	S	I	S	S	S	S
12	S	S	S	R	S	S	R	R	R	R	S	R	R	R
13	S	S	S	S	S	S	R	R	R	R	I	S	S	S
14	I	S	R	S	S	R	R	R	I	S	S	R	R	R
15	I	S	R	I	I	R	R	I	R	S	S	R	R	R
Salmonella	**IPM**	**MEM**	**CTX**	**CN**	**AK**	**CRO**	**TE**	**CAZ**	**FEP**	**ATM**	**CIP**	**SXT**	**AMC**	**FOX**
1	S	S	S	S	S	S	S	S	S	S	S	R	S	S
2	S	S	S	S	R	S	S	S	S	S	S	S	S	S
3	S	S	R	S	S	R	S	R	R	R	S	S	S	S

R: resistant, I: intermediate and S: sensitive

RESULTS

In order to identify ESBL genes in gram negative bacteria, multiplex PCR assays were performed on (n=15) strains of*E. coli*, (n= 6) strains of *Proteus*, (n=3) strains of *Salmonella* and (n=15) strains of *Pseudomonas*. ESBL genes were detected in (n=14) strains of*E. coli* and (n=3) strains of *Proteus*. β-lactamase genes were not observed among *Salmonella* and *pseudomonas* strains.

TEM including TEM-1 and TEM-2, SHV-1 and OXA-1(figure 1) OXA-48 (figure 5) and OXA-30 and CTX variants (figure2) were mostly detected in *E. coli* strains (figure 1 and Table 1). TEM was detected in 4 isolates, SHV was observed in9 isolates and 7 isolates were found OXA-expressing (Table 1).

However, the expression of these genes was not observed in *Salmonella* and *Pseudomonas* and only one strain of *Proteus* was found TEM and OXA-expressing. Minor ESBL-producing isolates were VIM, OXA-like-48and GES in*E. coli* (Table 1 and figure5). However, none of them were detected in *Salmonella* and only one strain of *Salmonella* was found OXA-48 like -expressing and 2 strains of *Pseudomonas* were found GES-expressing.

Some of*E. coli* isolates were found DHA-expressing (6 strains)(Figure 3) and no amplification of this gene was observed in *Salmonella, Proteus*and*Pseudomonas* strains.

No amplification of KPC, VIM and IMP was observed except in one strain of *Pseudomonas* and one strain in *E. coli*.

CTX-M variants including group 1, group 2, group 9, CTX-M group 8/25ACC-1 and ACC-2 FOX-1 to FOX-5, MOX variants, CMY variants and VEB variants were not detected in all of bacterial isolates. Similarly, GES–1 to GES-9 and GES-11 and VEB-1 to VEB-6 were not expressed in all of bacterial isolates. All results are shown in Table 1.

The findings of susceptibility tests obtained 39 isolates of*E. coli, Proteus, Salmonella* and *Pseudomonas.* The majority of *E. coli* isolates were resistant to CTX, CRO, CAZ, ATM, SXT, FEB and AMC (Table 2). Antibiotics of IPM and MEM were shown effective at inhibiting the isolates of *E. coli, Proteus* and *Pseudomonas*. All isolates from these bacteria have been found sensitive to these antibiotics except one strain from each of *Proteus* and *Pseudomonas* has been show resistant (Table 2).

Similar to*E. coli, Pseudomonas* was also found resistance to CTX, CRO, CAZ, ATM, SXT and AMC. Moreover, no resistance towards IPM, MEM and AK was found in strains of *E. coli, Salmonella* and *Pseudomonas* except one strain of each of *Salmonella, Proteus* and *Pseudomonas* (Table 2).All *Proteus* strains (n=6) were found resistance to TE and 5 isolates are resistant to CAZ and CRO. The majority of *Proteus* isolates were found resistant to FEB antibiotic. The effectiveness of CTX, AMC and SXT antibiotics were resisted by *Proteus*, only 3 strains of *Proteus* isolates were resistant to these antibiotics(Table 2).

Six strains of*E. coli* and (n= 6) stains of *Pseudomonas* were found resistance to FOX. However, strains of *Salmonella* and *Proteus* were found sensitive to FOX antibiotic. Some strains of *E. coli* exhibited intermediate response toward CRO (n=3) and TE (n=4)(Table 2).

DISCUSSION

PCR is a technique used for rapid detection of different genes of ESBL. In the current study, some of the Gram negative bacteria were found to express ESBL genes.*E. coli* and *Salmonella* and very few strains of *Proteus* expressed ESBL genes while no strain in *Pseudomonas* was found positive to ESBL genes. SHV and OXA-1-like were found commonly expressed by*E. coli* strains isolated from restaurant workers in Wasit, Iraq, the rates of expression of these genes were 60% and 46% respectively. These findings are similar to previous studies conducted in Germany, Russia, Poland, Turkey and other countries that demonstrated *E. coli* is expressing to these genes[10–12]. However, few strains of *E. coli* were found positive for TEM expression (26%). Nevertheless, previous study reported that TEM was most common than SHV in *Klebsiella* [13]. The former was found prevalent along with OXA-1-like and SHV in *E. coli* isolated from foot ulcers of diabetic patients[12].

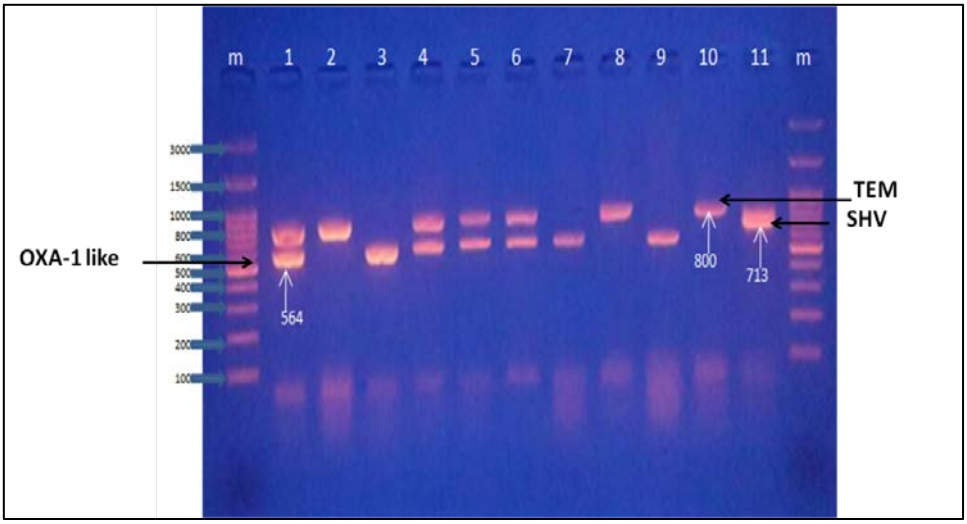

Figure 1: OXA-1, TEM and SHV in*E. coli*after PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lane 2: OXA-1-like (564bp) ,lane 10: TEM(800bp), lane 11; SHV (713bp).

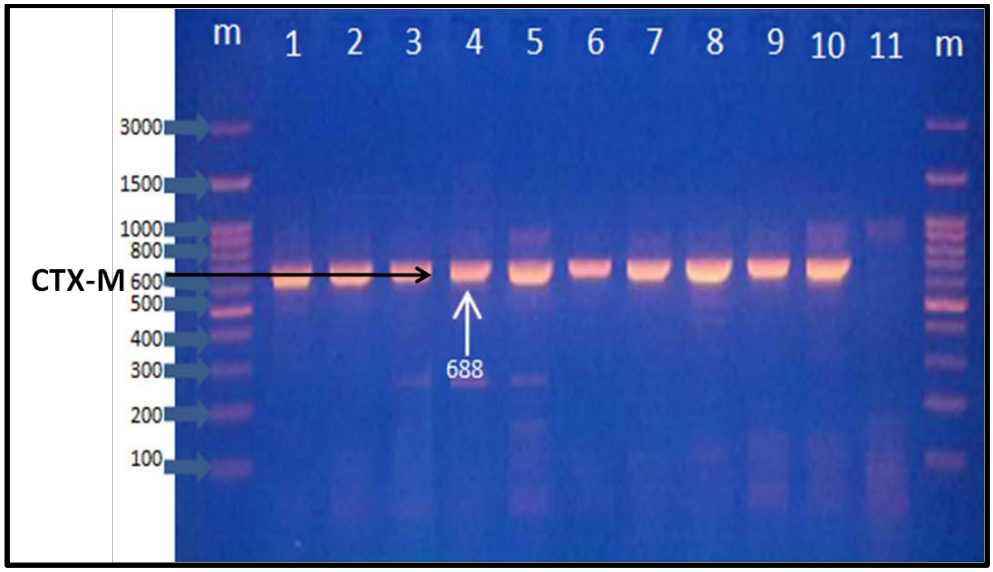

Figure 2:CTX-M in*E. coli*after PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lane1-10: CTX-M(688bp) ,lane 11: negative control.

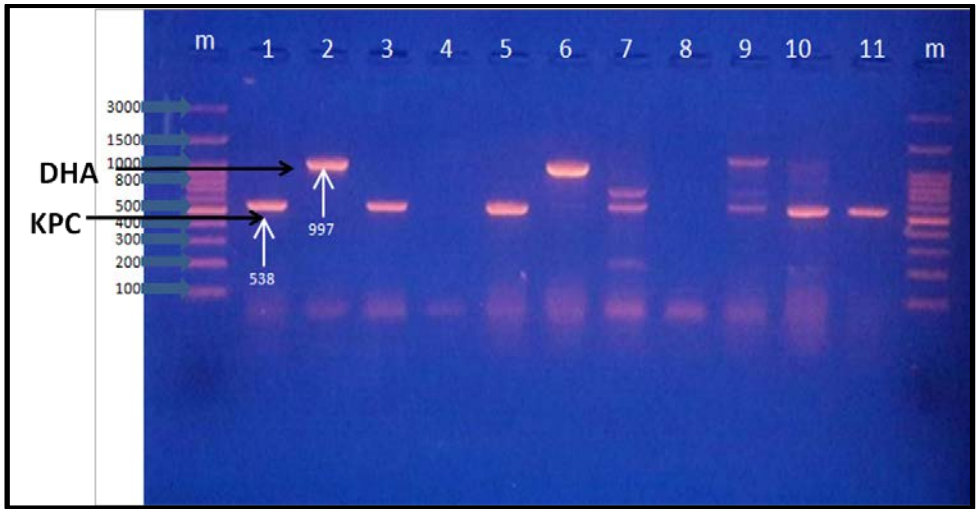

Figure 3: DHA and KPC in*E. coli*after PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lane2 and 6 (997bp, lane 1,3,5,10 and 11 : KPC (538bp). Control : [T1]

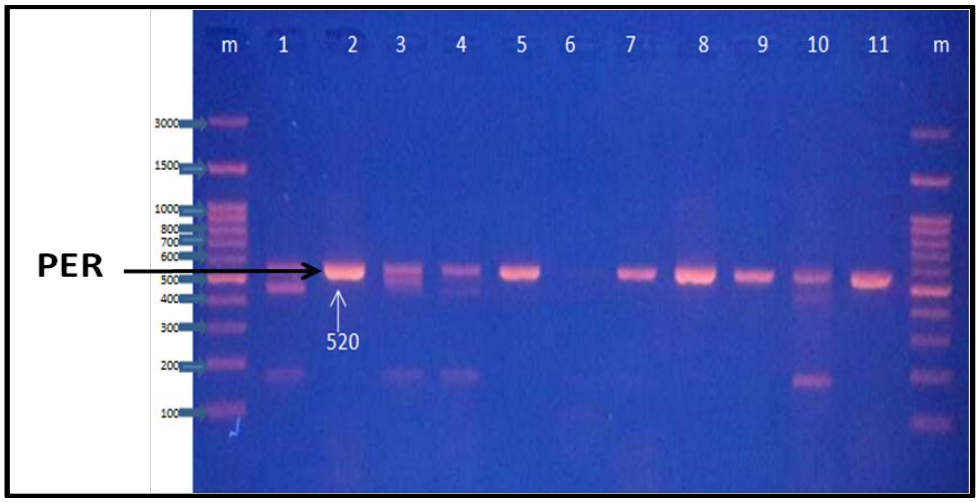

Figure 4: PERin*E. coli*after PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lanes1-5 and 7-11: PER (520bp). Lane 6: negative control.

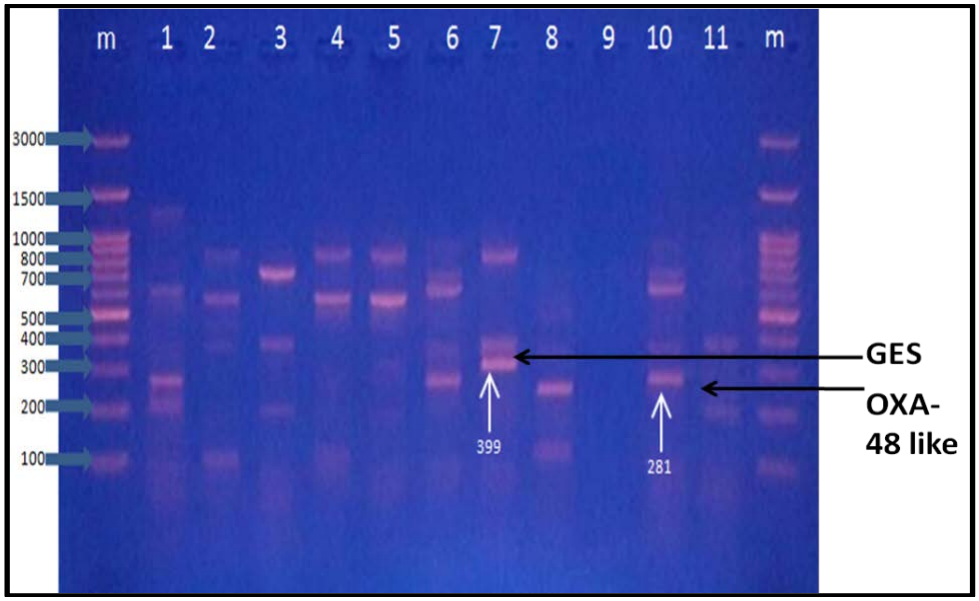

Figure 5: GES and OXA-48like in*E. coli*ofter PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lanes1-5 and 7-11: PER (520bp). Lane 6: negative control.

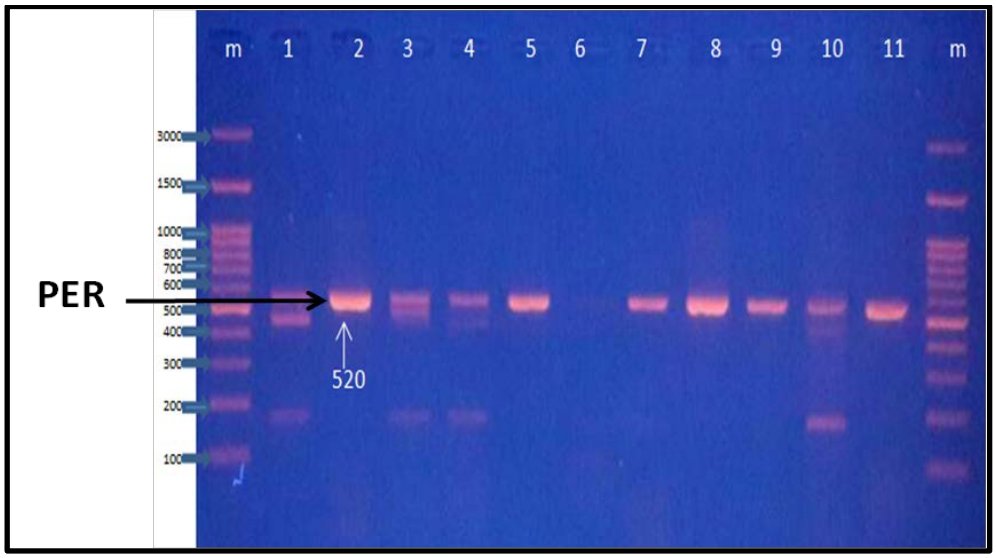

Figure 6: PER in*E. coli*ofter PCR on 1%Agarose gel electrophoresis. Lane: M, 100-bp DNA ladder; lanes1-5 and 7-11:PER (520bp)andLane 6: negative control.

In the present study, multiplex PCR detected 26% of*E. coli* isolates and 50% of *Proteus* species expressed CTX-M. The prevalence rate of ESBL expression is lower than of CTX-M gene detected in urinary *E. coli* (71.4%). The expression of DHA was investigated in*E. coli*. The findings showed that only (n=2) strains were positive. This result was not expected since this gene has been commonly observed in Enterobacteriaceae. DHA is belonged to CMY-2 group. The latter is consistently has not been found negatively expressed but not only in*E. coli* but also in *Pseudomonas*, *Proteus* and *Salmonella*.

ESBL-producing bacteria have become a serious public health concern due to their resistance to β-lactam antibiotics. ESBL-producers are globally considered the cause of outbreaks in Canada, France, and the United Kingdom[14]. Therefore, a number of antibiotics has been investigated by a sensitivity tests for *E. coli*, *Salmonella*, *Proteus* and *Pseudomonas*.

The sensitivity test for*E. coli* isolates in this study showed high sensitivity to IPM and MEM. This finding is consistent with previous studies that showed *E. coli* strains isolated from clinical samples being sensitive to IPM and MEM. This could be due to stability and high effectiveness of carbapenem against β-lactamase producing bacteria. Also, the results showed that *E. coli* isolates had high resistance rate to FEP(66.6%) and ATM(53.3%). High prevalence rate of SHV and OXA-1-like might be contributed to resistance rate of*E. coli* to CTX, CAZ and FEP antibiotics. It was found that TEM carrier strains of *E. coli* and SHV positive strains were able to produce β-lactamase. These genes are associated with CTX resistance. In the present study, *E. coli* strains were SHV and OXA -1-like positive and these isolates have been found resistance

to SXT antibiotic (60%). It is known that genes encoding ESBLs confer resistance to aminoglycosides, tetracyclines and chloramphenicol. Twenty-six percent of*E. coli* strains have been found resistant to tetracycline. This could reveal that*E. coli* strains harbouring ESBL genes of SHV, OXA-1-like, and PER-1 and PER-3, are TE and AMC resistant.

Our results showed that 60% of ceftazidime resistant *E. coli* has SHV positive(60%).This results are in agreement with a recent study that found there is strong association between TEM and HSV detection and ceftazidime resistance in *Klebsiella* pneumonia [15].

Salmonella had a negligible resistance to antibiotics used in this study. However, the results are not concrete be cause only 3 strains were tested. Nevertheless, all tested *Salmonella* strains were negative ESBLs genes.

The results of sensitivity tests showed that TEM positive *Proteus* strains have been associated with high resistance rate to CRO, TE and CAZ.

Unexpectedly, there was some strains showed resistance response to CTX, CRO, FEP, ATM, SXT, AMC and FOX despite of no detection of ESBLs genes. This could suggest that there are other genes that might contribute to production of β-lactamase.

Strains of*E. coli* showed high resistance rate to AMC combination (86%). Interestingly, this finding is comparable to a study that reported resistance rates of 97.5% in *E. coli* isolated from patients' samples[16]. Similarly, the resistance rate was recorded at 87.5% in *E. coli* strains isolated from biological samples[17][CM2].

Several studies reported that*E. coli* strains are resistant to MEM[18, 19]. These findings are not comparable to the results of this study showing that all of*E. coli* and *Salmonella* strains are sensitive to MEM and IPM[12]. Our results clearly exclude the presence of carbapenemase that is responsible for MEM resistance.

Two strains of *Proteus* showed intermediate response to IPM and one strain was resistant. Similarly, *Pseudomonas* has one strain resistant and two strains intermediate to IPM. The reduced susceptibility could be due to a reduce permeability to IPM. These observations were also found in *Klebsiella* pneumoniae isolated from hospitalized patients[20].

The results of studies investigating ESBL genes in*E. coli* and their effects on antibiotics resistance are inconsistent and showed remarkable variations[21–25]. The variation of results depended on geographical locations. For example, the results obtained are varied among countries (e.g. India, Iceland, Estonia, Romania, Iran[24], 25.2% Turkey[26]. It was reported 54.5% of*E. coli* isolates as ESBL producers in a hospital in India [21]. Detection of ESBL-producing strains is critically important as they confer the spread of antibiotic resistance among different bacteria. There are several factors contributing to the development of resistance patterns, including co-selection of multi-drug resistance phenotypes, moving the genetic elements among species, virulence factors, clonal spread of virulent strains, and transferable of plasmids harbouring ESBL genes - bearing plasmids that may facilitate the spread of ESBL and other resistances[27].

CONCLUSION

The degree of resistant of*E. coli*, *Salmonella*, *Pseudomonas* and *Proteus* varies to different β-lactam antibiotics. Drug resistance was found with positive expression of SHV, CTX-M, OXA and PER-1 and PER-2 genes. Negative ESBL genes were also found resistant to the tested antibiotics.

Molecular methods (PCR multiplex assay) are not enough to efficiently differentiate ESBL-producing bacteria, since species that have shown negative ESBL genes have been found resistance to the antibiotic tested in the present study. ESBL detection using molecular techniques (e.g. PCR and DNA sequencing), phonotypic assays and biochemical assaysare critically needed to detect ESBL and non-ESBL producing bacteria.

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