

RESEARCH ARTICLE

Molecular Detection of Complement Resistance Genes (*traT* and *iss*) in some Enterobacteriaceae Isolates

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Abstract

The current study was aimed to detect the existence of genes encode outer membrane proteins: transfer protein (*traT*) and increased serum survival (*iss*) which associated with resistance to complement bacterial lysis activity in different Enterobacteriaceae species isolates. Enterobacteriaceae isolates included in this study were (5) isolates of *E. coli*, *K. pneumonia*, *Salmonella typhi* and *Shigella dysentery*, (4) isolates of *Proteus vulgaris* and (2) isolates of *Serratia marcescens* which were isolated from different clinical infections. The *traT* and *iss* genes were revealed from whole DNA of 26 isolates of Enterobacteriaceae family, it was found that 21(80.7%) of Enterobacteriaceae isolates gave positive result for *traT* gene at 288bp while only 17(65.3%) of isolates gave positive result for *iss* gene at 258bp. It was observed that the *traT* gene was recognized among 100% of *Salmonella typhi* and *Serratia marcescens* strains and the prevalence of a *traT* gene was showed among 80% of *K. pneumoniae* and *Shigella dysentery* isolates whereas 75% of *Proteus vulgaris* and 60% of *E. coli* isolates contain the *traT* gene. The *iss* was found among 80% of both *E. coli* and *Shigella dysentery* isolates while it was observed in 60% of *K. pneumonia* and *Salmonella typhi* isolates and it was present only in 50% of *Proteus vulgaris* and *Serratia marcescens* isolates, as conclusion the study confirmed presence of *traT* and *iss* which linked to human complement resistance among local Enterobacteriaceae species and high rate of occurrence among extraintestinal isolates.

Keywords: Enterobacteriaceae, PCR, complement resistance, *traT*, and *iss*.

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INTRODUCTION

The Enterobacteriaceae family contains a large number of species that are causes for most common infections like nosocomial infections, urinary tract and wound infections, pneumonia, meningitis and septicemia (Ruiz *et al.*, 2002). The pathogenicity of this group is due to several factors include adhesins, hemolysin secretion, serum resistance and biofilm creation . Also, it is probable that the genotypes and phylogenetic origin of Enterobacteriaceae varies according to geographical areas (Johnson *et al.*, 2002 and Mart'inez *et al.*, 2006). Outer membrane proteins (OMPs) are a group of proteins exist in the outer membrane of Gram-negative bacteria. These proteins preserve the bacteria in a aggressive environment and also assistance in a number of activities including moving of solute through the impassable outer membrane and signal transduction .These proteins are also complicated in connection and invasion of the bacteria (Koebnik *et al.*, 2000). OMPs have different functions in the activation of the immune system of the host, such as resistance against antimicrobial peptides and activation of dendritic cells (Jeannin *et al.*,2000). Resistance to the bactericidal action of complement is a distinctive of all gram-negative bacteria that cause septicemia (Joiner, 1988). OMPs of some Gram-negative strains are possibly related with the sensitivity to the bactericidal action of complement (Futoma-Koloch *et al.*, 2006). Previously the role of OMPs in resistance to bactericidal activity of complement was confirmed in *S. typhimurium* and *Y enterocolitica*, *K. pneumoniae* *P. mirabilis* (Heffernan *et al.*,1994, Alberti *et al.*,1996 and Kaca *et al.*,2009). The bactericidal activity of complement is acquired by the transfer protein called *traT* which was showed in other studies in *E. coli*, *Salmonella*, *Shigella* and *Klebsiella* strains (Montenegro *et al.*, 1985, Fernandez-Beros *et al.*, 1990 and Wu *et al.*, 2007).

The increased serum survival protein (*iss*) has an action in defense to serum complement (Nolan *et al.*,2003). The *iss* has been known for its activity in virulence of extraintestinal pathogenic *Escherichia coli* strains (ExPEC) (Hassan, 2011). The *iss* gene is recognized as one of the most common virulence genes in extraintestinal pathogenic strains in poultry (Badouei *et al.*, 2015). The occurrence of immune resistance among

Enterobacteriaceae family was a cause to study the prevalence of genes associated with serum resistance among Enterobacteriaceae isolates in Babylon province as an indicator for appearance of resistance of Enterobacteriaceae infections .

MATERIALS AND METHODS

Isolation and Identification

Twenty-six clinical isolate of *Enterobacteriaceae* were collected from different clinical sources (urine , blood, stool and sputum) from patients in Al-Hilla teaching hospital /Babylon province during the period from November 2017 to March 2018. Specimens were cultured on selective media and recognized by biochemical tests as mentioned by Forbes *et al.*, 2007. Identification was confirmed by the automated method Vitek-2 system (BioMérieux, France).

Genetic detection of Virulence genes

The conventional PCR technique was used for recognition of virulence genes include (*traT* and *iss*).

Extraction of Bacterial DNA

The whole bacterial DNA was extracted from bacterial isolates by using Genomic DNA Mini Bacteria Kit that provided by the company (Geneaid, UK). The DNA solution was stored at -20°C till used in PCR.

PCR Amplifications

Recognition of virulence genes in *E. coli* ,*Klebsiella pneumonia*, *Shigella dysenteriae*, *Serratia marcescens*, *Salmonella typhi*, *Proteus vulgaris* isolates was achieved by amplifying conventional PCR. The PCR primers were provided by (Macrogen company, Korea). The product size and sequences of the PCR primers are showed in Table 1.

RESULTS

The distribution of bacterial genes according to the site of infection

A total of 26 Enterobacteriaceae isolates were isolated from different clinical cases and were diagnosed by using vitek -2 system , the number each isolates were (5) *E.coli*, (5) *K.pneumoniae*, (5) *Salmonella typhi*, (5) *Shigella dysentery*, (2) *Serratia marcescens* and (4) *Proteus vulgaris*. The prevalence of *traT* and *iss* genes was 100% in both of respiratory and intestinal isolates while the percent of *traT* and *iss* genes in blood

Table 1. The size and sequences of primers used in current study

Gene name	Primer Sequence	size (bp)	References
<i>iss</i>	F GGCAATGCTTATTACAGGATGTGC	258	(Hassan, 2011)
	R GAGCAATATACCCGGGCTTCC		
<i>traT</i>	F GGTGTGGTGCGATGAGCACAG	288	(El Fertas-Aissani, <i>et al.</i> , 2013)
	R CACGGTTCAGCCATCCCTGAG		

isolates were 100% and 50%, respectively whereas the prevalence of virulence genes in blood culture isolates which have (68.7%) and the occurrence of *traT* and *iss* genes observed in UTI isolates was 80 % and 60%, respectively (Table 1). The virulence-associated phenotypes including complement resistance, adhesion and invasion, and maintenance within macrophages in *S. typhimurium* have been caused by different members of OMPs group (Pulkkinen and Miller, 1991 and Heffernan *et al.*, 1994). The contribution of OMPs has been showed in bacterial serum-resistance by their inactivation of complement at the C3, C9, and C5b-9 steps (Wooley *et al.*, 1993 and Biedzka-sarek *et al.*, 2005). The *iss* gene was first recognized in a human septicemic *E. coli* strain (Nolan *et al.*, 1992). The *iss* and *traT* genes were present among 95.5% and 86.4% of *E. coli* strains of blood infections and only among 68.8 % and 43.8% of the intestinal infections, respectively (Fernandez-Beros *et al.*, 1990), this result is comparable with the current study. The occurrence of the *iss* gene was higher in tissue isolates that clarify the role of this protein in pathogenesis of a vain pathogenic *E. coli* APEC strains (Nolan *et al.*,

Table 2. The dissemination of genes among bacterial isolates according to the site of infection.

Source of isolates	<i>traT</i> n(%)	<i>iss</i> n(%)
Respiratory infections	2/2 (100%)	2/2 (100%)
Intestinal infections	7/10 (70%)	7/10 (70%)
Blood	4/4 (100%)	2/4 (50%)
Urinary tract infections	8/10 (80%)	6/10 (60%)
Total	21/26 (80.7%)	17/26 (65.3%)

1992 and Johnson *et al.*, 2008a). The *traT* genes were existent in most of the ETEC *E. coli* strains, this is may be due to ColV plasmids that are capable to transmission fully among varied *E. coli* strains, giving these strains some virulence-related characteristics (Fernandez-Beros *et al.*, 1990).

Molecular detection of virulence factors genes among Enterobacteriaceae isolates.

The *traT* and *iss* genes which encoded important virulence factors that are linked with human serum resistance were genetically scanned by using PCR technique for recognition of the existence of these genes in 26 isolates of Enterobacteriaceae family, it was found that 21(80.7%) of Enterobacterial isolates were contained *traT* gene at 288bp while only 17(65.38%) of isolates were contained *iss* gene at 258bp. A group of outer membrane protein (OMP) genes has been documented in the Enterobacteriaceae species including *Escherichia coli*, *Shigella flexneri*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus vulgaris*, *Proteus mirabilis*, and *Providencia stuartii*. One or two peptidoglycan-associated main outer membrane proteins were found in all studied strains (Hofstra *et al.*, 1980). The current data approved with other study which detect the commonness of *traT* in Gram negative bacteria, particularly *E. coli*, *Salmonella*, *Shigella* and *Klebsiella*, it was showed in a higher percentage among the *E. coli* strains isolated from the blood stream of patients with bacteremia /septicemia or from feces of patients with gastrointestinal infections (50-70%) while the incidence of *traT* in strains isolated from cases of urinary tract infections was variable (Montenegro *et al.*, 1985).

The PCR result observed in Figure 1 shows that 100 % of *Salmonella typhi* and *Serratia marcescens* isolates gave positive result for *traT* gene. OMPs has significant role in pathogenesis

of *S. typhimurium* and *Y enterocolitica* (Heffernan et al.,1994).

In the current study *traT* gene was identified in 80% *K. pneumoniae* and *Shigella dysentery* isolates, this result compatible with other study which found that *traT* gene was detected in (78.5%). of *K. pneumoniae* (Wasfi et al., 2016) while Kuo et al., 2017 (Kus et al., 2017)

detected *traT* gene in 11.3% of *K.pneumonia* isolates. 75% and 60% of *E. coli* and *Proteus* isolates were positive for *traT* gene, respectively (Fig. 1), this result parallel with other study by Wooly et al., 1993 who showed that 57.1% of *E.coli* isolates were contained *traT* gene and Wu et al., 2007 showed presence of *traT* genes in most of the *E.coli* O149 isolates. Fernandez-Beros

Table 3. The occurrence of virulence factors genes among bacterial species.

Species	Number	<i>traTn</i> (%)	<i>issn</i> (%)
<i>E.coli</i>	5	3/5(60%)	4/5(80%)
<i>Klebsiella pneumonia</i>	5	4/5(80%)	3/5(60%)
<i>Salmonella typhi</i>	5	5/5(100%)	3/(60%)
<i>Shigella dysentery</i>	5	4/5(80%)	4/5(80%)
<i>Proteus vulgaris</i>	4	3/4(75%)	2/4(50%)
<i>Serratia marcescens</i>	2	2/2(100%)	1/2(50%)
Total	26	21/26(80.7%)	17/26(65.38%)

et al.,1990 showed that the *iss* and *traT* genes were existent among 95.5 % and 86.4 % of the *E.coli* blood isolates and only among 68.8 % and 43.8 % of the intestinal isolates, correspondingly. Previously it was described a ColV plasmid, among *E.coli* isolates from blood and intestinal sources, ColV plasmids confer *Escherichia coli* with some virulence genes including *iss* and *traT* (Aguero et al., 1984 and Skyberg et al.,2008). ColV plasmids are capable to transmission completely among diverse *E. coli* strains, providing those strains with several virulence-linked features (Fernandez-Beros et al., 1990).

The occurrence of *iss* gene product at 258 bp in *E. coli* and *Shigella dysentery* isolates was 80% while it was 60% among *K. pneumonia* ,*Salmonella typhi* isolates and 50% of *Proteus vulgaris* and *Serratia marcescens* isolates as showed in Fig. 2. This result comparable with a study of Badouei et al., 2015 who found that the occurrence of the *iss* gene in a vain *E.coli* strains was 90.3% in the septicemic isolates and 64.3% among cecal isolates and also approved with a study of Vaz et al., 2017 who observed the occurrence of *iss* gene was ranged from 75 to 91% among the ExPEC strains and was ranged from 49 to 57% among the fecal

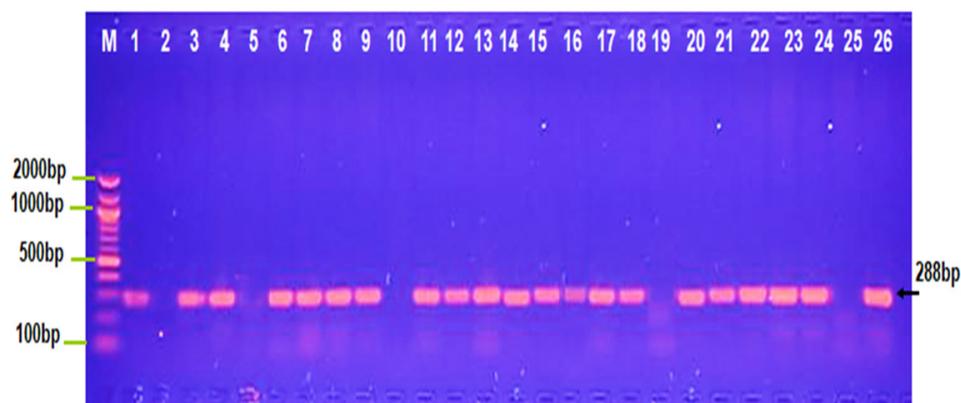


Fig. 1. Agarose gel electrophoresis image that shown the PCR product of a virulence factor gene (*traT*) at (258bp) in different bacterial isolates. Where M: Marker (2000-100bp), lane (1-5) *E. coli*, lane (6-10) *K. pneumonia* , lane (11-15) *Salmonella typhi*, lane (16-20) *Shigella dysentery* , lane (21-22) *Serratia marcescens* and lane (23-26) *Proteus vulgaris*.

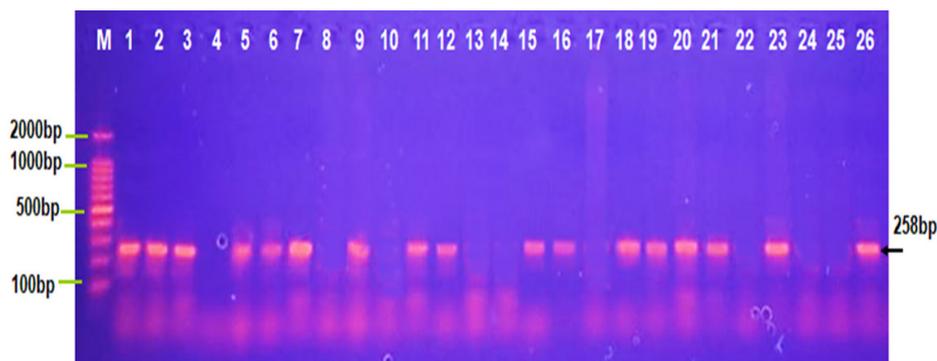


Fig. 2. Agarose gel electrophoresis image that shown the PCR product of a virulence factor gene (*iss*) at (258 bp) in different bacterial isolates. Where M: Marker (2000-100bp), lane (1-5) *E. coli*, lane (6-10) *K. pneumoniae*, lane (11-15) *Salmonella typhi*, lane (16-20) *Shigella dysenteriae*, lane (21-22) *Serratia marcescens* and lane (23-26) *Proteus vulgaris*.

strains, while other study showed that 30% of uropathogenic *E. coli* isolates were positive for *iss* gene and only 8% of *K. pneumoniae* isolates were contained *iss* gene (Hassan, 2015).

The *iss* gene was represented one of the most prevalent virulence genes in extra-intestinal pathogenic strains and three alleles of *iss* were recognized among *E. coli* isolates (Johnson *et al.*, 2008b). Human serum resistance among ExPEC permits persistence in the host's blood stream and can possibly distribute to cause human infections through the food chain is indistinct, it can colonize the intestinal area and prompt virulence gene transmission to human strains. For example, APEC strains and urogenital pathogenic *E. coli* (UPEC) causing human infections were revealed to share large genomic sequences (Johnson *et al.*, 2008a and Kariyawasam *et al.*, 2007).

The present study concluded that high distribution of genes encoded for outer membrane proteins responsible for human complement resistance among Enterobacteriaceae species isolated from different clinical infections and also these genes were detected among extraintestinal infections isolates.

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