

GENETIC DETECTION OF ISS, TRAT, AND OMPK36 GENES IN GRAM-NEGATIVE BACTERIA ISOLATED FROM DIFFERENT INFECTIONS IN NAJAF PROVINCE

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ABSTRACT

Background: The increase serum survival (iss) gene is associated with the synthesis of the O-antigen capsule, the transfer protein (traT) gene is linked to the synthesis of the K1 capsule, while the outer membrane proteint-36 (ompK36) gene is connected to antibiotic resistance. **Objectives:** The current study aimed to detection presence of iss gene, TraT gene, Ompk36 gene in different gram negative isolated. **Materials and Methods:** A total of 78 Gram-negative bacterial isolates were obtained from intestinal and extra-intestinal infections. All isolates were cultured on MacConkey agar and identified using the Vitek-2 system. Conventional PCR was employed for the amplification of the iss, traT, and ompK36 genes. Microsoft Excel was used for the statistical analysis of the data. **Results:** The iss, traT, and ompK36 genes were detected in the total DNA of 78 isolates from the Enterobacteriaceae family, which included 15 isolates each of E. coli, Klebsiella pneumoniae, Salmonella typhi, and Shigella dysenteriae, 12 isolates of Proteus vulgaris, and 6 isolates of Serratia marcescens. The iss gene was present in 51 out of 78 isolates (65%) at 258 bp, the traT gene was detected in 63 out of 78 isolates (80.7%) at 258 bp, and 45 out of 78 isolates (57.6%) tested positive for the ompK36 gene at 305 bp. **Conclusion:** The iss, traT, and ompK36 genes were detected among Enterobacteriaceae species isolated from various clinical infections, with a notably high occurrence in extraintestinal isolates.

KEYWORDS: iss, traT, ompk36, Enterobacteriaceae, Complement resistance proteins.

INTRODUCTION

Gram-negative bacteria are a group of bacteria characterized by a thin peptidoglycan layer and an outer membrane with three layers of lipopolysaccharides in their cell walls [1]. When Gram-negative bacteria stained by using the Gram stain technique, the bacterial cell wall retain the safranin dye, which gives their cell walls a pink or red color [2]. One of the Gram-negative bacterial families is enterobacteriaceae, which is regarded as one of the causes of many infections such as meningitis, pneumonia, UTIs, and nosocomial infections [3]. Numerous factors, including biofilm synthesis, serum resistance, hemolysin, and adhesins, represent the causes of Enterobacteriaceae pathogenicity. The phylogenetic and genotypic variation of Enterobacteriaceae may vary according to geographic regions [4]. One of the contributions of OMPs proteins is to protect bacteria in extreme conditions. These proteins also have

other functions, such as signal transduction and facilitating the movement of solutes through the otherwise impermeable outer membrane, these proteins are also involved in the adhesion and invasion of bacteria [5]. OMPs are regarded as antigens that trigger the host immune response, playing a role in activating dendritic cells and contributing to resistance against antimicrobial peptides [6]. The presence of outer membrane proteins (OMPs) in *Yersinia enterocolitica*, *Salmonella typhimurium*, *Proteus mirabilis*, and *Klebsiella pneumoniae* contributed to the bactericidal resistance activity of complement proteins [7]. The *Iss* gene very essential for o-antigen capsule that making protection for bacteria from bactericidal effects of complement proteins, where deleting of this gene leading to increasing of binding the complement proteins that contributed in membrane attack complex to bacterial surface [8]. The *Trat* gene associated with K1 capsule formation and responsible for inhibition of activity of classical pathway for complement system, consequently acquired the ability of evading from phagocytosis [9].

MATERIALS AND METHODS

Bacterial Isolation

The current study was conducted from January to September 2024 and included 78 Enterobacteriaceae isolates that were collected from different infections, including stool, sputum, urine, and blood. All samples were collected from Al-Furat Teaching Hospital.

Bacterial Identification

All bacterial isolates were identified through culture on MacConkey agar and biochemical tests. The identification of the samples was confirmed using the Vitek-2 system.

Bacterial DNA Extraction

The extraction of total bacterial DNA was performed according to the Presto™ Mini gDNA Bacteria Kit (catalog numbers GBB004, GBB100, GBB300). DNA purity was assessed using NanoDrop spectrophotometers at a wavelength of 260/280 nm.

Genetic detection of *Iss*, *Trat*, *Ompk36* virulence factor

The method for the detection of virulence genes (*iss*, *traT*, and *ompK36*) used in the current study was conventional PCR.

PCR Amplifications

The virulence genes in this study were detected in *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, and *Shigella dysenteriae* through gene amplification using the PCR technique. The primers used in this study were purchased from BIONEER, Korea. The sequences of the primers are described in Table 1.

Table 1 (Primer sequences and sizes of PCR products for the virulence genes.)

Genes Name	Primer Sequence		Size of PCR Product
Iss	F	GGCAATGCTTATTACAGGATGTGC	258
	R	GAGCAATATACCCGGGCTTCC	

Trat	F	GGTGTGGTGGCGATGAGCACAG	288
	R	CACGGTTCAGCCATCCCTGAG	
Ompk36	F	GAAATTTATAACAAAGACGGC	305
	R	GACGTTACGTCTATACTACG	

Statistical Analysis

A statistical analysis was conducted using Microsoft Excel. The results of the current study were analyzed accordingly [10].

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 1742 at 24/11/2022 to get this approval.

RESULTS

The gel electrophoresis results of PCR products from 78 Enterobacteriaceae isolates, including the virulence genes *iss*, *traT*, and *ompK36*, comprised 15 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 15 *Salmonella typhi*, 15 *Shigella dysenteriae*, 6 *Serratia marcescens*, and 12 *Proteus vulgaris*. The *iss* gene was present in 100% of respiratory infections, 70% of intestinal infections, 50% of blood infections, and 60% of urinary tract infections (UTIs). The *traT* gene was detected in 100% of respiratory infections, 70% of intestinal infections, 100% of blood infections, and 80% of UTIs. The *ompK36* gene was found in 50% of respiratory infections, 70% of intestinal infections, 25% of blood infections, and 50% of UTIs. See Table 2

Table 2 (Prevalence of *iss*, *traT*, and *ompK36* genes among bacterial isolates based on the site of infection.)

Source of infection	<i>Iss</i> gene	<i>Trat</i> gene	<i>Ompk36</i> gene
Respiratory infection	6/6 (100%)	6/6 (100%)	2/4 (50%)
Intestinal infection	21/30 (70%)	21/30 (70%)	21/30 (70%)
Blood	6/12 (50%)	12/12 (100%)	3/12 (25%)
UTI	18/30 (60%)	24/30 (80%)	15/30 (50%)
Total	51/78 (65 %)	63/78 (80.7 %)	41/78 (53.8 %)

The prevalence of virulence genes among bacterial isolates showed the presence of the *iss* gene in *Klebsiella pneumoniae* (9/15; 60%), *Escherichia coli* (12/15; 80%), *Salmonella typhi* (9/15; 60%), *Shigella dysenteriae* (12/15; 80%), *Proteus vulgaris* (6/12; 50%), and *Serratia marcescens* (3/6; 50%). The *traT* gene was detected in *Klebsiella pneumoniae* (12/15; 80%), *Escherichia coli* (9/15; 60%), *Salmonella typhi* (15/15; 100%), *Shigella dysenteriae* (12/15; 80%), *Proteus vulgaris* (9/12; 75%), and

Serratia marcescens (6/6; 100%). The *ompK36* gene was found in *Klebsiella pneumoniae* (6/15; 40%), *Escherichia coli* (15/15; 100%), *Salmonella typhi* (6/15; 40%), *Shigella dysenteriae* (9/15; 60%), *Proteus vulgaris* (6/12; 50%), and *Serratia marcescens* (3/6; 50%). See Table 3

Table 3 (Prevalence of *iss*, *traT*, and *ompK36* genes among different bacterial species.)

Source of infection	Number	Iss gene	Trat gene	Ompk36 gene
Klebsiella pneumoniae	15	9/15 (60%)	12/15 (80%)	6/15 (40%)
Escherichia coli	15	12/15 (80%)	9/15 (60%)	15/15 (100%)
Salmonella Typhi	15	9/15 (60%)	15/15 (100%)	6/15 (40%)
Shigella dysentery	15	12/15 (80%)	12/15 (80%)	9/15 (60%)
Proteus Vulgaris	12	6/12 (50%)	9/12 (75 %)	6/12 (50 %)
Serratia marcenes	6	3/6 (50%)	6/6 (100 %)	3/6 (50%)
Total	78	51/78(65 %)	63/78(80.7%)	45/78(57.6%)



Figure 1 (Agarose gel electrophoresis image illustrating the PCR product of the *iss* gene at 258 bp in different bacterial isolates. M: DNA marker (2000-100 bp); lanes 1-5: *Escherichia coli*; lanes 6-10: *Klebsiella pneumoniae*; lanes 11-15: *Salmonella typhi*; lanes 16-20: *Shigella dysenteriae*; lanes 21-22: *Serratia marcescens*; lanes 23-26: *Proteus vulgaris*.)

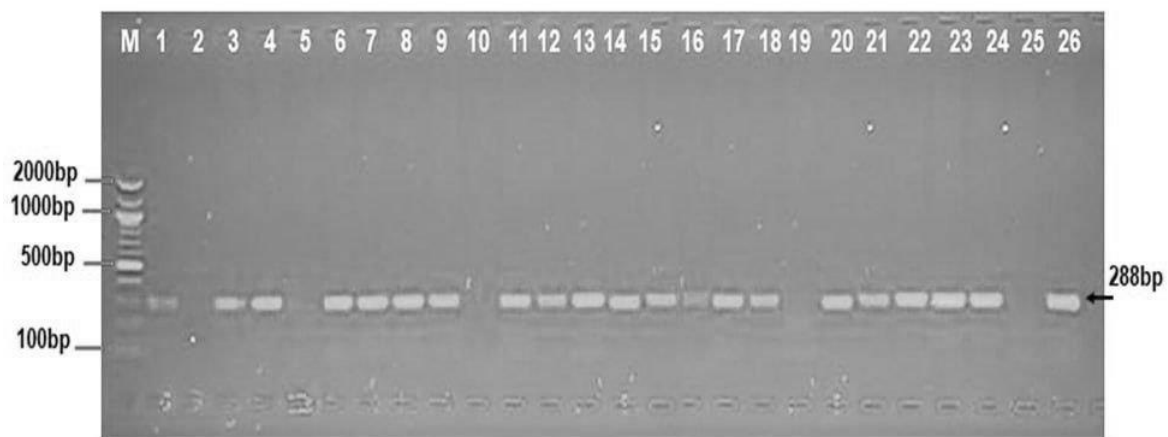


Figure 2 (Agarose gel electrophoresis image illustrating the PCR product of the *traT* gene at 288 bp in different bacterial isolates. M: DNA marker (2000-100 bp); lanes 1-5: *Escherichia coli*; lanes 6-10: *Klebsiella pneumoniae*; lanes 11-15: *Salmonella typhi*; lanes 16-20: *Shigella dysenteriae*; lanes 21-22: *Serratia marcescens*; lanes 23-26: *Proteus vulgaris*.)

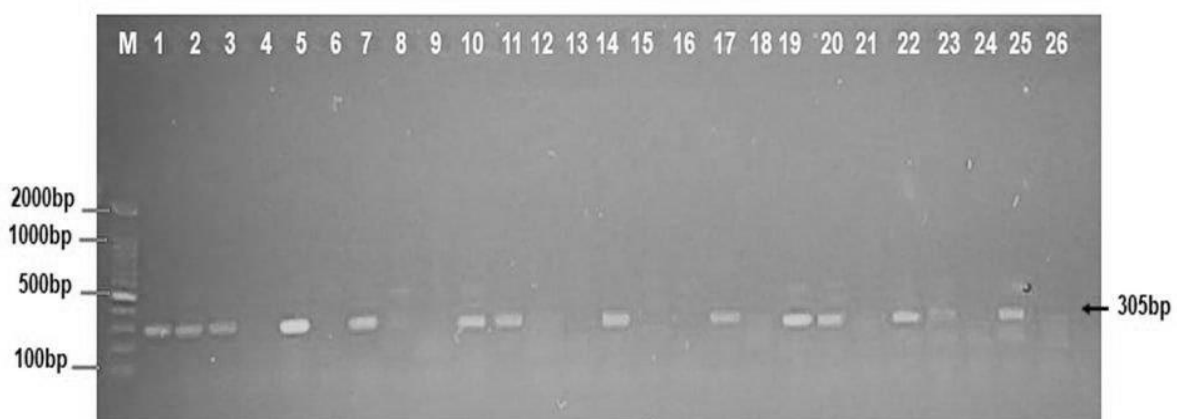


Figure 3 (Agarose gel electrophoresis image illustrating the PCR product of the *ompK36* gene at 305 bp in different bacterial isolates. M: DNA marker (2000-100 bp); lanes 1-5: *Escherichia coli*; lanes 6-10: *Klebsiella pneumoniae*; lanes 11-15: *Salmonella typhi*; lanes 16-20: *Shigella dysenteriae*; lanes 21-22: *Serratia marcescens*; lanes 23-26: *Proteus vulgaris*.)

DISCUSSION

A previous study by Biran et al. (2021) showed that extraintestinal pathogenic *Escherichia coli* (ExPEC) has the ability to evade serum bactericidal effects, making these strains of *E. coli* a significant and emerging clinical problem. ExPEC causes a variety of infections and is often highly resistant to antibiotics [11].

The results of the current study demonstrated the presence of the *iss* gene in 30 out of 78 Enterobacteriaceae isolates, distributed as follows: 100% (6/6) in respiratory infections, 50% (6/12) in blood infections, and 60% (18/30) in urinary tract infections. The *iss* gene was also detected in 70% (21/30) of intestinal infections. These findings agree with the results reported by Biran et al. in their study.

The results of a previous study by Rezaei et al. (2020) demonstrated that the frequency of the traT gene in *E. coli* was 90.6% in fecal isolates and 97.3% in urine isolates. In contrast, a study by Hasan et al. reported that the frequency of the traT gene in *E. coli* isolates from urine samples of patients with cystitis was only 31.7% (20 out of 63) [12,13]. In the current study, the traT gene was detected in 63 out of 78 Enterobacteriaceae isolates, distributed as follows: 100% (6/6) in respiratory infections, 100% (12/12) in blood infections, and 80% (24/30) in urinary tract infections. Additionally, the iss gene was detected in 70% (21/30) of intestinal infections.

The results of the current study indicate that 60% of *Klebsiella pneumoniae* isolates lacked the ompK36 gene. In comparison, a previous study by Nour reported that reduced expression of ompK36 was detected in 52.38% of *K. pneumoniae* isolates that produce carbapenemase [14].

Previous study David et al show the ompk36 gene presence only in *Klebsiella pneumoniae* isolates [15], while the current study demonstrated presence where the prevalence of ompk36 in *Escherichia coli* was 15\15 (100%), *Salmonella Typhi* 6\15 (40%), *Shigella dysentery* 9\15 (60%), *Proteus Vulgaris* 6\16 (50%) and *Serratia marcescens* 3\6 (50%).

CONCLUSION

The iss and traT genes were found to contribute to resistance against the complement killing effect, while the ompK36 gene was associated with antibiotic resistance. These genes were detected at a high prevalence among Enterobacteriaceae species isolated from various clinical infections, with a particularly high occurrence among extraintestinal isolates.

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