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Molecular Analysis of Some Virulence Genes of Escherichia Coli Isolates from Wound and Burn Samples in Kirkuk City

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ABSTRACT

Background: Gram-negative infections caused by non-spore producing E. coli that are facultatively anaerobic have been recognized as serious infections linked to higher mortality and significant costs associated with medical care.

Aim: The goal of this study was to determine whether or not a number of E. coli strains are capable of producing virulence genes by using a technique called real time PCR.

Materials and Methods: 155 people with burns and wounds had their cultures obtained, and susceptibility testing using swabs, tissue biopsies, and needle aspirations were performed. When compared to other techniques of specimen collection, swab cultures yield precise results and are a practicable, widely utilized, non-invasive, and cost-effective method. The two most recommended techniques for obtaining specimens are tissue biopsy and needle aspiration. Swab cultures, on the other hand, are appropriate since they are useful, widely applied, non-invasive, and reasonably priced. Samples from patients were collected in the consultation rooms, including swabs from burns and wounds. The sufferers had these taken from them. Before being identified, smear samples were initially cultivated in the lab on several selective media. Transport media-filled sterile collection containers containing these samples were brought to the lab.

Results: According to the study's findings, E. coli was found in 27.10% of the burns and wounds samples. According to the study's findings, the majority of patients with E. coli infections (71.43 percent) were females, while only 28.57 percent were males. According to the study's findings, 28.57 percent of patients diagnosed with E. coli infections were between the ages of 31 and 40. The investigation also indicated that all of the sputum samples containing E. coli isolates were from patients above the age of 40. Furthermore, 45 percent of urine samples were collected from patients aged 31 to 40, and 42.86 percent of patients had wound infections. When examined using the MTP technique, fifty percent of the E. coli isolates tested positive for being potent biofilm producers, according to the study's findings. The DNA of 12 distinct E. coli bacterial isolates was isolated and purified using a genomic DNA purification kit. The bacteria were cultured on chromogenic agar medium to produce these E. coli isolates. The results were detected using electrophoresis on agarose gel at a concentration of 1.5 percent. The gel was then exposed to ultraviolet light, which revealed the DNA as compact bands. The virulence gene was found in 95.24 percent of the E. coli isolates taken from burn and wound sites, according to the study's findings. The majority of E. coli isolates tested positive for amikacin, imipenem, and gentamycin, according to the study's findings. The majority of E. coli isolates, on the other hand, were resistant to the medications amoxicillin, levofloxacin, cefotaxime, ceftriaxone, pefloxacin, cefepime, and ampicillin.

Keywords- Molecular analysis, Virulence genes, Escherichia coli, Isolates, Wound samples, Burn samples.

I. INTRODUCTION

SSTIs, or skin and soft tissue infections, are among the most frequent types of infections in people of all ages. The vast majority of infections either go away on their own or can be treated with medication. In moderate or severe instances, hospitalization and parenteral therapy may be required [1,2]. Because they produce a favorable environment for bacterial development, burns are more persistent and richer reservoirs of infection than surgical wounds. This is mostly due to the fact that burns cover a larger area of the body and necessitate a lengthier hospital

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stay than surgical wounds [3]. Infection is a major cause of morbidity and mortality in hospitalized burn patients [4,5]. Sepsis is considered to be responsible for more than 75% of burn-related death, particularly in impoverished nations. Furthermore, burns unit overcrowding is a significant source of cross infection, necessitating constant monitoring of bacterial species and drug susceptibilities. This is required because significant changes in these data may be associated with changes in clinical treatment in terms of drug selection for therapy [6]. Pathogenic bacteria have a variety of virulence characteristics that allow them to colonize, invade, and infect their hosts [5]. Pathogenicity islands in E. coli are made up of two or more DNA segments that encode virulence factors like the Universal Stress Protein (USP) gene. This gene is important for bacterial survival during cell growth, adhesion, and motility. There are six varieties of USP, named A and C through G, and each is regulated differently under different stress circumstances. While the precise physiological role of USP is unknown, there is a strong relationship between the USPA gene and bacterial survival during growth arrest. According to research, USP provides a variety of physiological activities that essentially reprogram the cell to defend and escape when exposed to bacterial assault [7,8]. E. coli from individuals with pentatectis, a kind of uropathogenic E. coli, establish a bacterial surface that relies on cell surface molecules and varied structures, resisting elimination even after gentle washing and eventually causing pyelonephritis [9]. Furthermore, these bacterial cells inject chemicals that alter the physiology of the host's immune system[1]. The goal of this study was to look into the ability of several E. coli strains to produce virulence genes using real-time PCR.

II. PATIENTS AND METHODS

Cultures were collected from 155 people with wounds and burns, as well as susceptibility tests using swabs, tissue biopsies, and needle aspirations. Swab cultures are frequently used because they are practical, widely used, non-invasive, and cost effective, and they yield reliable results when compared to other specimen collection procedures. The most common methods for collecting specimens are needle aspiration and tissue biopsy. Swab cultures, on the other hand, are commonly accepted since they are convenient, extensively utilized, non-invasive, and affordable.

Before swabbing, the site must be cleansed with sterile normal saline or sterile water to avoid contamination with skin flora, necrotic tissue, or pus. Because wound infection develops only in viable wound tissue, only viable wound tissue, rather than necrotic tissue or pus, should be swabbed. A viable tissue sample of at least one centimeter squared is required for a C&S swab.

Collection of Samples: a. Aseptically collect wound and burn samples with sterile swabs or other appropriate collection procedures. b. Transfer the collected samples to the laboratory for further analysis in sterile containers. c. Label each sample correctly with unique identifiers for easy tracking and documentation.

2.1 Sample Preparation:

The collected wound and burn samples were transferred into sterile tubes containing adequate transport media in a sterile setting (e.g., sterile phosphate-buffered saline). To ensure proper mixing, the samples were gently vortexed or stirred. Serial dilutions of the samples were optionally done to get the ideal bacterial load for subsequent analysis.

A volume of 100 Micoliter) of the prepared samples was pipetted onto selective agar plates such as MacConkey agar or Eosin Methylene Blue agar for bacterial isolation and culture. To evenly disperse the sample across the agar surface, a sterile spreader or sterile cotton swab was utilized. To allow bacterial growth, the plates were incubated at the right temperature (typically 37°C) for a set period of time (e.g., 24-48 hours). Following incubation, the plates were visually inspected for the existence of bacterial colonies, and representative colonies with various morphologies were chosen for future investigation.

A bacterial solution was generated for DNA extraction by inoculating a single representative colony into an adequate volume of sterile distilled water or buffer. A commercial DNA extraction kit was used, and the manufacturer's instructions were followed. A spectrophotometer was used to quantify the extracted DNA, and agarose gel electrophoresis was used to determine its purity.

Amplification of the Universal Stress Protein (USP) pathogenicity genes of Escherichia coli was performed using Polymerase Chain Reaction (PCR). DNA template, primers specific to the target genes, PCR buffer, dNTPs, and DNA polymerase were used to create PCR reaction mixtures. PCR amplification was carried out under optimal cycle conditions, which included denaturation, annealing, and extension stages. To validate the PCR results, appropriate negative and positive controls were utilized. The PCR results were then tested for amplification of target genes using agarose gel electrophoresis.

To eliminate remaining primers and impurities before sequencing, the PCR products were purified using a DNA purification kit. To get sequence data for the amplified genes, the purified PCR products were submitted for Sanger sequencing or next-generation sequencing procedures.

2.2 Data Analysis:

a. Retrieve the obtained sequence data and execute quality control tests on the sequence. b. Align the sequences, detect polymorphisms, and examine the existence of virulence genes using relevant bioinformatics methods. c. Compare the acquired sequences to reference sequences from public databases to establish relatedness and discover distinct virulence profiles of E. coli isolates.

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Data Analysis and Reporting: a. Examine the molecular analysis results, including the presence or lack of certain virulence genes in the E. coli isolates.

b. Apply the findings to wound and burn infections, taking into account the potential consequences for virulence and antibiotic resistance.

c. Write a complete report summarizing the findings, including details on the methodologies, findings, and conclusions.

The primer sequences are used for amplifying these genes through PCR.

The AccuPrep® Genomic DNA Extraction Kit from Bioneer/Korea was used to extract genomic DNA from all E. coli isolates. The aim was to analyze the presence of the USP toxin gene in these isolates using specific forward (5′-ACATTCACGGCAAGCCTCAG-3′) and reverse (5′-AGCGAGTTCCTGGTGAAAGC-3′) primers.

III. PROCEDURE

3.1 Bacterial Cell Lysis

Transfer 1 mL of bacterial culture or sample into a microcentrifuge tube. Centrifuge the tube at 12,000 rpm for 1 minute to pellet the bacterial cells. Carefully remove the supernatant without disturbing the cell pellet. Add 200 µL of Lysozyme solution to the cell pellet and mix by gentle pipetting. Incubate the mixture at 37°C for 30 minutes to allow lysis of the bacterial cells.

3.2 Genomic DNA Binding

Add 200 µL of Gram+ Buffer to the lysed bacterial cell suspension and mix well by vortexing. Add 20 µL of Proteinase K solution to the mixture and mix gently. Incubate the mixture at 70°C for 10 minutes to digest proteins and inactivate endogenous nucleases.

3.3 DNA Binding to the Column

Place a spin column into a collection tube provided in the kit. Transfer the lysate into the spin column assembly and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through and place the spin column back into the collection tube.

3.4 Washing Steps

Add 500 µL of Wash Buffer I to the spin column and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Add 500 µL of Wash Buffer II to the spin column and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. Add 500 µL of Wash Buffer II to the spin column and centrifuge at 12,000 rpm for an additional 2 minutes to remove residual ethanol. Briefly centrifuge the empty spin column at maximum speed for 1 minute to dry the column membrane.

3.5 Elution of Genomic DNA

Place the spin column into a clean microcentrifuge tube. Add 50-100 µL of TE buffer or water directly onto the center of the spin column membrane. Incubate at room temperature for 1 minute. Centrifuge at 12,000 rpm for 1 minute to elute the purified genomic DNA.

IV. RESULTS

The study showed that *E. coli* isolated from 27.10% of burn and wound samples, Table 1.

Table 1: Prevalence of isolated *E. coli* from burn and wound samples

wound and burn samples	*S. aureus*		Other bacteria		No growth		Total	
	No.	%	No.	%	No.	%	No.	%
Results of culture	42	27.10	75	48.39	38	24.52	155	100

P. value: 0001

The study demonstrated that most of patients infected with *E. coli* were females (71.43%) and 28.57% were males, Table 2.

Table 2: Distribution of studied patients infected with *E. coli* according to gender

Source *E. coli* isolates		Sex		
		Female	Male	Total
Total	No.	30	12	42
	%	71.43	28.57	100

P-value: 0.002

According to the findings of the study, 28.57% of patients diagnosed with E. coli infections were in the age group of 31-40 years old. In addition, the study found that all of the sputum samples that included E. coli isolates were from patients who were at least 40 years old, and that 45% of urine samples came from people in the age group 31-40 years old, as well as 42.86% of patients who had wound infections. Table 3.

Table 3: Distribution of studied patients infected with *E. coli* according to age

Age groups (years)		Total
2-12	No.	3
	%	7.14
13-20	No.	3
	%	7.14
21-30	No.	10
	%	23.81
31-40	No.	12
	%	28.57
41-50	No.	6
	%	14.29
51-60	No.	8
	%	19.05
Total	No.	42
	%	100

P-value: 0.07

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The study showed that 50% of *E. coli* isolates were strong were biofilm producers as done MTP method, Table 4.

Table 4: Biofilm formation of *E. coli* isolates from clinical samples.

Source		Strong		Moderate		Weak		Negative	
		No.	%	No.	%	No.	%	No.	%
Total	42	21	50	13	30.95	5	11.90	3	7.14

Using a genomic DNA purification kit, the DNA of 12 different E. coli bacterial isolates was extracted and purified. These E. coli isolates were produced by culturing the bacteria on chromogenic agar medium. Electrophoresis performed on agarose gel with 1.5% concentration was used to detect the results. The gel was then illuminated with ultraviolet light, which shows the DNA in the form of compact bands.

The table displays the detection of USP virulence genes in E. coli isolates. Out of a total of 42 isolates, 40 (95.24%) tested positive for the USP virulence gene, while 2 (4.76%) tested negative. This indicates a high prevalence of the USP virulence gene in the E. coli isolates under investigation.

Table 5: Detection of USP virulence genes **gene in of *E. coli* isolates**

Source	USP **virulence gene**				Total	
	Positive		Negative			
	No.	No.	No.	%	No.	%
Total	40	95.24	2	4.76	42	100

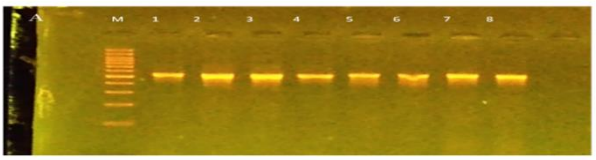

Figure 1: Detection of shiA gene in of *E. coli* isolates

According to the findings of the study, the majority of E. coli isolates exhibited sensitivity to amikacin, imipenem, and gentamycin, whereas the majority of isolates exhibited resistance to amoxicillin, levofloxacin, cefotaxime, ceftriaxone, pefloxacin, cefepime, and ampicillin (Figure 2).

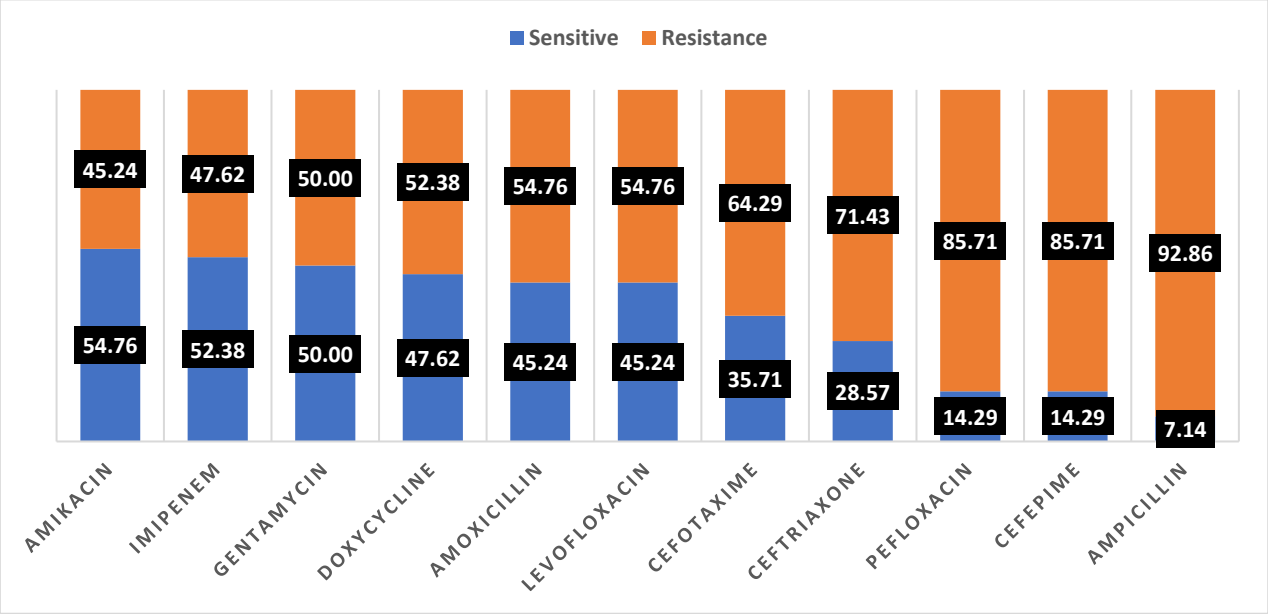

Figure 2: Antibiotic susceptibility of *E. coli* isolates.

V. DISCUSSION

Table 5 shows a significant incidence of the USP virulence gene in the E. coli isolates studied. The vast majority (95.24 percent) of the 42 isolates tested positive for the presence of this virulence gene, confirming its ubiquitous presence in the examined population[1]. Pathogens rely on virulence genes to develop infections, escape host defenses, and cause tissue damage. They encode virulence proteins such as toxins, adhesins, and invasins, which allow bacteria to colonize and damage host tissues. The presence of the USP virulence gene in such a high proportion of E. coli isolates suggests an elevated potential for pathogenicity and the ability to cause disease in the context of wound and burn infections [3]. Because females spend the majority of their time in the kitchen, where accidents are common, the incidence of burns was higher in females. Nasreen A. Siddiqui [11] made a similar observation. Scalding was the most prevalent cause of burns in the study conducted by Chien

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WC et al [12], but fire burns were the most common in our investigation. It's probable that this is due to socioeconomic circumstances in our society. Ansari Lari M et al. [13] discovered that the majority of patients were between the ages of 20 and 30. This finding was consistent with the findings of our study, which revealed that the age group most likely to be afflicted by burn injuries was between the ages of 20 and 30. According to our findings, a single isolate was detected in 57.85 percent of cases, whereas multiple isolates were found in 34.65 percent of cases. This is consistent with previous study [14]. The first and most crucial step in understanding bacterial pathogenesis and the interactions that occur within the host is the identification of virulence factors. 18 The presence of USP in this study was determined to be more similar to previous studies due to the fact that it was present in all of the isolates. (7,8,9) Furthermore, the analysis found that one USP was present in forty of the 42 E. coli samples. These findings demonstrated that USP genes were only found in samples obtained from burn victims and wound patients. Urine isolates of E. coli were found to be significantly more prevalent than other forms of infections in the current study. [Citation required] Because E. coli cells include virulence proteins and other components, they can attach to human epithelial cells and induce a rise in infection. This permits them to spread disease (10). The E. coli distribution findings revealed a higher prevalence in females compared to males, which was consistent with previous findings. Females had a higher prevalence of E. coli (11). The genotypic assay that used PCR to detect the USP virulence gene was performed in this investigation, and the results validated the gene's existence in all of the isolates acquired from the various clinical samples. Another study (15) concluded that the USP gene plays a function in the development of urinary tract infections (UTIs). In the second study, researchers sought to investigate whether USP, as a novel agent, is to blame for the DNA damage caused by E. coli in mammalian cells (5). Our findings, that 82 percent of E. coli isolates tested positive for the USP gene, are consistent with those of a different study from Mexico, which found 87.1 percent of such isolates to be positive (16), as well as the same study from Japan, which found 71.7 percent of such isolates to be positive. An earlier study in Japan found that 82% of E. coli isolates tested positive for the USP gene (15). Our results are in line with that study. The public's health has also been negatively impacted by the rise of antibiotic-resistant bacteria, such as Enterobacter (7, 17). This study demonstrates that certain strains of Escherichia coli are resistant to certain treatments. Confirming the high levels of resistance, an additional trial conducted in Iraq discovered complete resistance to ampicillin and aztreonam. There was a single case of ampicillin and aztreonam resistance in the previous Iraqi study, which is consistent with this trial. Although the resistance rate to ceftriaxone was lower at 52% (18), it is possible that antibiotic resistance will increase soon. Bacteria that create biofilms are more likely to be resistant to antibiotics, according to this research. This finding lends credence to earlier work that linked E. coli biofilm resistance to different environmental factors, this investigation corroborate those from earlier research (19). Compared to planktonic bacteria, harmful bacteria exhibit higher resistance, according to the research (11, 20). There was a correlation between resistance mechanisms and E. coli biofilms in the trial.

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