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Molecular detection of *tetB* gene in *Klebsiella pneumonia*, *Escherichia coli* and association between IL-10 gene polymorphism in burn patients

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ABSTRACT

This study aimed to check for the *tet*B gene in *K. pneumoniae,* and *E. coli* taken from burn wounds and to see if there is a link between *IL-10* gene variations and burn patients. One hundred samples of burn wound infections were collected and cultured for isolation and identification of *K. pneumoniae* and *E. coli* through morphological characterization, biochemical methods, and API 20E. The PCR technique was used to detect the *tet*B gene in all *K. pneumoniae* and *E. coli* isolates. The present results showed 25% of isolates identified as *E. coli*, while 80% of isolates identified as *K. pneumoniae* from burn samples after identification by many tests. The results of the PCR technique documented that 52% of E. coli and 45% of *K. pneumoniae* isolates harbored the *tet*B gene. A specific PCR fragment of 781 bp was designed to amplify a specific portion of the *IL-10* gene. The current results recorded a genetic polymorphism of the *IL-10* gene, rs1800896 (T327C), in burn patients. The results of this study are consistent with HWE with highly significant results (P = 0.000, 0.0002 and 0.000) for all groups, burn and control, respectively.

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Introduction

Burn patients are considered particularly prone to developing both systemic inflammatory response syndrome (SIRS) and complicated sepsis (Finnerty et al., 2009). The increase in cytokine production, both in humans and in experimental models, seems to play an important role in the pathophysiology of sepsis and septic shock during and after burn injury (Yeh et al., 2000; Finnerty et al., 2009).

Klebsiella pneumoniae is a pathogenic bacterium belonging to the Enterobacteriaceae family, which is widespread in the natural environment and benignly colonizes the gastrointestinal tracts of humans and animals. It is known for causing human nosocomial infections, such as urinary tract infections, intra-abdominal infections, and upper respiratory tract infections (Nouri et al., 2020, Fouad 2022; Al Jader & Ibrahem 2022,).

K. pneumoniae is an opportunistic pathogen capable of causing a wide range of diseases in humans and various animal species. Notably, the prevalence of antibiotic resistance has increased among

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Enterobacteriaceae, including *K. pneumoniae* isolates (Davis and Price, 2016). Due to the extensive use of antibiotics in humans, veterinary medicine, and agricultural practices, the emergence of *K. pneumoniae* strains harboring various resistance genes has increased considerably. Multidrug-resistant bacteria of different species, in both human and animal pathogens, have become an increasing worldwide problem.

The multidrug resistance of Enterobacteriaceae is frequently due to the acquisition of resistance genes from a shared pool (Partridge, 2011). These genes appear to have been captured from the chromosomes of various species by mobile genetic elements such as insertion sequences (IS) (Harmer and Hall, 2016), transposons (Liu et al., 2017), and integrons (Kaushik et al., 2018).

Escherichia coli, a member of the Enterobacteriaceae family, constitutes part of the normal commensal bacterial flora of animals and humans (Tajbakhsh et al., 2016). Antimicrobial resistance genes can easily spread among bacterial organisms via mobile genetic elements such as plasmids and transposons (Randall et al. 2004, Auhim & Rasheed 2025). Commensal *E. coli* spp. live with their human hosts in a mutually beneficial manner and generally do not cause disease. However, they may induce disease if the host's immune system is impaired or if the natural gastrointestinal barriers are breached, consequently playing a critical role in many infections (Bunduki et al., 2021). Due to its commensal nature, E. coli is sensitive to many commonly used antibiotics. However, *E. coli* taxa can accumulate resistance-associated genes through horizontal gene transfer (Poirel et al., 2018).

K. pneumoniae infections are difficult to treat due to the presence of certain virulence factors and the emergence of antibiotic resistance (Ranjbar et al., 2016). Tetracycline resistance is generally caused by the acquisition of a tetracycline resistance (tet) gene, as these genes are associated with primary resistance mechanisms, including active efflux pumps, ribosomal protection, and enzyme inactivation (Koo and Woo, 2011). To date, more than 40 different resistance genes have been identified (Roberts, 2005). In Gram-negative bacteria, the most important resistance mechanism involves the efflux pump system, which is encoded by tetracycline resistance genes tet(A), tet(B), tet(C), tet(D), and tet(G) (Chopra and Roberts, 2001).

Proinflammatory interleukins such as IL-6 increase early after burn injury and are counterbalanced by anti-inflammatory cytokines such as IL-10. An unbalanced immune response is thought to account for a substantial part of mortality resulting from severe burns. Therefore, it is not surprising that, according to reports from other groups, we recently observed increased plasma levels of IL-6 and IL-10 in burn patients, particularly in those developing severe sepsis (Pileri et al., 2008; Yeh et al., 2002).

Polymorphisms in cytokine genes with transcriptional relevance in vitro have been identified. Modifications in cytokine production are associated with genetic variations in the promoter region. Polymorphisms within the coding region may lead to differences in activity, whereas polymorphisms in the 3' untranslated region are associated with changes in mRNA stability (Schroder et al., 2003). Furthermore, a single nucleotide polymorphism (SNP) in the promoter region at position -1082 (G to A substitution) of the IL-10 gene (chromosome 1q31-32) has been associated with differential IL-10 production and with susceptibility to sepsis and severe prognosis after septic shock (Stanilova et al., 2006; Hassan and Degaim, 2024).

This study aimed to detect the presence of the *tetB* gene in *K. pneumoniae* and E. coli isolates recovered from burn wounds, as well as to investigate genetic polymorphisms of the IL-10 gene in burn patients using PCR and DNA sequencing.

Material and Methods

Sample Collection

One hundred burn swabs were collected from burn patients at Al-Hussein Teaching Hospital in Thi-Qar province during the period from March to November 2020. All samples were directly streaked onto MacConkey agar and incubated at 37°C for 24 hours. Selected isolated colonies were then grown on selective media and further identified by morphological characteristics, biochemical tests, and the API 20E system.

Fifty blood samples were collected from burn patients and 50 from healthy individuals as a control group. A total volume of 2 ml of blood was collected using sterile syringes and subsequently transferred into blood collection tubes containing EDTA.

Extraction of Genomic DNA

Isolates of *K. pneumoniae* and *E. coli* were inoculated into nutrient broth (NB) (LAB, United Kingdom) and incubated at 37°C for 24 hours. The chromosomal DNA of both bacterial species was extracted from fresh cultures using the DNA Bacteria Plus Kit (Geneaid, Korea) according to the manufacturer's instructions. In addition, DNA from the blood samples of burn patients and the control group was extracted using the gSYNC™ DNA Extraction Kit (Geneaid, Korea) following the manufacturer’s protocol.

PCR Detection of tetB and IL-10 Genes

The specific primer pairs for the *tetB* gene were as follows:
Forward: 5'- TTCGGCATTCTGAATCTCAC -3'
Reverse: 5'- ATGATCTAACCCTCGGTCTC -3'
(Van den Bogaard et al., 2001).

The PCR cycling program for the *tetB* gene was as follows: initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 60 seconds, annealing at 56°C for 90 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes (Abraham and Jefferson, 2010).

The primer sequences for the *IL-10* gene were as follows:
Forward: 5'- GGTAGTGCTCACCATGACCCC -3'
Reverse: 5'- ACACTGGAAATGCCCTCCATC -3'
The PCR program for the *IL-10* gene was as follows: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 57.5°C for 30 seconds, extension at 72°C for 35 seconds, and a final extension at 72°C for 5 minutes.
The final volume of each PCR reaction tube was 20 µl, consisting of 12.5 µl Master Mix, 1 µl of each forward and reverse primer specific to the target gene, 3 µl of DNA template, and the remaining volume completed with nuclease-free water.

The PCR products were visualized using 2% agarose gel electrophoresis. The presence of a 634 bp band was considered a positive result for the *tetB* gene, and a 781 bp band indicated the presence of the *IL-10* gene.

Interpretation of Sequencing Data

The sequencing results of the *IL-10* gene from 100 samples (50 from burn patients and 50 from the control group) were edited, aligned, and analyzed alongside reference sequences using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). Each detected variant within the investigated gene was annotated using SnapGene Viewer version 4.0.4 (https://www.snapgene.com).

Checking the rs1800896 SNP

The observed SNP was submitted to the dbSNP database to confirm its originality. Each identified SNP was highlighted according to its position in the reference genome. The presence of the previously reported SNP was confirmed by locating its corresponding dbSNP position. The dbSNP position for each detected SNP was then documented.

Statistical Analysis

All statistical analyses were performed using IBM SPSS software version 22. The Chi-square test was used to compare genotype and allele frequencies. Furthermore, the Hardy-Weinberg equilibrium for the single nucleotide polymorphisms (SNPs) in both groups was evaluated.

Results

The results of the present study showed that 25 out of 100 (25%) isolates were identified as *E. coli*, while 80 out of 100 (80%) were identified as *K. pneumoniae* from burn samples, based on morphological characteristics, biochemical tests, and the API 20E system.

The molecular results of the current study demonstrated that 13 out of 25 (52%) *E. coli* isolates and 36 out of 80 (45%) *K. pneumoniae* isolates harbored the *tetB* gene. Figure 1 shows the molecular size (634 bp) of the *tetB* gene in both bacterial species.

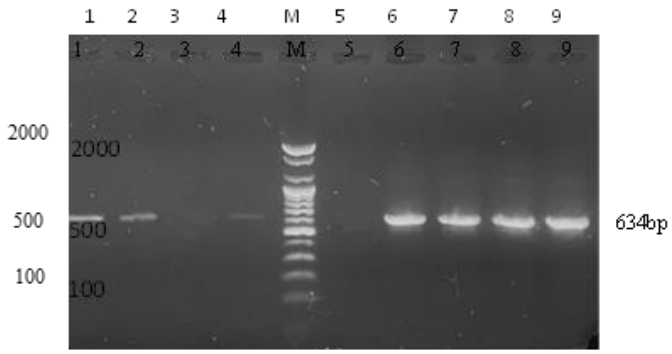

Fig 1. Agarose gel electrophoresis of *tet*B gene amplification, M: ladder, 1-2,4, 6-9 : positive results; 3,5: negative result.

The PCR results for the IL-10 gene showed that 100% of DNA samples extracted from burn patients harbored this gene. The molecular size (781 bp) of the IL-10 gene is shown in Figure 2.

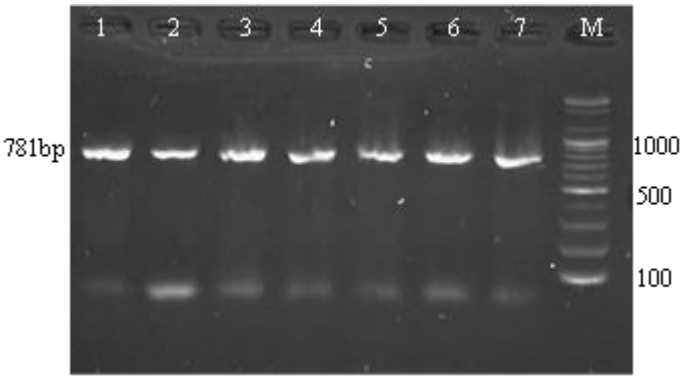

Fig 2. Agarose gel electrophoresis of *IL-10* gene amplification, M: ladder, 1-7 : positive results.

The IL-10 gene sequences showed more than 99% sequence homology between the amplified samples and the intended reference target sequence, which partially covers the IL-10 gene. The observed DNA variations in the analyzed samples were compared with the retrieved DNA sequence (GenBank accession no. NC_000001.11). The alignment results of the 781 bp samples revealed the presence of only one DNA variation in both the case and control groups, compared to the corresponding reference sequence (Figure 3).

To calculate Hardy-Weinberg equilibrium (HWE), the genotype and allele frequencies of the IL-10 T>C SNP are shown in Tables 1, 2, and 3. The results of this study were consistent with HWE, showing highly significant results (P = 0.000, 0.0002, and 0.000) for all groups: burn patients and controls, respectively.

The targeted high-frequency SNP was detected in the investigated samples, where the T nucleotide was replaced by a C nucleotide at position 327 of the amplified PCR fragment, referred to as 327T>C or T327C. Three polymorphic patterns were observed: TT, TC, and CC. The homozygous TT genotype was detected in cases (10/50) and controls (22/50), while the heterozygous TC genotype was observed less frequently in both cases (10/50) and controls (8/50). Interestingly, the homozygous CC genotype was detected in the majority of samples: cases (30/50) and controls (20/50), as shown in Table 1.

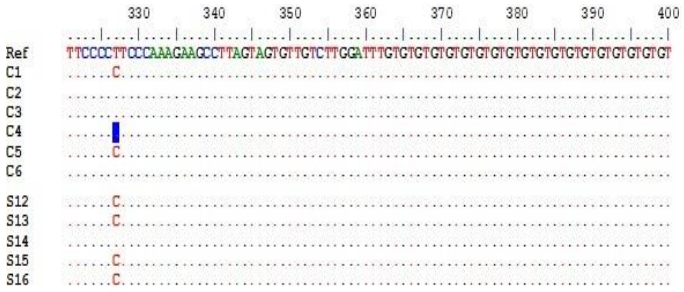

Fig 3. DNA sequences alignment of several samples with their corresponding reference sequences of the 781bp PCR fragments of the analyzed IL-10 gene

Table 1 Genotype frequency of rs1800896 T>C IL10 among burn patients and control.

rs1800896 SNP	Patients No:50	%	Control No:50	%	OD	95% CI	P. value
TT	10	20%	22	44%	0.3182	0.1307 to 0.7748	0.0117
TC	10	20%	8	16%	1.3125	0.4706 to 3.6603	0.6033
CC	30	60%	20	40%	2.2500	1.0108 to 5.0083	0.0470

Table 2 Allele frequency of rs1800896 T>C *IL10* among burn patients and control

SNP	Allele Frequency	Burn patients		Control		OR	95 % CI	p. value
		No.	%	No.	%			
rs1800896	T	30	30%	52	52%	0.3956	0.2214 to 0.7068	0.0017
	C	70	70%	48	48%			

Table 3 Hardy-weinberg equilibrium in of rs1800896 T>C *IL-10* among burn and control

rs1800896	All groups 100		Burn patients 50		Control 50	
	Observed	Expected	Observed	Expected	Observed	Expected
TT	32	16.81	10	4.5	22	13.52
TC	18	48.38	10	21	8	24.96
CC	50	34.81	30	24.5	20	11.52
HWE	P=0.41 ,q=0.59		P=0.25, q=0.75		P=0.52 q=0.48	
P. value	0.000		0.0002		0.000	

The allele frequency of the IL-10 T>C SNP between both groups showed a statistically significant association between the IL-10 gene 327 T>C and burn patients (p-value = 0.0017), as shown in Table 2.

The present results of HWE for the IL-10 T>C SNP among controls showed a highly significant correlation between the expected and observed genotypes, as shown in Table 3.

Discussion

Antimicrobial resistance is considered one of the most significant public health problems of the 21st century and is linked to factors related to the overuse or misuse of antimicrobials in human and veterinary practices, as well as environmental pollution. Addressing antimicrobial resistance effectively requires close collaboration under a "one health" approach, taking into account the interconnections between human health, animal health, and the environment (Sakkas et al., 2019).

The present results recorded that 80% of isolates were identified as *K. pneumoniae*. This study disagreed with the results of Ranjbar et al. (2019), who showed that 44.22% of isolates were identified as *K. pneumoniae* from hospital-acquired infections. Additionally, the study by Derkhshan et al. (2014) recorded that the majority of *K. pneumoniae* isolates were from urine.

The current results recorded 25% of isolates identified as *E. coli* from burn patients' samples. A local study performed at two hospitals in Duhok showed 17% (25/147) of burn samples identified as *E. coli* based on morphological, biochemical, and molecular analyses (Al-Sarhan and Çam, 2023).

The results of Azzopardi et al. (2014) revealed that Gram-negative infections are the most prevalent in burn surgery; also, *E. coli* plays a significant role in infection cases in burn centers. The risk of infection-associated mortality is high in burn patients (Lachiewicz et al., 2017); therefore, characterization of *E. coli* in burn wounds is of utmost importance.

The PCR results for detection of the *tetB* gene showed that 45% and 52% of *K. pneumoniae* and *E. coli* isolates, respectively, harbored the *tetB* gene.

Due to the increasing antibiotic resistance, awareness of microbial resistance forms and mechanisms of resistance transmission among bacterial infections can be an effective strategy to avoid microbial resistance transmission (Sedighi et al., 2020). Also, Al Kareem et al. (2020) and Degaim et al. (2021) used PCR techniques to identify and detect virulence factors and antibiotic resistance genes in different bacterial species.

The association between tetracycline resistance genes (tetA and *tetB*) and class II integrons (intII) might be related to the co-transfer of both tetracycline resistance genes. Likewise, the positive association between the presence of intI and intII genes and tetracycline resistance was reported (Rezaee et al., 2012; Degaim et al., 2019).

The present study disagreed with the study by Shin et al. (2015), which showed that tetracycline-resistant *E. coli* isolated from humans and animals in numerous countries carried *tetB* (45.1%). They suggested that these genes are important for the development of tetracycline resistance. Essentially, tet(A) and/or tet(B), encoding efflux mechanisms, have been described as the most common tetracycline resistance determinants in E. *coli* (Schwaiger et al., 2010; Hu et al., 2013).

Some studies demonstrated that bacteria and bacterial genes might move easily among humans, animals, and the environment; also, the hazard of antimicrobial resistance transmission from the environment to humans should be measured according to resistant bacteria and resistance genes circulating in the environment (Martínez et al., 2018).

The study by Fatima et al. (2021) recorded that all *K. pneumoniae* tetracycline-resistant strains had the *tetB* gene, while the study by Asghari et al. (2021) showed that only 12.6% of *K. pneumoniae* isolates had the *tetB* gene.

Both tet(A) and tet(B) genes are among several tetracycline determinants in *E. coli* that encode energy-dependent membrane-associated efflux proteins (Roberts, 2005).

The current results do not agree with the study of Torkan et al. (2015), which noted that only 21.4% of isolates had the tet(B) gene and suggested that the tet gene might be the predominant tetracycline resistance gene harbored by pathogenic E. coli. Additional tetracycline resistance genes, thought to confer resistance through ribosomal protection and enzymatic inactivation, could also be harbored by the tetracycline-resistant gene-positive isolates.

The current DNA sequencing results recorded a significant association of rs1800896 T>C SNP of the IL-10 gene in burn patients and the control group. The severity of the initial injury and the subsequent development of complications are major factors affecting the prognosis of burn patients (Grieb et al., 2010). It has been shown that approximately 75% of the variation in IL-10 production is genetically determined; in fact, IL-10 production appears to be controlled at the transcriptional level (Crawley et al., 1999; Schroder et al., 2003).

Also, Pileri et al. (2008) reported that patients after burn injury frequently show elevated circulating levels of IL-10 that appear to correlate with the development of septic complications.

Increased IL-10 production is associated with decreased resistance to infection and poorer prognosis (Giannoudis et al., 2000; Khudhair et al., 2021).

The DNA sequencing analysis of IL-10 (rs1800896) T>C SNP recorded that the homozygous CC pattern was detected in the majority of samples: burn patients (60%) and controls (40%). The results of Palumbo et al. (2012) demonstrated that the IL-10 -1082 GG genotype was associated with higher IL-10 production measured at day 3 in burned patients who developed septic complications. In the study by Lowe and colleagues, an association was recorded between the IL-10 -592A/-819T allele, decreased IL-10 levels, and increased risk for mortality (Lowe et al., 2003).

The allele frequency of IL-10 T>C SNP among burn patients and the control group showed a significant relationship ($p = 0.0017$). Also, the C allele was detected at a higher percentage in burn patients (70%) compared to the control group (48%). This study documented that rs1800896 involves a T nucleotide replaced by a C nucleotide at position 327 of the amplified PCR fragment. IL-10 is a known anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines and chemokines (Mosser and Zhang, 2008). During the response to burn trauma, levels of IL-10 are known to increase significantly to regulate the effects of the pro-inflammatory response (Finnerty et al., 2006).

In IL-10 gene polymorphism studies, stratifying patients by IL-10 genotypes and presence of sepsis, it was observed that septic patients bearing the -1082G/G genotype had an increased risk for sepsis development compared with non-septic patients bearing -1082A/* genotypes (Palumbo et al., 2012).

The results of Huebinger et al. (2010) described a reduced risk of death in burn patients bearing a low IL-10 production-associated genotype, while Palumbo et al. (2012) showed that high and genetically determined IL-10 production might predict severe burn complications. Likewise, Huebinger et al. (2010) showed that the -592 A/-819T allele in the IL-10 promoter region was significantly associated with decreased mortality risk. Given the common inflammatory cellular response to burn trauma, biologic feedback mechanisms to regulate inflammation are necessary.

Conclusion

This study reveals that *Klebsiella pneumoniae* and *Escherichia coli* are common culprits in burn wound infections, with many strains carrying the tetracycline resistance gene *tetB*. Additionally, we found a strong link between a specific genetic variation in the IL-10 gene (rs1800896 T>C) and burn patients, which may influence how their bodies respond to inflammation and infection. These insights highlight the need to keep a close eye on antibiotic resistance and genetic factors to better manage infections and improve recovery for burn patients.

Availability of data and material

Data are accessible upon request.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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