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Research Article

ISOLATION, MOLECULAR CHARACTERIZATION, AND ANTIMICROBIAL RESISTANCE TEST OF UROPATHOGENIC *ESCHERICHIA COLI* WITH ASSESSMENT OF INTERLEUKIN-10 IN RHEUMATOID ARTHRITIS PATIENTS WITH UTI

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ABSTRACT

Objectives: It was aimed to isolate *Escherichia coli* bacteria in rheumatoid arthritis (RA) patients with urinary tract infection (UTI), examination of the antimicrobial sensitivity test, molecular detection of *16SrRNA*, *UidA* virulence gene of the pathogens, and assessment of the level of interleukin (IL)-10 in the sera of RA patients.

Methods: Two hundred and sixteen (116 RA patients with suspected UTI and 100 healthy controls without RA or UTI) were enrolled, midstream urine samples (MSU) and blood were collected from different ages and both sexes. All isolates were diagnosed based on microscopic examinations and morphological characteristics using suitable media. The diagnosis was confirmed by the Vitek2 system. Molecular identification was carried out by the polymerase chain reaction (PCR) technique. The evaluations of the immune marker were determined through an enzyme-linked immunosorbent assay (ELISA) test, which was the first investigation in Iraqi RA patients.

Results: The results showed that *E. coli* had the highest percentage, representing 25.8%. Molecular methods, such as the PCR technique, were used to identify the *E. coli* by *16sRNA* and *UidA* virulence genes; therefore, 26 of the isolates (86.66%) were identified through the presence of both genes. Testing antibiotic sensitivity against 13 different antimicrobial agents showed that *E. coli* isolates were highest resistance with high significant differences ($p \leq 0.01$) for rifampicin25 (83%), ampicillin/sulbactam 20 (67%), cefotaxime 19 (63%), trimethoprim/sulfamethoxazole 17 (57%), ciprofloxacin 16 (53%), gentamicin 12 (40%), while there is intermediate resistance to cefotrixone 10 (33%) with non-significant susceptibility rate 20 (67%), ($p=0.068$). In contrast, the isolates showed high susceptibility to imipenem 29 (79%), meropenem and amikacin 28 (94%), piperacillin/tazobactam 27 (90%), and tigecycline 26 (87%) respectively, with highly significant differences ($p=001$). In addition, high significant sensitivity to cefotazdime 21 (70%), ($p=0.0001$). The current study demonstrated that *E. coli* possessed a multi-drug resistance against most of used antibiotics. Molecular detection of 26 bacterial isolates containing *16SrRNA* and *UidA* virulence genes using a conventional PCR test. Five milliliters of venous blood sample from each patient under aseptic technique, the blood samples were collected from the same people from whom urine samples were collected for immunological analysis, such as rheumatoid factor, erythrocyte sedimentation rate, C-reactive protein, and anti-CCP, results exhibited positive reaction and clear elevation levels of antibodies in the serum of RA-infected patients. Analysis of IL-10 in patients' sera, which was separated from 5 mL of venous blood sample for (30 patients with positive bacterial growth cultures of *E. coli* and 8 healthy controls), and the level was measured using the ELISA technique. The level of IL-10±SE increased non-significantly ($p \geq 0.05$) in RA patients with *E. coli* UTI (con 24.95 pg/mL±2.64 vs. 18.39 pg/mL±2.52), respectively, in comparison to healthy controls. The mean level of IL-10 reached 25.6 pg/mL differs significantly in RA patients' era with UTI who were infected with *E. coli.*

Conclusion: *E. coli* is the most predominant multidrug-resistant pathogen responsible for UTIs in RA patients, who had virulence genes to enhance the immune reaction, and IL-10 may have a role in RA risk.

Keywords: Urinary tract infection, Polymerase chain reaction, *Escherichia coli, UidA* genes, Antimicrobial susceptibility.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality. It is a systemic arthritic and autoimmune condition that affects millions of people worldwide. RA mainly attacks small joints (hands and feet); its symptoms as hyperplasia of the synovial membrane, increase in macrophages, high levels of pro inflammatory cytokines, activation of catalytic enzymes responsible for degradation of extracellular matrix, and the expression of autoantibodies, such as rheumatoid factor (RF), anti-citrullinated protein antibody (ACPAs), and C-reactive protein (CRP) which can precede the clinical manifestation of RA by many years [1]. RA causes the degradation of articular and periarticular tissues, distortion and impaired function of the tissues, and consequently, permanent disability [2]. It is believed that RA development is dependent on genetic factors, HLA polymorphisms, and environmental factors such as smoking and infectious agents, which induce autoimmunity by molecular mimicry, cross-reactions, or the formation of immunological complexes [3]. It is assumed that one of the most common bacteria linked with the etiopathogenesis and development of RA is *Escherichia coli* [4]. It is a member of the Enterobacteriaceae, normal flora in the human and animal gut, and pathogenic in patients with immune system disorders. It is the main pathogen responsible for 90% of infectious diseases and affects up to 150 million people with UTIs worldwide [5,6]. Particular sets of virulence factors of bacterial strains play a role in the pathogenesis process, antimicrobial resistance, and host immunoevasion [7-9]. Furthermore, it employs several mechanisms that disturb the host cellular process and the resistance for different groups of antibiotics including target modification, production of enzymes, alteration of metabolic pathways, production of

enterotoxin, verotoxin, colicins and siderophores, type-1 pili, motility, the efflux pumps, capsule, fimbriae, toxins, lipopolysaccharide (LPS), pathogenicity islands and biofilm production [10,11]. *E. coli* undergoes mutation and horizontal gene transfer to generate uropathogenic *E. coli* (UPEC) capable of colonizing and ascending to the urinary tract and expressing type 1 pili, to mediate adherence and invasion of human bladder epithelial cells, causing UTIs [12,13]. The bacterial replication is associated with inflammation such as neutrophils present in the urine of infected patients (it is a hallmark of UTIs), as well as the pro-inflammatory cytokine interleukin (IL)-6 (which is important in the development of local tissue damage) and the chemokine IL-8 (a potent neutrophil chemotactic molecule). Bladder epithelial cells have a receptor for lipopolysaccharide (the inflammatory molecules) from Gram-negative bacteria, and secrete the ILs in response to LPS to activate the innate immune response [14]. Moreover, the deployment of an array of toxins provides UPEC with the means to inflict extensive tissue damage, facilitating bacterial dissemination as well as releasing host nutrients and disabling the immune effector cells, but UPEC can suppress or evade immune system, lead to recurrent infections [15,16]. Furthermore, it has genes mediated by mobile genetic elements carrying additional resistance to various groups of antibiotics, resulting in multidrug resistance (MDR) treatment failure and mortality [17,18]. Studies hypothesized that exposure to specific strains of *E. coli* could play a role in early-stage sero-positive RA pathogenesis according to RF status. The production of toxins by colonizing *E. coli* may cause an inflammatory response, a pathway for UTIs symptoms also increased production of ILs in the infected patient [19]. A variety of infectious diseases can result from localized bacterial infections in the joints; these destructive joint diseases, including autoimmune diseases like RA, are linked to an increased prevalence of bacterial arthritis [5]. IL-10 is an anti-inflammatory and immune regulation cytokine, or human cytokine synthesis inhibitory factor, it inhibits the activity of Th1 cells, NK cells, and co-stimulatory molecules on macrophages in the lymph nodes [20,21]. It enhances B-cell survival, proliferation, and antibody production. IL-10 is produced by mast cells, counteracting the inflammatory effect that these cells have at the site of an allergic reaction [22]. IL-10 is upregulated in synovial fluid and joints of RA patients, and its capacity to inhibit cellular immunity and deactivate macrophages, which is behind joint and synovial inflammation, with the ability to enhance the proliferation of synovial cells and to induce pro-inflammatory cytokines (IL-6) and chemokines (IL-8) [23]. In addition, it can directly regulate innate and adaptive Th1 and Th2 responses by limiting T-cell activation and differentiation in the lymph nodes, also suppressing pro-inflammatory immune responses, leading to impaired pathogen control, tissue damage, and reduced immune pathology [24]. This study aimed to isolation of *E. coli* bacteria in RA patients with UTI; examine the antimicrobial sensitivity test, molecular detection of *16SrRNA*, *UidA* virulence gene of the pathogens, and assessment the level of IL-10 in the sera of RA patients.

METHODS

Collection of samples

Two hundred and sixteen midstream urine samples (MSU) were collected from (100 healthy control without RA or UTI and 116RA patients suffering from UTIs of all ages and both sexes from different hospitals. The samples were collected from patients during the period from September 2023 to the end of March 2024 from (Baghdad Teaching Hospital, Medical City, and Al Nuaman, Teaching Hospitals) in Baghdad/Iraq. The study received ethical approval from the Genetic Engineering and Biotechnology Institute for Postgraduate Studies at the University of Baghdad., Authorization was also granted by the Iraqi Ministry of Health., Informed consent was obtained from all participants prior to their involvement. Data collection was carried out by distributing questionnaires to these participants. The urine sample was cultured; bacterial isolates were identified by methods of routine tests depending on their morphological and physical characteristics on the culture media and colonymorphology on Bloodagar, Nutrient agar, and MacConkey agar. The colonies on the blood agar are microscopically identified as *E. coli*, and upon the laboratory manual tests that were used, biochemical tests including catalase test, oxidase, and urease reaction. The identification card for Gram-negative bacteria was used to confirm the identification of all clinical isolates of *E. coli* using the Vitek2 system [25-27].

Antimicrobial susceptibility testing

All *E. coli* isolates were tested for antibiotic sensitivity against 13 different antibiotics as follows: Ampicillin/sulbactam, amikacin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefotrixone, rifampicin, imipenem, gentamicin, ciprofloxacin, tigecycline, meropenem, and trimethoprim/sulfamethoxazole.

Molecular method

Bacterial DNA extraction and polymerase chain reaction (PCR) protocol

The extraction of genomic DNA from bacterial growth for all 26 Viteck-2 diagnosed isolates was carried out according to the protocol of (EasyPure-Genomic DNAKit from Transgene-China). Genomic DNA was extracted from pure E. coli isolates following incubation in brain–heart broth for 24 h at 37°C. The concentration and quality of the extracted DNA were assessed using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) to ensure suitability for downstream applications. The detection of DNA bands using agarose gel electrophoresis with a concentration 1% and a voltage of 75 volts for 60 min [28].

Detection of E. coli 16SrRNA gene and UidA gene using conventional PCR

Specific primers of (*16SrRNA*) were used for detecting the *E. coli* bacteria and (*UidA)* virulence genes related to causing UTIs and RA, as shown in Table 1. The PCR amplification mixture, which is used for the detection of each gene, includes One NEB (England) Master Mix, 5× (12.5 µL), 3 µL of DNA template, 1 µL (1 mM) of each forward and reverse primer, and 7.5 µL of nuclease-free water to complete the amplification mixture to 25 µL as shown in Table 2. After preparing the reaction volume in a PCR tube, the mixture was spin down, and then, the PCR tube was placed in the PCR thermo cycler. The amplification reactions were started according to the program, as described in Table 3.

Immunological method

Blood sample collection

A total of 116 RA patients and 100 controls provided venous blood samples collected under aseptic conditions. Five milliliters of blood were drawn from each participant, from the same individuals who provided urine samples. Part of the sample was placed in ESR tubes

Table 1: The name, sequence, and product size of primers used for the detection of *16SrRNA* and *UidA* genes of *E. coli* by PCR in the study

Genes name	Sequences (5→3) Primer		Size (bp)	Ref.
16Srna E. coli	F	5'GATCATGGCTCAGAATTGAACG'3	660	In this study
	R	5'AATTCTACCCCCTCTACGAGA'3		
UidA E. coli	F	5'TATCTCTATGAACTGTGCGTC'3	350	In this study
	R	5'CCAATGCCTAAAGAGAGGTTA'3		

E. coli: Escherichia coli, PCR: Polymerase chain reaction

Table 2: Components of PCR master mix reaction for *16SrRNA* and *UidA* genes of *E. coli* in the study

Component	Volume (µL)	Final concentration
NEB (England) Master Mix, 5X	12.5	1X
Forward primer	1	1 Mm
Reverse primer	1	1 Mm
DNA template	3	25 ng
Nuclease-free dH_2O	7.5	
Final volume	25	

E. coli: Escherichia coli, PCR: Polymerase chain reaction

for measurement using the Westergren method. The remaining 3 mL were allowed to clot at 37°C, centrifuged at 25,000 rpm, and the serum was separated and stored at −20°C. Serum samples were later used to analyze RF, CRP, and ACPA, with RF measured by a routine turbidity-based serological assay [29]

Statistical analysis

The frequency of bacteria indicative of UTI was expressed as a percentage, cytokine data were reported as mean±S.E., and the differences between means were evaluated using a T-test. The difference was deemed significant when the probability (P) value was ≤0.05. These analyses utilized SPSS version 13. A one-way ANOVA test with a p<0.05 was deemed statistically significant.

IL-10 analysis

Using (32 patients and 8 control samples) to carry out the test, serum was separated from 2 mL of venous blood and examined using the enzyme-linked immunosorbent assay technique. The contents of IL-10 were determined for all 32 serum samples of RA patients with positive bacterial growth cultures of UTI (26 *E. coli*, 6 other bacterial growth, and 8 healthy controls without RA or UTI) in strict accordance**.**

RESULTS AND DISCUSSION

Two hundred and sixteen MSU were collected from (100 healthy controls without RA or UTI and 116 RA patients with suspected UTI) of all ages and both sexes. Samples were collected from different hospitals from September 2023 to the end of March 2024 from Baghdad Teaching Hospital, Medical City, Al Nuaman Teaching Hospitals in Baghdad/Iraq. The urine was cultured and identified by methods of routine tests: depending on their physical characteristics on the culture media and colony morphology on Bloodagar, Nutrient agar, and MacConkey agar plates, as shown in Fig. 1. In addition, all 30 isolates gave negative results for oxidase, catalase, and positive results for the urease test. From 216 urine samples, preliminary results showed growth of *E. coli* in 30 samples, while only 26 isolates were confirmed as *E. coli* isolates by the Vitek-2 system. This may be due to the contamination of the culture or the mixed growth during the incubation.

The diagnostic results of the study showed highly Significant differences as *E.coli* had the highest percentage representing 25.8%, followed by *P.mirabilis* and other bacteria with prevalence rates (6% and 17.3%) respectively (p≤0.01). A study by Sathiamoorthi Thangavelu *et al*., (2022) [14] indicated that *E. coli* a cause and the most frequent of UTIs among patients, with a rate (28.6%). According to Marrs et al. [11] and Adamus-Bialek et al. [18], Escherichia coli—particularly strains with high infection rates—remains the predominant cause of urinary tract infections. In addition, the results are in agreement with previous research by Al-Saadi and Abdullah [30], which revealed that *E. coli* formed 32% of the organisms [30].

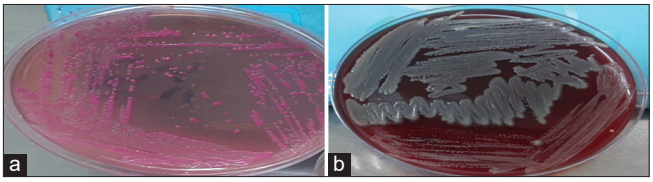

Fig. 1: (a) *Escherichia coli* on MacConkey agar, (b) on blood agar

Table 3: The optimal PCR program for the detection of *16SrRNA* and *UidA* genes amplification of *E. coli* in the study

Steps	Cycles	Genes amplification conditions			
		16SrRNA E. coli	**Time**	***UidA E. coli***	**Time**
Initial denaturation	1	94°C	5.00 min	94°C	5.00 min
Denaturation	30 (16SrRNA)	94°C	30 s	94°C	30 s
Annealing	30 (*UidA)*	51°C	45 s	51°C	45 s
Extension	30 (16SrRNA) 30 (*UidA)*	72°C	45s	72°C	45 s
Final extension	1	72°C	7 min	72°C	7 min

E. coli: Escherichia coli, PCR: Polymerase chain reaction

Antimicrobial susceptibility testing

The antibiotic susceptibility tests were carried out for all the *E. coli* isolates using VITEK 2 method (AST-GN Kit) to detect the antibiotic susceptibility in accordance with the recommendations of the manufacturer's instructions (bioMérieux, France). All identified *E. coli* were exposed to 13 different antimicrobial agents, as shown in Table 4. The results of the antibiotic sensitivity test exhibit that most of *E. coli* isolates had MDR profiles. The highest resistance with high significant differences (p≤0.01) was to rifampicin with a prevalence rates 25 (83%), ampicillin/sulbactam 20 (67%) respectively, and non-significant resistance to cefotaxime was 19 (63%) (p=0.14), but trimethoprim/sulfamethazol had non-significant resistance of 17 (57%) (p=0.46), also *E. coli* exhibited non-significantly resistance to ciprofloxacin with a percentage of 16 (53%), (p=0.006). In addition, the isolates showed approximately moderate resistance with a prevalence rate of 12 (40%) and susceptibility of 18 (60%) to gentamicin, with non-significant differences (p≥0.27). The results of this study agree with the researchers by Marrs *et al.* [11], AL-Hamdani and AL-Hashimy [29] that showed the percentage of resistance to rifampicin was (25%), which is an important antibiotic in this group with high ability and effectiveness against Gram-negative bacteria, also *E. coli* has the ability of antimicrobial resistance and immune evasion due to a variety of virulence factors such as capsule, toxins, pilli and biofilm formation. Likewise, *E. coli* isolates showed resistance to ampicillin/sulbactam with a percentage of 67% and cefotaxime 63%. This result agrees with the study recorded by Girlich *et al.* [31]. While there is intermediate resistance at ceftriaxone 10 (33%), with non-significant susceptibility 20(67%) (p=0.068). These observations are in agreement with the research by Sabir *et al.* [32]. Meanwhile, the *E. coli* isolates exhibited high susceptibility to imipenem 29 (79%), meropenem and amikacin 28 (94%), piperacillin/tazobactam 27 (90%), and tigecycline 26 (87%), respectively, with highly significant differences (p=001); otherwise, cefotazdime had high significant sensitivity rate 21 (70%), (p=0.0001). The current study demonstrated that *E. coli* possessed a high level of resistance against rifampicin and ampicillin/sulbactam with high susceptibility to imipenem, meropenem, and amikacin, respectively. These findings were in agreement with those mentioned by Yazdi *et al.* [33]. Another research found the pattern of *E. coli* had a high rate of susceptibility to the imipenem, meropenem, and

Table 4: The results of the antibiotic susceptibility test for 26 samples of uropathogenic *E. coli* bacterial isolates from RA patients with UTI

Antibiotic groups	R (%)	S (%)	I (%)	χ	p-value
Ampicillin/Sulbactam	20 (67)	7 (23)	3 (10)	15.80	0.0001
Amikacin	1 (3)	28 (94)	1 (3)	48.60	0.0001
Piperacillin/Tazobactam	3 (10)	27 (90)	0	19.20	0.0001
Cefotaxime	19 (63)	11 (37)	0	2.13	0.14
Ceftazidime	5 (17)	21 (70)	4 (13)	18.20	0.0001
Cefotrixone	10 (33)	20 (67)	0	3.33	0.068
Rifampicin	25 (83)	2 (7)	3 (10)	33.80	0.0001
Imipenem	0	29 (97)	1 (3)	26.13	0.0001
Gentamicin	12 (40)	18 (60)	0	1.20	0.27
Ciprofloxacin	16 (53)	12 (40)	2 (7)	10.40	0.006
Tigecycline	4 (13)	26 (87)	0	16.13	0.0001
Meropenem	2 (6)	28 (94)	0	22.53	0.0001
Trimethoprim/ Sulfamethoxazole	17 (57)	13 (43)	0	0.53	0.46

Total number of isolates=26. R: Resistance, S: Susceptibility, I: Intermediate. (p≤0.01) highly significant, P≥0.05: Non-significant. UTI: Urinary tract infection, *E. coli: Escherichia coli*

amikacin [34]. Furthermore, the variation of pathogens may be due to the limited numbers of isolates and the abuse in countries from which those isolates were isolated. Research agrees with the study by Alhadidi *et al.* [35] and suggests that *E. coli* pathogens resist more than 3 types of antibiotics, which is recorded as multidrug-resistant. MDR was defined as a resistance to at least 3 antibiotics *E. coli* is one of the most common uropathogenic bacteria; it expresses several virulence factors to break the inertia of the mucosal barrier, like its ability to adhere to the host epithelial cells, which is a precondition for the creation of infectious diseases, mostly through the expression of fimbriae [36]. Emergence of antibiotic resistance among UPEC strains can occur through several mechanisms, and the MDR strains limit the treatment options. This made them a worldwide concern as it significantly reduced the efficiency of the first-line treatment agent against this pathogen and increased the cost of treatment, morbidity, and mortality in countries [37]. Therefore, species identification, awareness, and education programs are necessary for the right diagnosis of diseases, proper selection, and wise use of available antibiotics will help in reducing the bacterial vigor of resistance and control of infections.

Molecular identification of *E. coli* by *16SrRNA* gene

The genomic DNA was extracted from 26 bacterial isolates of positive culture, which were diagnosed positively by the Vitek2 system. The total DNA was extracted, and the concentration of DNA was determined. The results of gel electrophoresis showed the presence of DNA bands at the same level for all isolates in agarose gel (Fig. 2).

All 26 phenotypically positive isolates of *E. coli* strains were subjected to molecular diagnosis using the specific initiator of the *16SrRNA* gene-specific primer with molecular size 660 bp to confirm its diagnosis by PCR. The results were detected by gel electrophoresis on 2% agarose and exposed to U.V light in which, the results showed that 26 bacterial isolates belong to *E. coli* that had positive amplification for *16SrRNA* geneas, as shown in Fig. 3, and it shows closely result for biochemical diagnosis, so this tool was rapid and accurate with high identification genomic rate (100%) [38]. Several studies referred to the use of *16SrRNA* for the identification of *E. coli* isolated. *16SrRNA* was described as having a high discriminatory power for the identification of bacteria and to differentiate between closely related genera because it exists in almost all bacteria, often existing as a gene cluster or operon, and also the function of this gene has not changed over time [39]. The current results were similar to the study performed by Khalaf *et al.* [40] in Baghdad/Iraq, as the samples were identified as *E. coli,* and it depends on the *16SrRNA* gene 100%.

PCR has become a very rapid and reliable tool for the molecular biology-based diagnosis of a variety of infectious diseases. It has been applied for the detection of microorganisms from microbial cultures and tissues, and directly from clinical samples. The pairwise comparison of PCR versus VITEK-2 indicated that there was no difference between these two techniques in the identification of *E. coli* [41]. This result is compatible with Baqer [42], who showed that the use of the Vitek2 system is more accurate in the diagnosis of *E. coli*.

Detection of *E. coli UidA* gene using conventional PCR

All isolates identified by the Vitek-2 system assay, only 26 isolates (86.66%) showed a positive result in the detection of *16SrRNA* gene by PCR amplification. All isolates were confirmed to be *E. coli* using *UidA* virulence gene-specific primer with molecular size 350 bp, and the results were detected by gel electrophoresis on 2% agarose for 1 h and exposed to ultraviolet light. Results indicated that *E. coli* isolates had positive amplification for *UidA* virulencegeneas shown in Fig. 4. The results of the current study agree with the diagnosis of the study conducted by Ejrnaes [4], Ali and Al-Dahmoshi [43]. In addition, the results of the current study agree with the results of the local study conducted by the research [7] that showed the *UidA* gene encodes the 1-glucuronidase enzyme, and most of the *E. coli* isolates possessed *UidA* gene. The B-glucuronidase is the first enzyme of the hexuronide–hexuronate pathway in *E. coli* and is encoded by the *UidA*

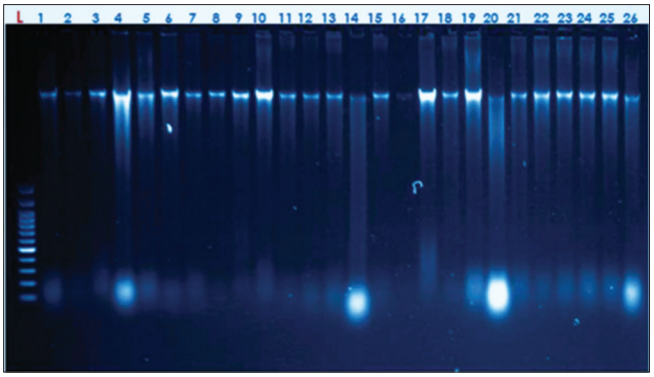

Fig. 2: Genomic DNA extraction electrophoresis for *Escherichia coli* (1%) agarose gel at 75 volt for 60 min

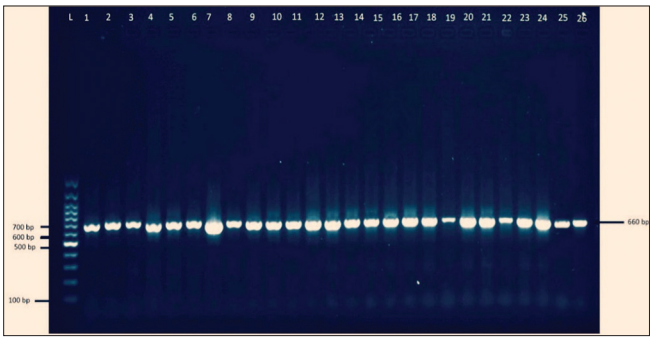

Fig. 3: The agarose gel electrophoresis of polymerase chain reaction amplified products for 16sRNA gene of *Escherichia coli* isolates, Lane L: 1000 bp DNA ladder, lanes (1–26): DNA of positive result with expected size (660 bp), (2% Agarose, 75 Vol for 1 h)

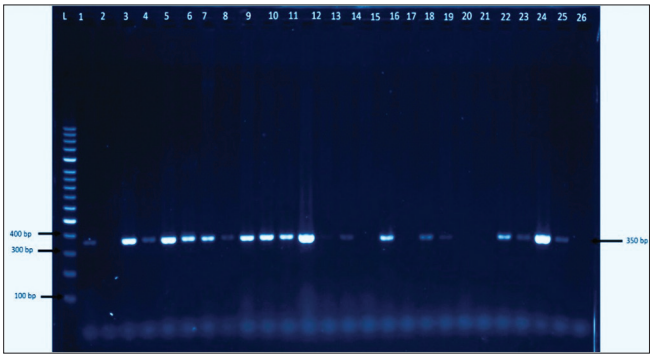

Fig. 4: The agarose gel electrophoresis of PCR amplified products for detection the *UidA* virulencegene of *Escherichia coli* isolates. Lane L: 1000 bp DNA ladder; lanes (1–26): DNA of *E. coli* of *UidA* gene with expected size (350 bp), (2% agarose, 75 vol for 1 h)

gene. Therefore *E. coli* encoding *UidA* gene has been used as the target for *E. coli* detection using molecular techniques, as it is present in the vast majority of *E. coli* strains, but should be used with other genotypic traits unique to *E. coli* as *16SrRNA.* Likewise, another variable such as the physiological condition of the bacteria could be responsible for the non-expression of the enzyme activity [44]. Therefore, *E. coli*-specific gene *UidA*, was added as a control/reference gene. However, the study conducted another study in the Netherlands and Germany by Brons JK, *et al.* [44], as the result with a prevalence rate of (86.66% vs.14.4%) is in agreement with a previous study which found that out of a total of all collected clinical samples the *E. coli* marker gene *UidA* was present in 97.7% of all strains and the remaining 2.3% were belonged to phylogenetic group which is known to be quite divergent from all other *E. coli* groups. Therefore, the presence of these marker genes in

a given sample as the criterion for positive identification of UPEC, in addition to detecting all samples as *E. coli* dependent on the *16SrRNA* gene 100% [44,45].

Immunological analysis

Blood parameters tests

There is strong evidence that this organism is associated with the development of urinary tract infections in patients with RA, ranging from recurrent subclinical *E. coli* urinary tract infections to full-blown RA [29,46]. Therefore, the researchers performed several blood tests, including (RF, ESR, CRP, RF, and ACPA). All of these tests showed elevated levels in the serum of patients whose urine cultures tested positive for *E. coli*. As shown in Table 5a, the mean ESR was significantly higher in the study group than in the control group (61.50 mm/h vs. 16.97 mm/h, T=36.668), both highly significant values (p≤0.01), but the mean ± SE was significantly higher in the *E. coli* infection group (55.5 mm/h ± 2.45). Similarly, the mean CRP value was significantly higher in the case group than in the control group (39.57 mg/L vs. 9.45 mg/L, T = 12.394), both highly significant values (p≤0.01). There was no significant increase in CRP values ±S.E. in the *E. coli* infection group. This is consistent with the results of Nama *et al.* [47], which showed that ESR, CRP, and RF values are essential for the diagnosis of inflammatory diseases. Although there is no clear consensus on when to perform one, the other, or both tests, laboratory tests such as ESR and CRP have been an important part of the medical toolkit for many years. CRP has become the most widely used serological marker for measuring acute disease activity [46,48]. Similarly, all samples were positive in the RF test, indicating that latex particles in the serum of RA patients infected with *E. coli* showed visible agglutination reactions, which is consistent with the results of previous studies [49]. These results [50,51] also showed that RA patients with positive RF had significantly elevated levels of *E. coli* antibodies. In addition, the mean concentrations of antibodies against citrullinated proteins/peptides (ACPA) and ACPA concentrations in RA patients were significantly higher than those in the non-RA group (p≤0.01) (control group) (59.55 EU/mL vs. 19.20 EU/mL, T=19.015), but the mean ± SE of ACPA in patients with *E. coli* infection was not significantly increased (73.06 EU/mL±2.83), as shown in Table 5b. Studies have also shown that RA patients have slightly elevated anti-*E. coli* antibodies and anti-citrullinated protein antibodies (ACPA) are more common and more active in the synovial joints of patients with RA [52]. The two most important autoantibodies in RA, RF and anti-citrullinated protein antibodies provide unique clinical and pathophysiological manifestations. They appear before the onset of disease symptoms, implicate the pathogenesis of RA, and predict a more severe disease course. Therefore, the combination of the two markers can improve the accuracy of RA diagnosis [46].

IL-10 levels analysis

The level of IL-10 differs significantly in RA patients' sera with UTI according to the bacterial infection, as investigated in the study. IL-10 is a part of the immune response to urinary tract infection (UTI) due to *E. coli* and other bacterial infections, and it is important in the early control of infection in the bladder. Using the ELISE technique test, the level of IL-10 was measured for 32 patients with a positive bacterial growth cultures of 26 *E. coli*, 6other bacterial growth, and 8 healthy controls. Results showed that IL-10 level ±SE increased non significantly (p≥0.05) in RA patients with UTI of *E. coli* (con24.95 pg/mL±2.64 vs. 18.39 pg/mL±2.52), respectively, in comparison to healthy controls as mentioned in Table 6. These results are in agreement with the study conducted by Abd-Alwan *et al*., 2022 [53] that showed IL-10 level±SE elevated significantly in RA patients with UTI more than control (con.214.91 pg/mL±14.09 vs 6.36 pg/mL±0.94). In addition, research of Ad'hiah *et al*. (2019) [54] explained that cytokines like IL-10 are under genetic control that regulates most aspects of innate and adaptive immune responses including inflammation, activation, migration, proliferation of cells, apoptosis, and hematopoiesis. Their action has synergistic or antagonistic effects as a result of environmental factors and complex host-pathogen interactions. Likewise, the mean level of IL-10 differs non-significantly among RA patients' sera who were infected with *E. coli* (total no. 26 isolates) when it gave mean level of IL-10 (conc.25.6 pg/ml). Otherwise, the mean level of IL-10 in RA patients who infected with *P. mirabilis* and other bacteria(9.9 pg/mL and 27.1 pg/ml) respectively, these non-significant differences appear when the comparison between the concentrations of IL-10 (pg/mL) which exhibit non-significant variation with total least significant difference (31.38) as shown in Table 7. Defining the mechanism of engagement of the immune system by the bacteria that enables the protective IL-10 response is critical to exploring how we might exploit this mechanism for new infection control strategies. Results agree with the research indicating that IL-10 increased significantly in UTI patients as an immune modulatory cytokine, especially who infected with *E. coli* (total no.18 isolates), and the IL-10 levels ranged between 74.45–413.84 pg/mL) with (S.D.=95.94) [53]. Furthermore, IL-10 plays a central role in limiting the host immune response to pathogens, preventing host damage, and maintaining normal tissue homeostasis [55]. In RA patients with UTIs, IL-10 may protect the host against exaggerated immune responses that produce inflammation and tissue damage [56]. Results revealed that the bacterial infection is an

Table 5a: Mean comparison of immunological parameters among study group (n=116 vs. 100)

Cases	ESR	S.E.	CRP	S.E.	ACPA	S.E.
Patients	61.50	1.08	39.57	2.40	59.55	1.99
Control	16.97	0.54	9.45	0.37	19.20	0.72
T	36.668		12.394		19.015	
p-value	0.0001 (P≤0.01) Highly significant		0.0001 (P≤0.01) Highly significant		0.0001 (P≤0.01) Highly significant	

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ACPA: Anti-citrullinated protein antibody, SE: Standard error

Table 5b: Mean comparison of immunological parameters among rheumatoid arthritis patients with urinary tract infection of the study group according to the type of infection (n=30)

Cases	ESR	S.E.	CRP	S.E.	ACPA	S.E.
E. coli	55.5	2.45	64.93	3.25	73.06	2.83
T	2.28		1.182		1.012	
p-value	0.047 p≤0.01: Highly significant		0.24 p≥0.05: Non-significant		0.31 p≥0.05: Non-significant	

E. coli: Escherichia coli, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ACPA: Anti-citrullinated protein antibody, SE: Standard error

Table 6: Mean comparison of IL-10 conc. among patients and control of the study (n=32 vs. 8)

Cases	Conc. pg/mL	S.E.	T-test	p-value
Patients=32	24.95	2.64	1.199	0.23
Control=8	18.39	2.52		

p≥0.05: Non-significant. SE: Standard error

Table 7: Mean comparison of IL-10 Conc. among rheumatoid arthritis patients with UTI of *E. coli* in the study's group (n=32 vs. 8)

Cases	No. 32 samples	Conc. (pg/mL)	LSD
E. coli	26	25.6	31.38
P. mirabilis	4	9.9	N.S.
Others	2	27.1	

UTI: Urinary tract infection, *E. coli: Escherichia coli*, LSD: Least significant difference

important reason that is sensed by and responsible for the induction of IL-10, particularly in the response to UPEC, and suggest that an excess of these cytokines and other inflammatory agents may cause the body to create a so-called cytokine storm [56]. Danilo G. Moriel *et al*., in 2016 indicated that UPEC is the primary cause of UTI and is generally treated empirically [6]. In addition, the dimeric structure of the specific gene (*IrmA*) in UPEC displays similarity to those of human cytokine receptors, including the IL-10 receptor (IL-10R) binding domains and other cytokines, resulting in immunogenic in UTI patients and IL-10 plays an important role in the innate immune response to UTI especially UPEC infection [6]. The results are in agreement with a previous study of Drage *et al.*, 2019 [57], which revealed that the mean IL-10 concentration was significantly elevated in patients displaying UTI with *E. coli,* and they suggest a role for IL-10 in regulating bacterial persistence. However, the rapidly increasing incidence of UTIs caused by multidrug-resistant UPEC strains has led to limited available treatment options and highlights the urgent need to develop alternative treatment and prevention strategies.

CONCLUSION

RA is a significant clinical complication in systemic autoimmunity, particularly in relation to urinary tract infections (UTIs). The clinical investigations indicate that all 26 patients infected with *E. coli* exhibit a high incidence of urinary tract infections concomitant with RA. Furthermore, most bacterial isolates demonstrate MDR, as *E. coli* possesses mechanisms for antimicrobial resistance and immune evasion through various virulence factors, including capsules, toxins, pili, and biofilm formation. The identification of 26 bacterial isolates harboring 16SrRNA and UidA virulence genes through conventional PCR. IL-10 is crucial in the innate immune response to urinary tract infections, particularly UPEC infections. Therefore, these findings highlight the need to reconsider and update empirical treatment strategies for UTIs in healthcare settings. This continuous evolvement in antimicrobial resistance patterns necessitates national surveillance studies to monitor and ensure safe and effective empiric therapy. Further studies with larger samples are important to redefine the scope of UTI, autoimmunity, and the need for further investigation into the role of IL-10 and cytokines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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