

How to Cite:

Rahim, R. I., & Abdulla, N. Y. (2022). Phenotypic and genotypic study of some virulence factors for main bacteria which caused to UPEC. *International Journal of Health Sciences*, 6(S4), 6817–6828. <https://doi.org/10.53730/ijhs.v6nS4.10308>

Phenotypic and genotypic study of some virulence factors for main bacteria which caused to UPEC

Rabab Ibrahim Rahim

ALDiwaniya hospital, Iraq

Nibras Yahya Abdulla

Department of Biology, Faculty of Science, Kufa University, Iraq

Corresponding author email: nibras.alsallami@uokufa.edu.iq

Abstract--The study was aim to isolating and identification of bacteria from urinary tract infection. The present study includes 240 samples collected from patients suffering from urinary tract infection during the period extended from September 2021 to December 2021 (female and male). From 240 specimens 160 (66.6%) samples were females and 80 (33.3 %) were males .Out of the total 240 specimens only 195 (81.3%) specimens showed significant bacterial growth ,135(69.2%) of female specimens and 60 (30.7%) from male specimens. The result of our study found that the many bacterial species are responsible for causes urinary tract infection , they have been included 9 bacterial species and the most common pathogen was *Escherichia coli* 85 (43.5%) , *Klepsiella pneumonia* 32 (16.4%) ,*Enterobacter*19(9.7%), *S. aureus* 20(10.2%) , *S. sarophyticus* 10 (5.1%) *Proteus mirabilis* 9 (4.6%), *Pseudomonas aeruginosa* 9 (4.6%), *Citrobacter koseri* 9(4.6%) and *Salmonella typhi* 2 (1%). In this study, some of virulence factor were detected by phenotypic methods while others detected by phenotypic and then genotypically by using PCR techniques. The phenotepic investigated include biofilm formation, capsule produced, hemolysis and enzymes which were protease, DNase, and gelatinase. The genotypic investigated include four genes distributed as follows: The genes of Biofilm includes (*agn43/flu* , *bcsA*).The gene of capsule's (*kpsMII*) .The gene of protease (*ompT*).

Keywords---phenotypic, genotypic UTI, E. coli, virulence factors.

Introduction

Infections of the Urinary Tract are regarded as one of the most serious illnesses, ranking second among bacterial infections in the medical community, and they are prevalent, according to estimates of the number of individuals afflicted. Around 150 million individuals every year" The cost-estimation technique for identifying and treating urinary tract infections is critical since the expenses are substantial, and laboratory testing is required to achieve a state of recovery (Medina and Castillo-Pino , 2019). According to one research in the United States, the number of UTI patients in applied departments, health departments, and emergency rooms is over 8 million cases per year, with an estimated cost of \$1 billion. The kidneys, kidneys, ureters, bladder, and urethra are among the organs of the urinary system that collect, store, and discharge urine from the body. At all ages and for both sexes, data has been collected. The presence of microbial causes in the urinary system is characterized as a wide spectrum of symptoms ranging from minor discomfort to bacteremia. Bacteremia and sepsis are both life-threatening conditions (Sikora and Zahra, 2022). Urine is sterile, meaning it is free of germs, but it also includes fluids, salts, and waste products, as well as contamination from a variety of flora on the skin. As it goes through the urethra, the germs congregate and grow inside the urethra. UTIs are caused when bacteria enter the bladder or kidneys and grow in the urethra, with the most common variety being "bladder infection," sometimes known as a bladder infection (WHO, 2014). Many virulence factors are present in bacteria that cause urinary tract infections, which assist them, bind to the surfaces of epithelial cells lining the urinary system. The bacteria attach themselves to the lipopolysaccharide receptors on the surface of the urothelial cells. Many virulence features relevant to urinary tract infection are present in these *E. coli* strains, including the development of particular adhesions and toxins (Martinson and Walk, 2020).

Methodology

Bacteria

Urinary samples collected from diabetes patients in diabetes center in AL-Sader Hospital ,after collected the samples each sample processed as the following

1. Urinary sample centrifuged in 3000rpm for 5m.
2. Discharged the supernatant layer and take pellet layer .
3. Take some drops from a pellet layer and cultured on blood agar, MacConkey agar and Mannitol salt agar.
4. After growth the bacteria on this media the bacteria diagnosed by biochemical test.

Biofilm formation

Tissue culture plate method (TCP)

Detection of biofilm formation by tissue culture plate method (TCP) assay according to (Lizcano et al., (2010).

Congo red Agar method (CRA)

Detection of biofilm formation by Congo red Agar method (CRA) assay according to (Freeman *et al.*,1989).

Protease, Gelatinase, Hemolysin and DNase production

Detection of Protease, Gelatinase, Hemolysin and DNase production were prefer by culture on Skim – Milk agar, nutrient gelatine agar, blood agar and dnase agar respectively .

Extraction of Genomic DNA

DNA of The isolated bacteria was prepared by boiling method. Briefly, colonies were suspended in 100 microliters of sterile distilled water and boiled at 100C in the water bath for 15 minutes than rapidly cooled at -20°C for one hour, then centrifugation and the supernatant were preserved for the used in the amplification-processes (Shah *et al.*, 2017).

Gene amplification

The primers used in the PCRs are described in table 1.The DNA extract of bacterial isolates were subjected to primers genes as following: 8µL Master mix, 5µL DNA template, 1.5µL for each primers, 4µL Deionized water (dd water) by using PCR. The protocol was used depending on Promega Biosystem manufacturer's instruction. Single reaction (final reaction volume 20 µl). PCR was performed in an Eppendorf cycler using the following program: 5 mins denaturation at 94°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 52°C, and 30 sec at 72°C, with a final extension of 7 mins at 72°C(*kpsMTII*). Denaturation (94 °C for 30 sec), annealing (52 °C for 30 sec) and extension (72 °C for 90 sec), followed by one cycle consisting of 5 min at 72 °C in a thermal cycler(*ompT*). 3 min at 95°C, 30 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, and a final extension for 5 min at 72°C(biofilm gene).

Table 1: Primers used in this study

Gene	Primer sequence (5'-3')	PCR product size	Reference
<i>agn43/flu</i>	F:GGGTAAAGCTGATAATGTCG R:GTTGCTGACAGTGAGTGTGC	508	Ulett, (2007)
<i>bcsA</i>	F: GCTTCTCGGCGCTAATTTG R:GAGGTATAGCCACGACGGTG	826	Juliane,(2017)
<i>kpsMTII</i>	F: GCGCATTGCTGATACTGTTG R:CATCCAGACGATAAGCATGAGCA	272	Souza,(2019)
<i>ompT</i>	ATCTAGCCGAAGAAGGAGGC R:CCCGGGTCATAGTGTTCATC	559	Zhao,(2009)

Result and Discussion

Distribution of the specimens collected from Urinary Tract Infection

A total 240 specimens collected from the hospitals and private laboratories from patients with Urinary Tract Infection during the period extended from September 2021 to December 2021 (female and male), 160 (66.6%) samples, were females and 80 (33.3 %), were males .Out of the total 240 specimens only 195 (81.3%) specimens showed significant bacterial growth ,135(69.2%) of female specimens and 60 (30.7%) from male specimens table (2) .Our results were closed for the result of (.Middelkoop *et al.*,2021) who found the ratio infection in female was 65% and in male 35% .The women are more susceptible than men due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Awanesset *al.*, 2000).

Table 2: Distribution of the specimens from Urinary Tract Infection Specimens

No.	Type of patient	Number of sample	%	Number of Sample that bacterial growth appeared	%
1	Female	160	66.6	135	69.2
2	Male	80	33.3	60	30.7
Total		240		195	

Distribution of Bacterial Species that Causes of Urinary Tract Infections

Bacterial isolates were diagnosed by using the bacterial cultured , the biochemical tests and Vitek 2 system ,the results showed present nine bacterial species included *Escherichia coli* 85 (43.5%) , *Klebsiella pneumonia* 32 (16.4%) ,*Enterobacter*19 (9.7%), *S.aureus* 20(10.2%) , *S. sarophyticus* 10 (5.1%) *Proteus mirabilis* 9 (4.6%), *Pseudomonas aeruginosa* 9 (4.6%), *Citrobacter koseri* 9(4.6%) and *Salmonella typhi* 2 (1%), figure (1) . The finding does not correlate with the findings of Iyevhobu *et al.*, (2020) which reported that *Staph.aureus* was the most frequently occurring organism (40%) and *Proteus mirabilis* being the second most frequent organism (20%).

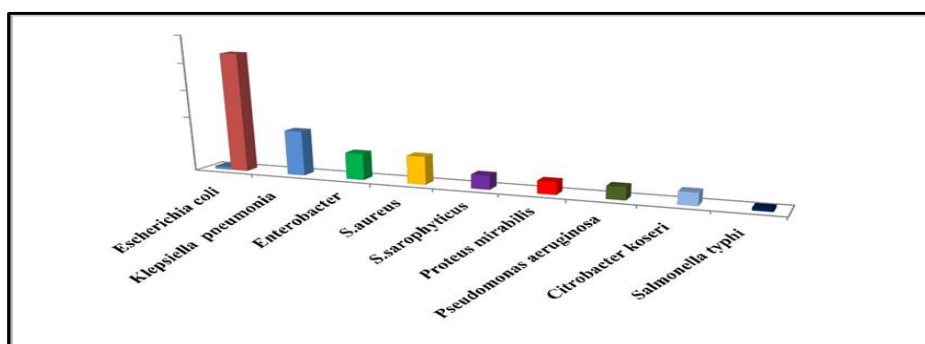


Figure 1: Percentage of frequency the bacterial species that causes Urinary Tract Infection

Phenotypic Detection of Some Virulence Factors for *E. coli*

In this study, some of these VFs were detected by traditional phenotypic methods while others detected by phenotypic and then genotypically by using PCR techniques. These tests were applied on most common bacteria in UTI which was *E.coli*. The investigated include biofilm formation, capsule produced, hemolysis and enzymes which were protease, DNase, and gelatinase.

Biofilm Production

Congo-Red Agar Method (CRA)

The results showed that 90% from *E. coli* produced strong and moderate slime layer these isolated distribution between 76.6% isolates produced strong slime layer indicated by formation of black colonies with dry crystalline consistency and 13.3% isolates were moderate for slime producer indicated by formation of brown or semi black colonies, while 10% isolates did not produce slime layer indicated by formation of pink colonies as shown in figure (2). The slime layer works on the bacterial cell packaging, forming thin, living membranes known as biofilm its act as a buffer that inhibits the antibiotic influence within the bacteria cell and thus confers resistance (Al-Khafaji, 2018).

Micro Titer Plates (MTP) Method

The present study showed that 95% from *E.coli* produced strong and moderate biofilm these isolated distribution between 36.6% of *E.coli* isolates appeared high biofilm formation and 58.3% of *E.coli* isolates showed moderate biofilm formation, while 5% isolates did not produce biofilm (Table 3).Our result was agree with result of (Tessa *etal.*,2020) which found that about 94% of *E. coli* had the ability to produce the biofilm.

Table 3: Distribution of *E. coli* Isolates According to the Types of Biofilm

Stander rang of OD	Biofilm	<i>E. coli</i> No. (%)
<0.120	Non	5%
0.120-0.240	Moderately	58.3%
>0.240	High	36.6%

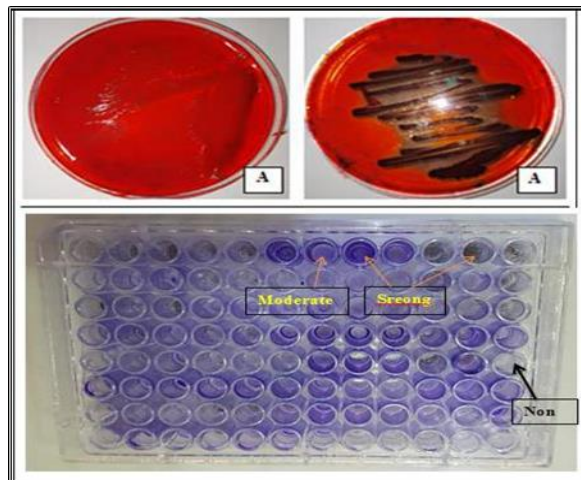


Figure 2: Virulence factors of *E. coli* (A): on Congo red agar Biofilm production isolates, (B): Biofilm formation of *E. coli* on microtiter plate.

Detection of capsule producing

The results recorded in (Table 4), showed that 25% of isolates were capsulated *E. coli* and 75% of isolates were non capsulated. The results showed that high number of isolates no capsulated. The role of the capsule in protecting UPEC from phagocytosis has been defined, however, its role in resistance to serum-mediated killing is unclear, with several conflicting reports in the literature. Bacterial lipopolysaccharide (LPS) plays a primary role in mediating UPEC resistance to the bactericidal activity of human serum (Totsika, *et al.*, 2012).

Production of hemolysin

Results showed that 55 % of *E. coli* isolates were positive to hemolysin production and gave beta-hemolytic on blood agar while only 45% of *E. coli* were non producer hemolysin (Table 4). Our result with agreed Nguyen (2019) who has reported that 50% of isolates produce hemolysin. The function of hemolysin has been associated with exfoliation of uroepithelial cells in human bladder organoids as well as inhibition of the proinflammatory cytokine interleukin-6 (IL-6) from human bladder epithelial cells and peritoneal macrophages (Hilbert, *et al.*, 2012).

Production of Protease

Results showed that 78.3 % of *E. coli* isolates were positive to protease production and gave clear zone around the colonies (figure 3) while only 21.6% of *E. coli* were non producer protease (Table 4). Our result agreed with (Isabelle, *et al.*, 2019) who has reported that 74% of isolates produce protease, protease contribute to virulence by cleaving a variety of proteins and peptides (Isabelle, *etal.*, 2019).

Production of Gelatinase

The results showed that 33.3% of *E. coli* isolates produced gelatinase enzyme and 66.6% of isolates not produce gelatinase enzyme (Figure 3 and Table 4). This

result agreement with Iswarya *et al* , (2019) who pointed out that 34% of *E.coli* gelatinase. The gelatinase is an important enzyme that increases the pathogenicity of bacteria as the enzyme works to break down the gelatin into the basic units from amino acids (Monireh, *et al.*,2021).

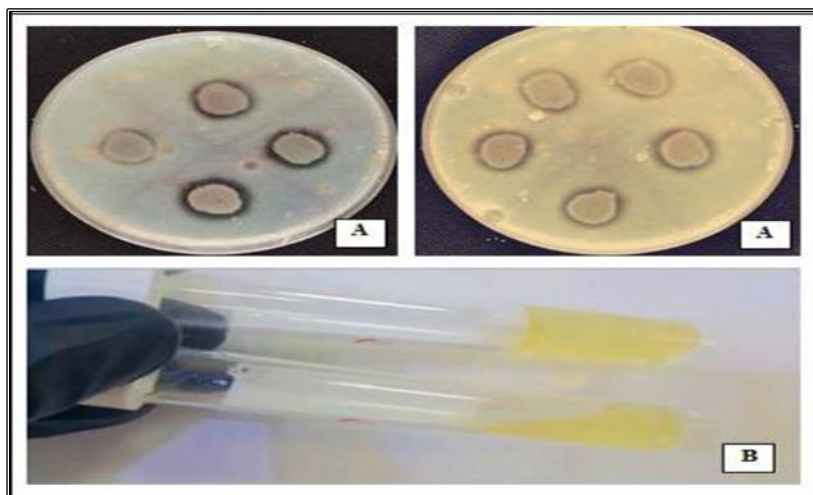


Figure 3: Virulence factors of *E. coli* (A): Production of Protease, (B): Production of Gelatinase

Production of DNase

Results showed that none of the isolates gave positive result on DNase agar (Table 4-4).

Table 4: The Percentage of Some Virulence Factors Produced by *E. coli*

Virulence Factors	Positive (%)	Negative (%)
Capsule	(25%)	(75%)
Hemolysin	(55 %)	(45%)
Protease	(78.3 %)	(21.6%)
Gelatinase	(33.3%)	(66.6%)
DNase	(0%)	(100%)

Virulence factors genes detection

In the current study we selected 30 *E.coli* isolated and used genes related to virulence factors, with four genes distributed as follows:

1. The genes of Biofilm includes (*agn43/flu* , *bcsA*).
2. The gene of capsule's (*kpsMII*) .
3. The gene of protease (*ompT*) .

agn43/flu gene

The result showed that the *agn43/flu* gene was detected in 18/30 (60%) *E. coli* as in Figure (4). *agn43* encodes a 1,039 amino acid product with a 52 amino acid N-terminal signal sequence. *Agn43/flu* is important for biofilm formation in *E. coli* cells grown in glucose-minimal medium (Henderson, and Owen, 1999). *Agn43/flu* (and flagella-driven motility) is required for the aggregating phenotype seen in *E. coli* grown at 37°C to mid-exponential phase; *Agn43/flu* dependent aggregation promotes resistance to oxidative stress and biofilm formation (Hasman, *et al.*, 1999). Our result was agreement with Juliane *et al.*, (2017) they reported that the gene of *Agn43/flu* was approved in 58.3% of the samples.

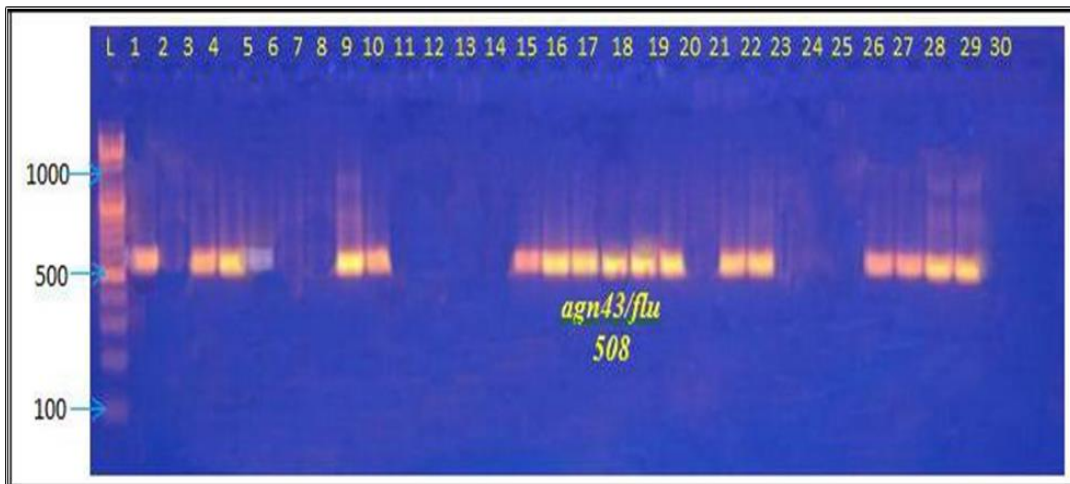


Figure 4: PCR amplification products of *E. coli* isolates that amplified with *agn43/flu* gene primers with product 508 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1, 3, 4, 5, 8, 9, 14, 15, 16, 17, 18, 19, 21, 22, 25, 26, 27, 28, 29) show positive results with the *agn43/flu* gene.

bcsA gene

The outcome revealed that the *bcsA* gene was present in 22/ 30 (73.3%) *E. coli* as in figure (5). This result was disagreement with Juliane *et al.*, 2017 which showed *bcsA* gene present in (100%) of *E. coli*. The deletion of the *bcsA* gene, which is required for cellulose formation, resulted in a large increase in curli-dependent adhesion, which is consistent with this observation. We discovered that increasing cellulose synthesis enhanced tolerance to desiccation, indicating that cellulose's role is connected to environmental stress resistance rather than biofilm formation. The curli/cellulose network is commonly produced in enterobacteria at low growth temperatures (32 C), but not at 37 C (Gualdi, *et al.*, 2008).

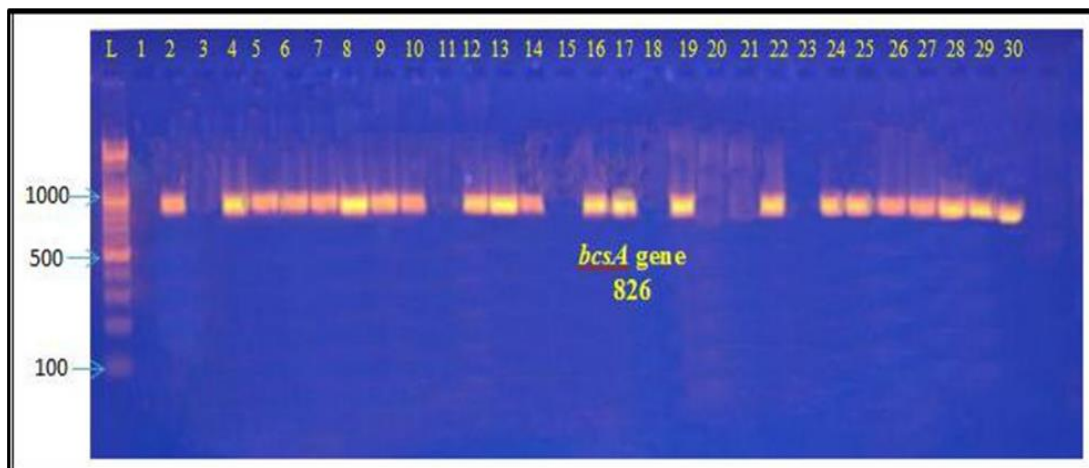


Figure 5: PCR amplification products of *E. coli* isolates that amplified with *bcsA* gene primers with product 826 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (2, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 16, 17, 19, 22, 24, 25, 26, 27, 28, 29, 30) show positive results with the *bcsA* gene.

kpsMII gene

The result revealed that the *kpsMII* gene was detected in 7/30 (23.3%) *E. coli* as in Figure (6). Our result was disagreement with Gabrielle et al., 2019 which showed *kpsMII* gene was detected in (56%) of *E. coli*. Extraintestinal pathogenic *E. coli* (ExPEC) polysaccharide capsules are essential both pathogenetically and taxonomically (Johnson, and O'Bryan, 2004).

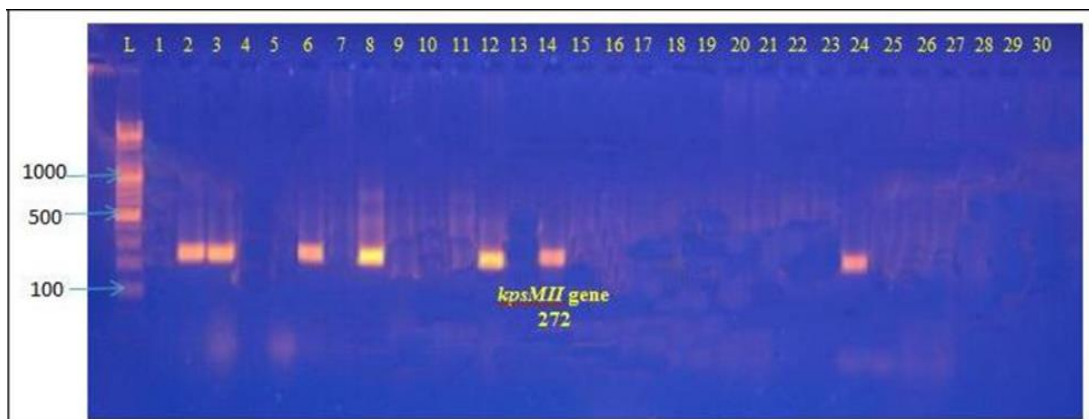


Figure 6: PCR amplification products of *E. coli* isolates that amplified with *kpsMII* gene primers with product 272 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (2, 3, 6, 8, 12, 14, 18, 24) show positive results with the *kpsMII* gene *ompT* gene

The result showed that the *ompT* gene was detected in 20/30 (66.6%) *E. coli* as in Figure (7). This result was agreement with Soheila et al., 2019 which reported present *ompT* gene (63%) of *E. coli*. The product of the *ompT* gene contributes to virulence by inactivating host defense proteins, cleaving host cell-surface peptide

to expose them or form receptors for the pathogen, and protamine inactivation, among other things (Stumpe, et al., 1998 ; Santo, et al., 2006).

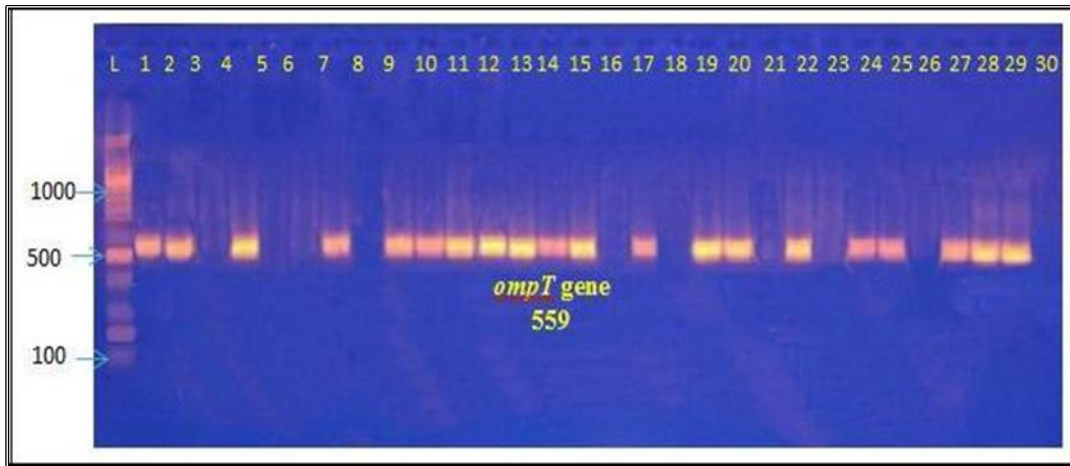


Figure 7: PCR amplification products of *E. coli* isolates that amplified with *ompT* gene primers with product 559 bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1, 2, 4, 5, 7, 9, 10, 11, 12,13, 14, 15, 17, 19, 20, 22, 24, 25, 27, 28, 29) show positive results with the *ompT* gene.

Reference

- Al-Khafaji, A. N. (2018). Isolation and Identification of Methicillin Resistance Staphylococcus aureus and Detection their Ability to the Production of Virulence Factors. *Journal of University of Babylon for Pure and Applied Sciences*, 26(8), 100-111.
- Awaness, A.M., Al-Saadi, M.G and Aadoas, S.A. (2000): Antibiotics resistance in recurrent urinary tract infection; *Kufa. Med. J*, 3: 159.
- Dhami, R. Ilah M. A.-A., Kadhim, B. M., & Abdullhusein, H. S. (2020). A serological study to diagnose the causes of recurrent viral and immune miscarriage in aborted women who attend the shatrah general hospital. *International Journal of Health & Medical Sciences*, 3(1), 42-47. <https://doi.org/10.31295/ijhms.v3n1.131>
- Freeman, D. J.; Falkiner, F. R.; and Keane, C.T. (1989). New method for detecting slime production by coagulase negative staphylococci. *J. Clin. Pathol.* 42:872-874.
- Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. *Infect Immun* 75:3233–3244. <https://doi.org/10.1128/IAI.01952-06>.
- Gabrielle ,M.; Estevan ,R.; Alaor, M.;Maria, V.; Marcus ,V. et al.(2019). Comparative Study Of Genetic Diversity, Virulence Genotype, BiofilmFormation And Antimicrobial Resistance Of Uropathogenic Escherichia coli (UPEC) Isolated From Nosocomial And Community Acquired Urinary Tract Infections. *Infection and Drug Resistance*,12 : 3595—3606.
- Gualdi, L. ; Tagliabue, L. ; Stefano, B. ; Teresa, I. ; Cristina De, C. and Paolo L. (2008). Cellulose modulates biofilm formation by counteracting curli-mediated

- colonization of solid surfaces in *Escherichia coli*. *Microbiology* (2008), 154, 2017–2024, DOI 10.1099/mic.0.2008/018093-0.
- Hasman, H., Chakraborty, T., & Klemm, P. (1999). Antigen-43-mediated autoaggregation of *Escherichia coli* is blocked by fimbriation. *Journal of bacteriology*, 181(16), 4834–4841. <https://doi.org/10.1128/JB.181.16.4834-4841.1999>.
- Henderson, I. R. & Owen, P. (1999). The major phase-variable outer membrane protein of *Escherichia coli* structurally resembles the immunoglobulin A1 protease class of exported protein and is regulated by a novel mechanism involving Dam and oxyR. *Journal of bacteriology*, 181(7), 2132–2141.
- Hilbert, D.W., Paulish-Miller TE, Tan CK, Carey AJ, Ulett GC, Mordechai E, Adelson ME, Gygas SE, Trama JP. 2012. Clinical *Escherichia coli* isolates utilize alpha-hemolysin to inhibit in vitro epithelial cytokine production. *Microbes Infect* 14:628–638.
- Isabelle, D., James, A. T., Jean-Mathieu, L., et al (2019). Identification and characterization of OmpT-like proteases in uropathogenic *Escherichia coli* clinical. *Microbiologyopen*, 18 (11).
- Iswarya , J. ; Indira, A. ; Swasti, C. ; Arun, J. ; Rani ,D. S. ; and Jude, J. F. (2019). Urinary Neutrophil Gelatinase Associated Lipocalin – A Sensitive Marker for Urinary Tract Infection in Children. *Indian J Nephrol.* ; 29(5): 340–344.
- IYEVHOBU, K.; OSHIO ,K.; OBODO, B.; OBHIOZELE ,B.; OMOLUMEN ,L. E.; and IROBONOSEN, O.(2020). Urinary Tract Infection among Male and Female Students in Ambrose Alli University, Ekpoma, Edo State, Nigeria. *Global Scientific Journal* , 8 (4) :712-717.
- Johnson, J. R., & O'Bryan, T. T. (2004). Detection of the *Escherichia coli* group 2 polysaccharide capsule synthesis Gene kpsM by a rapid and specific PCR-based assay. *Journal of clinical microbiology*, 42(4), 1773–1776.
- Juliane, S.; Alexander ,B. ;Jörg ,N.; Michał ,B.;Jörg ,W.et al.(2017). Genotypic and Phenotypic Characteristics Associated with Biofilm Formation by Human Clinical *Escherichia coli* Isolates of Different Pathotypes. *Applied and Environmental Microbiology*,83(24).
- Lizcano, A; Chin, T; Sauer, K; Tuomanen, E. I., and Orihuela, C. J. (2010). Early biofilm formation on microtiter plates is not correlated with the invasive disease potential of *Streptococcus pneumoniae*. *Microbial pathogenesis*, 48(3-4): 124-130.
- Martinson, J. and Walk, S. T. (2020). *Escherichia coli* Residency in the Gut of Healthy Human Adults. *EcoSal Plus*, 9(1), 10.1128/ecosalplus.ESP-0003-2020. <https://doi.org/10.1128/ecosalplus.ESP-0003-2020>
- Medina, M. and Castillo-Pino, E. (2019). An introduction to the epidemiology and burden of urinary tract infections. *Therapeutic advances in urology*, 11, 1756287219832172. <https://doi.org/10.1177/1756287219832172>.
- Middelkoop S.J.M.; van Pelt L.J.;Kamping G.A.; Maatend J.C.and Stegeman C.A.(2021). Influence of gender on the performance of urine dipstick and automated urinalysis in the diagnosis of urinary tract infections at the emergency department. *European Journal of Internal Medicine*,87 : 44-50.
- Monireh,M. ,Mehdi D.,Behnaz,K.,Mohammad,H., Hassan ,R.(2021). Colorimetric and label free detection of gelatinase positive bacteria and gelatinase activity based on aggregation and dissolution of gold nanoparticles. *Journal of Microbiological Methods*,191.

- Nguyen ,T. N.; Minh-Duy ,P. ;Brian, M. F.et al.(2019). Complex Multilevel Control of Hemolysin Production by Uropathogenic Escherichia coli. American Society for Microbiology,10(5).
- Santo, E. ; Macedo, C. and Marin, J.M. (2006).Virulence factors of uropathogenic Escherichia coli from a university hospital in Ribeirao Preto, Sao Paulo, Brazil. Rev. Inst. Med. Trop. S. Paulo.;48(4):185-188.
- Shah, P. K. ; Santella, A. ; Jacobo, A. ; Siletti, K. ; Hudspeth, A. J. ; Bao, Z. (2017). An In Toto Approach to Dissecting Cellular Interactions in Complex Tissues.
- Sikora, A. and Zahra, F. (2022). Nosocomial Infections. [Updated 2021 Aug 10].In: StatPearls [Internet]. *Treasure Island (FL): StatPearls Publishing.*
- Soheila, N.; Morad, R. and Zahra, N.(2019). Extra-intestinal pathogenic Escherichia coli from human and avian origin: Detection of the most common virulence-encoding genes.Veterinary Research Forum.,10 (1) 43-49.
- Souza, G.M.; Neto E.; Silva A.M.; Iacia M.V. ;Rodrigues ,M.V.and Pereira ,V.C.(2019). Comparative Study Of Genetic Diversity, Virulence Genotype, Biofilm Formation And Antimicrobial Resistance Of Uropathogenic Escherichia coli (UPEC) Isolated From Nosocomial And Community Acquired Urinary Tract Infections. Dove Medical Press Ltd, 12 :3595—3606.
- Stumpe, S. ; Schmid, R. ; Stephens, D.L. ; Georgiou, G. and Bakker, E.P. (1998). Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of Escherichia coli. J. Bacteriol.;180(15):4002-4006.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, 5(1), i-v. <https://doi.org/10.53730/ijhs.v5n1.2864>
- Tessa, E. F.; Erin, E. R.; Christopher, P. and Bryan , B. (2020). Evaluation of Biofilm Production by Escherichia coli Isolated From Clinical Cases of Canine Pyometra. Topics in Companion Animal Medicine,39.
- Totsika, M, Moriel, D. G. ; Idris, A. ; Rogers, B. A. ; Wurpel, D. J. , et al. (2012). Uropathogenic Escherichia coli mediated urinary tract infection. Curr Drug Targets 13: 1386–1399.
- Ulett, G.C.; Valle, J.; Beloin ,C.; Sherlock, O.; Ghigo, J.-M.; Schembri, M.A.(2007).
- WHO, (2014). Safe management of wastes from health-care activities, 2nd edition.
- Zhao L, Gao S, Huan H, et al.(2009). Comparison of virulence factors and expression of specific genes between uropathogenic Escherichia coli and avian pathogenic E. coli in a murine urinary tract infection model and a chicken challenge model. Microbiology; 155(5): 1634-1644.