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# A Study of Urinary Tract Infections Prevalence, Antibiotics Resistance, and Biofilm Formation Capability of the Bacterial Causal Agents

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## ABSTRACT

The aim of this study was to evaluate the incidence of UTI in Salahadin governorate/Iraq, identify the causative agents, their antibiotic sensitivity and their ability to form biofilms; such studies are mandatory to determine the empirical therapy of such cases. A total of 650 urine samples were collected. Two hundred and fifty samples were cultured, as suspected of having UTI, and 193 samples (77.2 %) showed growth (Positive culture), while 57 samples (22.8 %) were negative cultures. Gram-positive bacteria were the predominant cause of UTI (66.7%), while Gram-negative species were found in 33.3%. *Staphyloccus spp.* were the predominant Gram-positive genus to isolate from UTI patients, while *E. coli* was the predominant among Gram-negative bacteria. The isolates were tested for antibiotic susceptibility and biofilm formation. The most effective tested antibiotic on the Gram-positive isolates was Nitrofurantoin followed by Chloramphenicol (resistance percentage 19 and 20% respectively), and the least effective one was Azithromycin (64% resistance). While the most effective tested antibiotic on the Gram-negative isolates was Amikacin (only 8% resistance), and the least effective one was erythromycin (98% resistance). Biofilm detection by Congo red agar (CRA) method and microtiter plate (MTP) assay were done; the results showed that by CRA method 81.3% of isolates were biofilm formers. While 96.7% of isolates were detected as biofilm formers by microtiter plat assay. Generally, biofilm formation was more predominant among Gram-positive isolates than Gram-negative ones.

## Introduction

Urinary tract infection (UTI) is the infection that leads to an inflammatory response in the urinary tract epithelium [1]. In communities and hospitals, one of the most commonly encountered infections is urinary tract infections (UTIs). Clinically, UTIs can be complicated or uncomplicated. Uncomplicated UTIs affect individuals which are otherwise healthy with no abnormalities in the structure of the urinary tract. On the other hand, complicated UTIs occur in individuals with abnormalities in the structure of the urinary tract, immunosuppression, or with indwelling urinary catheters [2]. Most of the UTIs are of bacterial origin, but some are due to fungi and seldom to viruses' infection[3].

For the last three decades bacterial resistance has been going on among UTI-causing pathogens and the available reports and data confirm that the elevation in resistance to commonly utilized antibiotics is a sequel of inappropriate use of the antimicrobials. The surfacing of resistance amongst the UTI pathogens is an issue of intense concern and requires urgent attention in order to derive appropriate remedies to overcome the trouble [4].

Bacterial biofilms play a significant role in UTIs, being the cause of both acute and persistent infections. Up to 80% of all infections involve biofilm-forming bacteria, and mainly in the urinary tract, biofilm can become a serious problem. The antimicrobial resistance shown by biofilms is one of

the most important concerns of these structures. Biofilm can be resistant to antibiotics up to 1,000-fold more than planktonic cells as a result of several mechanisms [5].

**Methodology**

**Samples collection and bacterial identification**

Urine samples were collected during the period from 1/12/2020 to 1/2/2021 from out and inpatients with different health issues admitted to Al-Alam and Salahadin General Hospitals. The method of urine collection was clarified to patients carefully. Each urine sample cup was labelled with name, age, sex, and time of collection[6]. Samples were analyzed using the dipstick and sediment microscopic examination, and those suspected of UTI were submitted to the microbial culture method. All samples were inoculated on blood agar as well as MacConkey agar, incubated at 37°C for 24 hours and then inspected for bacterial growth[7]. Bacterial colonies were primarily classified by Gram-stain morphology and then definitively identified depending on standard culture and biochemical characteristics of isolates by using the Vitek-2 system (bioMerieux) according to the manufacturer's instructions.

**Antimicrobial Susceptibility Testing**

This test was performed by the Kirby-Bauer method on Mueller Hinton agar (HiMedia, India) depending on Clinical Laboratory Standards Institute (CLSI, 2021) guide and according to Bahador *et al.* (2019).[8,9] Antibiotics that were tested in our study are mentioned in table1.

**Table 1: Antibiotic discs utilized in this study**

| Antibiotic group | Antimicrobial agent (Code) | Dose /Disc | Antibiotic group | Antimicrobial agent (Code) | Dose /Disc |
|---|---|---|---|---|---|
| Penicillins | Oxacillin (OX) | 5µg | Aminoglycosides | Amikacin (AK) | 10µg |
| | Ampicillin (AM) | 25µg | | Gentamicin (CN) | 10µg |
| β_lactamase inhibitor combination | Amoxicillin+ clavulanic acid (AMC) | 20/10µg | Fluoroquinolones | Ciprofloxacin (CIP) | 10µg |
| Folate pathway inhibitor | Trimethoprim (TMP) | 10µg | Phenicol | Chloramphenicol (C) | 30µg |
| Macrolides | Erythromycin (E) | 10µg | Glycopeptides | Vancomycin (VA) | 30µg |
| | Azithromycin (AZM) | 15 µg | Carbapenems | Meropenem (MEM) | 100µg |

**Detection of biofilm formation**

**a) Congo red agar (CRA) method**

CRA plates were inoculated with bacteria and incubated at 37°C /24 hrs. The plates were examined at 24 and 48 hrs. to view the color of grown colonies. Black colonies were recorded as a positive result whereas non-slime producing strains formed red colonies; color shades between black and red were interpreted as different slime production intensities [10,11].

**b) Microtiter plate (MTP) method**

MTP assay is the most broadly used and regarded as the standard test for the detection of biofilm formation. The test was done as designated by Christensen *et al.*(1985) with an alteration in incubation time which was lengthened to 24 hrs., as described by O'Toole and Kolter (1998).[12,13] Bacterial isolates were incubated in nutrient broth at 37°C/24 hrs.; bacterial growth was diluted in 1:200 and inoculated into micro-titer plates (96 flat-bottom well micro-titer plates). After 24 hrs., PBS buffer was used to wash the wells 2-3 times and left to air-dry. Subsequently, crystal violet solution (0.4 %) was added as a stain for 10 min. Afterwards, the plates were washed-off by sterilized distilled water and left to dry at room temperature. Then, ethanol 70% was added to quantify bound bacteria. Finally, the absorbance at 490 nm was determined; an OD of 490 nm > 0.12 was considered as a biofilm forming sample. Heavy biofilm OD > 0.240, Moderate biofilm OD= 0.120-0.240, and No or weak biofilm OD < 0.120 [14]

## Results and discussion

**Incidence of UTI**

In the current study, a total of 650 urine samples were collected. After early diagnosis, two hundred and fifty samples were cultured as suspected of UTI, 193 samples (77.2 %) showed growth (Positive culture), while 57 samples (22.8%) were negative cultures. Two hundred and twenty samples (88 %) were from females and 30 were from males (12%). One hundred and eighty-eight females (75.2%) were married and 37 of them (14.8%) were pregnant. Samples ranged in age from 5-62 years (Table 2). The major rate of UTI (39.2 %) was seen in (21-30) years group, shadowed by (31-40) years (24%).

**Table 2: Age groups in the present study**

| Age group | Number of samples | Percentage |
|---|---|---|
| **Less than ten years old** | 10 | 4 % |
| **10-20** | 32 | 12.8 % |
| **21-30** | 98 | 39.2 % |
| **31-40** | 60 | 24 % |
| **41-50** | 25 | 10 % |
| **51-60** | 15 | 6 % |
| **Older than 60** | 10 | 4 % |
| **Total** | 250 | 100 % |

The differences in isolation rate are due to differences in the type and volume of the study population, and duration of the study. The percentage of negative cultures despite being positive in urinalysis may be

due to infection with anaerobic bacteria, fungi or viruses that are not obtainable in routine culture methods used in this study[15]. Females are more predisposed to have UTIs than males because the urethra is closer to the anus and much shorter. Sexual contact improves ascending of the organisms, as well, vaginal microbiota play a serious role in boosting colonization of coliforms in the vagina and this may cause UTI.[16,17] In the course of pregnancy, alterations in the urinary tract predispose women to infections. Those alterations include Ureteral dilation, because of the pressure on ureters from the gravid uterus; Hormonal effects of progesterone, which also may cause smooth muscle relaxation leading to dilation, and vesicoureteral reflux increases; Immunocompromising can be another reason for the increased frequency of UTIs seen in pregnancy[18].

**Etiological bacterial agents**

After excluding samples that are suspected of being contaminated, Gram staining reviled that one hundred isolates (66.7%) were Gram-positive, while 50 isolates (33.3%) were Gram-negative. Types and percentages of isolated bacteria are shown in table 3.

**Table 3: Number of bacterial isolates and their percentages**

| Bacterial species | Number of isolates | Percentage |
|---|---|---|
| *Staphylococcus aureus* | 32 | 21.33 % |
| *Enterococcus faecalis* | 23 | 15.33 % |
| *Escherichia coli* | 20 | 13.33 % |
| *Staphylococcus haemolyticus* | 14 | 9.33 % |
| *Klebsiella oxytoca* | 11 | 7.33 % |
| *Staphylococcus epidermidis* | 11 | 7.33 % |
| *Staphylococcus saprophyticus* | 8 | 5.33 % |
| *Streptococcus agalactiae* | 6 | 4 % |
| *Klebsiella pneumoniae subspp. Pneumonia* | 5 | 3.33 % |
| *Citrobacter freundii* | 5 | 3.33 % |
| *Serratia fonticola* | 5 | 3.33 % |
| *Micrococcus luteus* | 4 | 2.67 % |
| *Pseudomonas aeroginosa* | 2 | 1.33 % |
| *Proteus mirabilis* | 2 | 1.33 % |
| *Kocuria rosea* | 1 | 0.67 % |
| *Kytococcus sedentarius* | 1 | 0.67 % |

Abd-Alwahab and Thalij (2015) study results agree with ours, her isolates were 68.5% Gram-positive and 31.5% were Gram-negative bacteria from UTI patients in Tikrit [19]. Our results are close to those of Abdullah (2008) in Musol whose isolates were 56.7% Gram-positive and 43.4% Gram-negative bacteria [20]. These results disagree with those of Mahdi (2020) who reported that 92% of her isolates were Gram-negative and only 8% were Gram-positive bacteria in Tikrit [21]. Al-Tikrity (2016) had the opposite of our results, he found that 66% of the isolated bacteria were Gram-negative and 34% were Gram-negative in UTI patients in Erbil [22].

**Antibiotic resistance of Gram-positive bacteria**

Generally, the most effective tested antibiotic on the Gram-positive isolates was Nitrofurantoin followed by Chloramphenicol, and the least effective one was Azithromycin, as illustrated in table 4.

**Table 4: Antibiotics Resistance for Gram-positive bacterial isolates**

| Bacteria | No. of isolates | Antibiotics No. of resistant isolates (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **F** | **C** | **OX** | **MEM** | **TMP** | **AK** | **CIP** | **AMC** | **AZM** | CN |
| ***Staphylococcus aureus*** | 32 | 5 (15.6) | 5 (15.6) | 22 (68.8) | 6 (18.8) | 11 (34.4) | 10 (31.3) | 4 (12.5) | 10 (31.3) | 19 (59.4) | **15 (46.9)** |
| ***Staphylococcus haemolyticus*** | 14 | 4 (28.6) | 5 (35.7) | 8 (57.1) | 4 (28.6) | 5 (35.7) | 4 (28.6) | 4 (28.6) | 7 (50) | 8 (57.1) | **4 (28.6)** |
| ***Staphylococcus epidermidis*** | 11 | 2 (18.2) | 3 (27.3) | 7 (63.3) | 2 (18.2) | 6 (54.5) | 3 (27.3) | 4 (36.4) | 4 (36.4) | 8 (72.7) | **4 (36.4)** |
| ***Staphylococcus saprophyticus*** | 8 | 3 (37.5) | 2 (25) | 5 (62.5) | 6 (75) | 5 (62.5) | 5 (62.5) | 5 (62.5) | 4 (50) | 6 (75) | **4 (50)** |
| ***Enterococcuus faecalis*** | 23 | 2 (8.7) | 4 (17.4) | 9 (39.1) | 5 (21.7) | 10 (43.5) | 11 (47.8) | 4 (17.4) | 10 (43.5) | 14 (60.9) | **12 (52.2)** |
| ***Streptococcus agalactiae*** | 6 | 0 | 0 | 4 (66.7) | 1 (16.7) | 1 (16.7) | 2 (33.3) | 2 (33.3) | 1 (16.7) | 6 (100) | **2 (33.3)** |
| ***Micrococcus luteus*** | 4 | 3 (75) | 0 | 1 (25) | 0 | 3 (75) | 0 | 1 (25) | 0 | 2 (50) | **0** |
| ***Kocuria rosea*** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| ***Kytococcus sedentarius*** | 1 | 0 | 1 (100) | 1 (100) | 0 | 1 (100) | 1 (100) | 1 (100) | 0 | 1 (100) | **1 (100)** |
| **Total resistance percentage** | **100** | **19** | **20** | **57** | **24** | **42** | **36** | **25** | **36** | **64** | 42 |

The current results were close to other studies at some points and disagree at others. For example, Nigussie and Amsalu (2017) found that their Gram-positive isolates were 100% sensitive to

nitrofurantoin, showed a high resistance ratio to ciprofloxacin (50.0%), trimethoprim-sulphomethoxazole (43.8%) and almost 25% resistance to oxacillin and amoxicillin +clavulanic acid were detected[23]. Woldemariam *et al*. (2019) reported that among Gram-positive organisms, *Staph. aureus* was 100% susceptible to ciprofloxacin and nitrofurantoin; Coagulase negative Staphylococci (CONS) were highly sensitive to amoxicillin +clavulanic acid and nitrofurantoin 6/7(83.3%); Vancomycin (83.3%) was the drug of choice with superior effectiveness on *Enterococcus spp*., whereas 2/6 (33.3%) of the *Enterococcus spp*. were nitrofurantoin resistant.[24] Petca *et al*. (2020) agree with our result that nitrofurantoin showed major effectiveness against Gram-positive bacteria, they reported 3.3% resistance among *Enterococcus spp*., and only 0.8% resistance among *Staphylococcus spp*.[25] Al-Asady *et al*. (2022) also reported (80%) sensitivity to nitrofurantoin among Gram-positive uropathogens [26]. Assafi *et al*. (2015) found that nitrofurantoin-positive bacterial isolates showed sensitivity to nitrofurans, while isolates were resistant to the penicillin group [27].

**Antibiotic resistance of Gram-negative bacteria**

Generally, the most effective tested antibiotic on the Gram-negative isolates was amikacin, and the least effective one was erythromycin, as illustrated in table 5.

**Table 5: Antibiotics Resistance for Gram-negative bacterial isolates**

| Bacteria | No. of isolates | Antibiotics No. of resistant isolates (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **AK** | **E** | **VA** | **CN** | **F** | **C** | **CIP** | **TMP** | **AM** | AZM |
| ***Escherichia coli*** | 20 | 1 (5) | 20 (100) | 18 (90) | 4 (20) | 3 (15) | 0 | 3 (15) | 15 (75) | 20 (100) | 7 (35) |
| ***Klebsiella oxytoca*** | 11 | 0 | 11 (100) | 11 (100) | 6 (54.5) | 4 (36.4) | 0 | 2 (18.2) | 3 (27.3) | 11 (100) | 1 (9.1) |
| ***Klebsiella pneumonia*** | 5 | 3 (60) | 5 (100) | 5 (100) | 4 (80) | 5 (100) | 4 (80) | 4 (80) | 3 (60) | 5 (100) | 4 (80) |
| ***Citrobacter freundii*** | 5 | 0 | 5 (100) | 5 (100) | 2 (40) | 4 (80) | 2 (40) | 0 | 2 (40) | 4 (80) | 1 (20) |
| ***Serratia fonticola*** | 5 | 0 | 4 (80) | 5 (100) | 4 (80) | 2 (40) | 1 (20) | 0 | 3 (60) | 3 (60) | 3 (60) |
| ***Pseudomonas aeroginosa*** | 2 | 0 | 2 (100) | 2 (100) | 1 (50) | 2 (100) | 1 (50) | 0 | 2 (100) | 2 (100) | 0 |
| ***Proteus mirabilis*** | 2 | 0 | 2 (100) | 2 (100) | 1 (50) | 1 (50) | 1 (50) | 0 | 2 (100) | 2 (100) | 2 (100) |
| **Total resistance percentage** | **50** | **8** | **98** | **96** | **44** | **42** | **18** | **18** | **60** | **94** | 36 |

When comparing the current results to other studies, it found that they match or are close to ours at some points and disagree at others. For example, Al-Zahrani *et al*. (2019) found that the rate of antibiotic resistance was highest for ampicillin (85.6%), nitrofurantoin resistance (48.8%), and resistance to ciprofloxacin (17.9%).[28] While Demir and Kazanasmaz (2019) reported high resistance to ampicillin (87.1%), the lowest resistance was to nitrofurantoin (21.4%) and amikacin (4.2%)[29]. Assafi *et al*.(2015) found that Gram-negative bacteria were sensitive to carbapenems and aminoglycosides, and the majority of bacteria were unaffected by penicillins [27]. Gram-negative bacterial isolates were 100% resistant to ampicillin in Nigussie and Amsalu's (2017) study, and highly resistant to gentamicin (58.8%), while all of the Gram-negative isolates were nitrofurantoin sensitive[23]

**Detection of Biofilm formation**

**A) Congo red agar "CRA" method (Slime layer production)**

One hundred and twenty-two (81.3 %) of the total bacterial samples showed positive slime formation on CRA. Thirty-four isolates (22.67%) were moderate biofilm formers that appear as black-dark grey colonies (sometimes with a clear zone around the colonies), 57 isolates (38%) were weak biofilm formers that appear as dark red colonies (maroon), 24 isolates (16%) were considered as non-biofilm producers that appear as pale red colonies (close to pink).

Eighty-two percent of the Gram-positive isolates were biofilm formers, and only 18% were non-biofilm producers; the majority of isolates (53%) were weak biofilm formers, 14% moderate and 15% heavy biofilm formers by this method. While, 88% of the Gram-negative isolates were biofilm formers, and just 12 % were non-biofilm formers. Forty percent of isolates were heavy biofilm formers, the same (40%) for moderate biofilm formers, and only 8% were weak biofilm formers by this method.

**B) Microtiter plate (MTP) method**

Totally, 145 isolates (96.7%) were biofilm formers (50% moderate, 46.7% heavy biofilm producers) and only five isolates (3.3%) were non-biofilm formers by this method. All but 2 isolates (98%) of Gram-positive bacteria were biofilm formers by this method, and half of them were heavy biofilm formers. All but 3 isolates of the Gram-negative bacteria were biofilm formers (40% heavy and 54% moderate biofilm formers) by the MTP method. Tables 6 and 7 illustrate the percentages of biofilm formation of each bacterial type by both methods.

The difference in thickness of biofilms can be credited to variations in isolates' capability to synthesize biofilms, the initial number of cells that have the ability for adherence, or differences of the quality and quantity of auto-inducers quorum sensing signaling molecules (QS) system produced from each isolate [30].

**Table 6: Results of biofilm formation of Gram-positive bacteria**

| Bacterial type | No. of isolates | MTP method Isolates (%) | | | CRA method Isolates (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Heavy biofilm OD>0.240 | Moderate biofilm OD= 0.120-0.240 | No or weak biofilm OD<0.120 | Heavy biofilm | Moderate biofilm | Weak biofilm | No biofilm |
| *Staph. aureus* | 32 | 17 (53.1%) | 15 (46.9%) | - | 7 (21.9) | 7 (21.9) | 15 (46.9) | 3 (9.4) |
| *Staph. haemolyticus* | 14 | 12 (85.7%) | 2 (14.3%) | - | 2 (14.3) | - | 10 (71.4) | 2 (14.3) |
| *Staph. epidermidis* | 11 | 7 (63.6%) | 4 (36.4%) | - | 1 (9.1) | - | 5 (45.5) | 5 (45.5) |
| *Staph. saprophyticus* | 8 | 3 (37.5%) | 5 (62.5%) | - | 1 (12.5) | 1 (12.5) | 5 (62.5) | 1 (12.5) |
| *E. faecalis* | 23 | 10 (43.5%) | 13 (56.5%) | - | 4 (17.4) | 4 (17.4) | 14 (60.9) | 1 (4.3) |
| *Strep. agalactiae* | 6 | 1 (16.7%) | 5 (83.3%) | - | - | 2 (33.3) | 3 (50) | 1 (16.7) |
| *M. luteus* | 4 | - | 4 (100%) | - | - | - | 1 (25) | 3 (75) |
| *Kocuria rosea* | 1 | - | - | 1 (100%) | - | - | - | 1 (100) |
| *Kytococcus sedentarius* | 1 | - | - | 1 (100%) | - | - | - | 1 (100) |
| Total percentage | 100 | 50% | 48% | 2% | 15 % | 14% | 53% | 18% |

**Table7: Results of biofilm formation of Gram-Negative bacteria**

| Bacterial type | No. of isolates | MTP method Isolates (%) | | | CRA method Isolates (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Heavy biofilm OD>0.240 | Moderate biofilm OD= 0.120-0.240 | No or weak biofilm OD<0.120 | Heavy biofilm | Moderate biofilm | Weak biofilm | No biofilm |
| *E. coli* | 20 | 7 (35%) | 12 (60%) | 1 (5%) | 9 (45) | 10 (50) | 1 (2) | - |
| *K. oxytoca* | 11 | 6 (54.5%) | 5 (45.5%) | - | 9 (81.8) | - | 2 (18.2) | - |
| *K. pneumonia* | 5 | 2 (40%) | 3 (60%) | - | 2 (40) | - | - | 3 (60) |
| *C. freundii* | 5 | 3 (60%) | 1 (20%) | 1 (20%) | - | 3 (60) | 1 (20) | 1 (20) |
| *Serratia fonticola* | 5 | 1 (20%) | 3 (60%) | 1 (20%) | - | 5 (100) | - | - |
| *P. aeroginosa* | 2 | 1 (50%) | 1 (50%) | - | - | - | - | 2 (100) |
| *Proteus mirabilis* | 2 | - | 2 (100%) | - | - | 2 (100) | - | - |
| Total percentages | 50 | 40% | 54% | 6% | 40% | 40% | 8% | 12% |

The results showed that by CRA method 81.33% of isolates were biofilm formers. While 96.7% of isolates were detected as biofilm formers by microtiter plate assay. In spite of the CRA method's fame, it is not recommended as a suitable method for the discovery of biofilm production, as it does not give reliable and dependable results as it is based on four colors scale (black, grey, maroon, and red) which causes different interpretations from one person to another[31].

## References

**[1] Ganesh, R.; Shrestha, D.; Bhattachan, B.; *et al.* (2019).** Epidemiology of urinary tract infection and antimicrobial resistance in a pediatric hospital in Nepal. *BMC Infect Dis*, 19, 420. https://doi.org/10.1186/s12879-019-3997-0

**[2] Flores-Mireles, A. L.; Walker, J. N.; Caparon, M. and Hultgren, S. J. (2015).** Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*, 13(5), 269–84. https://doi.org/10.1038/nrmicro3432.

**[3] James, M. (2018).** What's to know about urinary tract infections? School of Medicine, University of Illinois-Chicago. *Med News Today*, 2p.

**[4]Ouno, G. A.; Korir, S. C.; Joan, C. C.; Ratemo, O. D.; Mabeya, B. M.; Mauti, G. O.; *et al.* (2013).** Isolation, identification and characterization of urinary tract infectious bacteria and the effect of different antibiotics. *J Naturl Sci Res*, 3(6), 2224-3186.

**[5] Soto, S. M.(2014).** Importance of biofilms in urinary tract infections: new therapeutic approaches. *Adv Biol*, 2014,13.

doi:10.1155/2014/543974

**[6] Vandepitte, J.; Verhaegen, J.; Engbaek, K.; Rohner, P., Piot, P. and Heuck, C.C. (2003).** *Basic laboratory procedures in clinical bacteriology*. Geneva: World Health Organization.

**[7] Collins, H.C.; Lyane, M.P.; Grange, M. J. and Falkinham, O. J. (2004).** *Microbiological Methods*.(8th ed.). London: Arnold, a member of the hodder headline Group.

**[8] CLSI. (2021).** Performance Standards for Antimicrobial Susceptibility Testing; 31st edition. CLSI supplement M100. Clinical and laboratory institute, USA.

**[9] Bahador, N.; Shoja, S.; Faridi, F.; Dozandeh-Mobarrez, B.; Qeshmi, F. I.; Javadpour, S. and Mokhtary, S. (2019).** Molecular detection of virulence factors and biofilm formation in Pseudomonas aeruginosa obtained from different clinical specimens in Bandar Abbas. *Iranian Journal of Microbiology*, *11*(1), 25.

**[10] Jain, A. and Agarwal, A. (2009).** Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. *J Microbiol Methods*, 76, 88–92.

**[11] Arciola, C. R.; Baldassarri, L. and Montanaro, L. (2001).** Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. *J Clin Microbiol*, 39:2151–6.

**[12] Christensen, G.D.; Simpson, W.A.; Younger, J. A.; Baddour, L. M.; Barrett, F. F.; Melton, D. M. and Beachey, E. H. (1985).** Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol*, 22: 996-1006.

**[13] O'Toole, A. G. and Kolter, R. (1998).** Initiation of biofilm formation in Pseudomonas fluorescence WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. *Molecular microbiology*, 28:449.

**[14] Namvar, A. E.; Asghari, B.; Ezzatifar, F.; Azizi, G. and Lari, A. R. (2013).** Detection of the intercellular adhesion gene cluster (ica) in clinical Staphylococcus aureus isolates. *GMS Hyg Infect Control*, 8(1), Doc03.

**[15] Haghi-Ashteiani, M.; Sadeghifard, N.; Abedini , M.; Soroush, S. and Taheri-Kalani, M.** (2007). Etiology and antibacterial resistance of bacterial urinary tract infections in children's medical center, Tehran, Iran., ***Acta. Medica. Iranica.***, 45(2),153-157.

**[16] Hussein, A. K.; Palpitany, A. S. and Ahmed, H.S. (2014).** Prevalence of Urinary Tract Infection among Secondary School Students in Urban and Rural in Erbil: Comparative Study. *Kufa J for Nursing Sciences*, 4(3).

**[17] Kamel, H. F.; Salh, Q. K. and Shakir, A. Y.(2014).** Isolation of Potential Pathogenic Bacteria from Pregnant Genital Tract and Delivery Room in Erbil Hospital. *Diyala J of Medicine*, 7(1).

**[18] Gilstrap, L. C. and Ramin, S. M. (2001).** Urinary Tract Infections During Pregnancy. *Obstetrics and Gynecology Clinics of North America*, 28(3), 581–591. doi:10.1016/S0889-8545(05)70219-9

**[19] Abd-Alwahab, M. H. and Thalij K. M. (2015).** Determine the Ability of Bacterial Isolates from Urinary Tract Infections on L-Asparginase Production. Tikrit Journal of Pure Science, 5 (20), 1-11.

**[20] Abdullah, A. H. N. (2008).** Isolation and identification of oxacillin resistant Staphylococci from clinical and ecological samples from Al-khansaa hospital in Mosul city. MSc. thesis. College of education. Tikrit University.

**[21] Mahdi, F. M. (2020).** Evaluation of molecular impact of alcoholic extract for pomegranate peel and Trigonella foenum and some antibiotics on E. coli bacteria isolated from urinary tract infections. M.Sc. thesis. College of science. Tikrit University.

**[22] Al-Tikrity, I. A. L. (2016).** Detection of some Virulence Genes in *Escherichia coli* Isolated from Patients with Urinary Tract Infection in Erbil City. PhD thesis. College of science. Tikrit University.

[23]**Nigussie, D. and Amsalu, A. (2017).** Prevalence of uropathogen and their antibiotic resistance pattern among diabetic patients. *Turk J Urol*, 43(1), 85–92. doi: 10.5152/tud.2016.86155

**[24] Woldemariam, H. K.; Geleta, D. A.; Tulu, K. D.; Aber, N. A.; Legese, M. H.; Fenta, G. M. and Ali, I. (2019).** Common uropathogens and their antibiotic susceptibility pattern among diabetic patients. *BMC Infectious Diseases*, 19:43 https://doi.org/10.1186/s12879-018-3669-5

[25] **Petca, R. C.; Mareș, C.; Petca, A.; Negoiță, S.; Popescu, R.; Boț, M; *et al.* (2020).** Spectrum and Antibiotic Resistance of Uropathogens in Romanian Females. *Antibiotics*, *9*(8), 472. https://doi.org/10.3390/antibiotics9080472

[26] **Al-Asady, F. M.; Al-Saray, D. A. and Al-Araji, A. E. (2022).** Screening of urinary tract bacterial infections and their antibiogram among non-pregnant women admitted to Al-Sadiq hospital, Iraq. AIP Conference Proceedings, 2386 (1), 020006. https://doi.org/10.1063/5.0066884

**[27] Assafi, M. S. A.; Ibrahim, N. M. R.; Hussein, N. R.; Taha, A. A. and Balatay, A. A. (2015).** Urinary Bacterial Profile and Antibiotic Susceptibility Pattern among Patients with Urinary

Tract Infection in Duhok City, Kurdistan Region, Iraq. *Int J Pure Appl Sci Technol*, 30(2), 54-63.

**[28] Al-Zahrani, J.; Al Dossari, K.; Gabr, A. H.; Ahmed, A.; Al Shahrani S. A. and Al-Ghamdi, S. (2019).** Antimicrobial resistance patterns of Uropathogens isolated from adult women with acute uncomplicated cystitis. *BMC Microbiology*, 19, 237. https://doi.org/10.1186/s12866-019-1612-6

**[29] Demir, M. and Kazanasmaz, H. (2019).** Uropathogens and Antibiotic Resistance in the Community and Hospital-Induced Urinary Tract Infected Children. *J of Global Antimicrobial Resistance*, 20, 68-73. https://doi.org/10.1016/j.jgar.2019.07.019

**[30] Beenken, K. E.; Mrak, L. N.; Griffin, L. M.; Zielinska, A. K.; Shaw, L. N.; Rice, K. C. and Smeltzer, M. S. (2010).** Epistatic relationships between *sarA* and *agr* in *Staphylococcus aureus* biofilm formation. *PLoS One*, 5(5), 10790.

**[31] Knobloch, J. K.; Horsetkotte, M. A.; Rohde, H. and Mack, D. (2002).** Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*. *Med Microbial Immunol*, 191(2),101-6.

# دراسة انتشار التهاب المسالك البولية، مقاومة المضادات الحياتية، و قابلية المسببات البكتيرية على تكوين الغشاء الحيوي

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**الملخص**

كان الهدف من الدراسة هو تقييم نسبة الاصابات بالتهاب المسالك البولية في محافظة صلاح الدين/ العراق، تشخيص المسببات البكتيرية ومن ثم معرفة مقاومتها للمضادات الحياتية وقدرتها على تكوين الغشاء الحيوي. هذا النوع من الدراسات مهم جداً لمعرفة العلاجات الاساسية لمثل هذه الامراض. في الدراسة الحالية تم جمع 650 عينة ادرار وتم زرع 250 عينةً، اظهرت 193 منها نمواً (77.2%) بينما 57 عينة (22.8%) لم تظهر نمواً. البكتريا الموجبة لصبغة كَرام كانت المسبب الرئيس لالتهابات المسالك البولية ضمن عينة الدراسة الحالية مسؤولةً عن 66.7 % من الحالات في حين تسببت البكتريا السالبة لصبغة كَرام ب 33.33% من الحالات. كانت بكتريا *Staphylocccus spp.* الاكثر عزلاً من البكتريا الموجبة، و بكتريا *E.coli* كانت الاكثر عزلاً ضمن البكتريا السالبة لصبغة كَرام. أظهر المضاد الحيوي Nitrofurantoin أعلى فعاليةً على البكتريا الموجبة لصبغة كَرام يليه المضاد Chloramphenicol (نسبة المقاومة 19 و 20% على التوالي) أما المضاد الاقل فعاليةً فكان Azithromycin بنسبة مقاومة 64%. أما بالنسبة للبكتريا السالبة لصبغة كَرام فكان المضاد الحيوي Amikacin هو الاكثر فعالية تجاهها وبنسبة مقاومة 8% فقط، في حين أظهر المضاد Erythromycin أقل فعالية ضد هذه البكتريا بنسبة مقاومة 98%. تم التحري عن قدرة البكتريا على تكوين الغشاء الحيوي باستخدام طريقتي اكَار الكونغو الأحمر وطبق المعايرة المتعدد. حيث اظهرت النتائج ان 81.3% من العينات كانت مكونة للغشاء الحيوي بطريقة اكَار الكونغو الاحمر بينما اظهرت 96.7% من العينات قابليتها على تكوين الغشاء الحيوي بطريقة اطباق المعايرة. بصورة عامة كانت البكتريا الموجبة لصبغة كَرام اكثر قدرة على تكوين الغشاء الحيوي من البكتريا السالبة.