Serological and Molecular detection of *Staphylococcus aureus* isolated from UTI patients

https://doi.org/10.32792/utq/utj/vol14/4/5

Ahmed A. R. Abbas* Saad S. Hamim*
hamim_pa@sci.utq.edu.iq
***Ministry of health-Thi-Qar health office.**
***Department of pathological analysis-College of science-Thi-Qar University.**

Abstract

Urinary tract infections (UTIs) are one of the commonest infections encountered by clinicians despite the widespread availability of antimicrobial agents. Such Infections caused by different bacterial pathogens that can be acquired through both hospitals and the community. The present study is aimed to isolation and diagnosis of *Staph. aureus* , detecting the optimal antimicrobial for the treatment of these infections. A total of (600) mid-stream urine samples were collected during the period from August to December, 2018 from patients who were complained from UTIs at AL-Hussain Teaching Hospital in AL-Nasiriyah City, Southern Iraq. The study included isolation and diagnosis of *Staph. aureus* based on morphological, microscopic characterization, biochemical tests and confirmed by API-20 and Vitek2 systems. In addition , all *Staph. aureus* isolates were subjected to the serological diagnosis of protein A by using a latex agglutination test and convention PCR technique was used to detect the presence of the 16S rRNA gene (353bp). 380 (63.33%) were positive isolates for bacteriological examination, the *Staph. aureus* was identified with 50 samples (13.5 %). In addition, all *Staph. aureus* isolates were assayed for antimicrobial susceptibility against 14 selected antibiotic discs by using the disc diffusion method. All isolates were completely resistant to Penicillin (P), Oxacillin (Ox), and Ampicillin (Amp). While the most effective antibiotics were Nitrofurantoin, Gentamycin, isolates were susceptible to these antibiotics in 76%, and 60%, respectively. Also, In the analysis of the nucleotides sequence of the partial 16S rRNA gene, the results were

showed that these bacteria actually related to *Staph. aureus* ,according to current result, three of these isolates registered globally in the NCBI Gen bank, and the accession numbers of these isolates are (MK910079.1),(MK910080.1), and (MK910081.1).

Keywords: *Staphylococcus aureus*, Urinary tract infections,
Identification,
Serological and Molecular diagnosis

1. Introduction

Urinary tract infections (UTIs) are infections caused by the presence and growth of microorganisms anywhere in the urinary tract and may be the single most common human bacterial infections [1]. The urinary tract includes the organs that collect, store and release urine from the body, including kidneys, ureters, bladder, and urethra. UTIs are among the most common human bacterial infections in the community or hospital settings and has been reported in both sexes in all age groups [2]. The prevalence and incidence of UTI in women are higher than in men, which is probably the result of several clinical factors, including anatomical differences, hormonal effects and behavioral patterns [3]. About 150 million people worldwide develop UTI each year, with high social costs [4]. UTI accounts for a large portion of the workload in clinical microbiology laboratories and enteric bacteria (*Escherichia coli* in particular) remain the most common cause of UTI, although the distribution of UTI - allowing pathogens is changing [5]. Some other pathogens are associated with UTIs like *Staphylococcus*, *Staph. aureus* which is a major human pathogen and a widespread contaminant in hospitals. Although *Staph. aureus* isolation from urine samples is often secondary to Staphylococcal bacteremia that occurs elsewhere (e.g. endocarditis cases), In some patients, *Staph. aureus* causes colonization and infection in the urinary tract. Instrumentation of the urinary tract and presence of an indwelling catheter increase the risk of *Staph. aureus* carriage of the urinary tract. Majority of cases *Staph. aureus* bacteriuria is not associated with symptoms of urinary tract infection because bacteriuria nearly universally occurs concomitantly with long-term urinary catheterization [6]. The susceptibility of *Staph. aureus* to the penetration of the body's defenses and tissue invasion and possession of virulence factors such as toxin and

enzyme production, including high antibiotic resistance [7]. In the past few decades, *Staph. aureus* is considered as one of the most common nosocomial infections due to various virulence factors, acquisition of antibiotic resistance genes, poor sanitation, and hygiene of health care setting and discriminate use of antibiotics [8]. Also, *Staph. aureus* has a number of identifying properties, including free coagulase, clumping factor (bound coagulase), thermonuclease, and protein A [9]. The present study aimed to investigate the molecular characterization of 16S RNA gene in *Staph. aureus* isolates from UTI patients.

2. Materials and Methods

2.1 Samples collection

Samples were collected from 600 UTI patients from both sexes with different ages, based on the symptoms and which diagnosed by physician and microscopic examination of urine, Patients had taken care and medications in AL- Hussein Teaching Hospital in AL- Nassiriyah City South of Iraq , during the period from August to December, 2018.

2.2 Isolation, Identification & biochemical tests of *Staph. aureus*

Collected specimens were inoculated on several types from culture media which included blood agar, mannitol salt agar, and *Staph* 110 agar base according to standard methods. *Staph. aureus* was identified depending on the morphological features of culture media. Isolates were stained by Gram stain to detect their response to stain, shapes and their arrangement[10]. The biochemical tests used include Catalase, Coagulase, Oxidase and Novobiocin [11].The bacterial diagnosis was confirmed by API system and Vitek2 compact (BioMerieux, France).

2.3 Antibiotic susceptibility test for *Staph. aureus* isolates

All *Staph. aureus* isolates were subjected to antibiotic susceptibility by using disc diffusion method [12]. The inhibition zone diameters were measured and interpreted according to [13].

2.4 Serological diagnosis

All isolates were subjected to a serological diagnosis of protein A by using a latex agglutination test. This test consists of yellow latex particles that have been coated with fibrinogen and rabbit immunoglobulin G (IgG) specific for *Staph. aureus* . This test was performed according to the directions of the manufacturing company (Remel, UK).

2.5 Molecular detection

1. DNA extraction and purification: DNA was extracted and purified according to the company manufacturer instructions (Geneaid / Korea).

2. *Staph. aureus* isolates were subjected to the detection of the 16S rRNA gene by conventional PCR technique using specific primer pairs (Table 1). The amplification was conducted in a thermal cycler (BioRad / USA) which has been programmed with the following conditions: an initial denaturation step for 5 min. at 95°C with one cycle, 30 cycles of amplification were performed as follows: denaturation at 95°C for 30 sec annealing at 58°C for 30 sec and extension at 72°C for 1 min followed by a final extension step at 72°C for 5 min. These conditions were designed by the researcher in this study.

3. DNA sequencing: Three PCR products of the 16S rRNA gene were selected for the sequencer. The sequencing of gene reverse and forward primers was done in Macrogen, Korea outside of Iraq. Basic Local Alignment Search Tool analysis (BLAST) was lead to a blast algorithm. The sample sequences designated as (No1, No2 and No3) were edited, aligned and compared with the reference sequences by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) with MEGA6 software. Thus, the MEGA version 6.0 was used to construct phylogenetic tree**.**

The PCR primers were designed online and provided by (Macrogen, Korea) using NCBI Gene Bank and primers 3 plus [14] as follows (Table1).

Table 1 : Primer sequences of 16S rRNA gene in *Staph. aureus* .

Gene	**Primer Sequences (5'-3')**		**Product size (bp)**
16S rRNA *Staph. aureus*	F*	GTTGACTGCCGGTGACAAAC	353
	R*	GCTGTTACGACTTCACCCCA	

F: forwared, *R: reverse, T: thymine, C: cytosine, G: guanine, A: adenine

*

3. Results

3.1 Isolation of *Staph. aureus*

From a total of 600 urine samples from both sexes who suffered from UTI infections with different ages. During the period from August to December, 2018, were collected and tested. The results of the present study showed that 380 (63.5%) isolates noted positive growth of pathogen collected from UTI patients while 220 samples (36.5%) showed no significant growth. Only (50) samples were given growth *Staph. aureus* with 13.5% while 50% were other types of bacteria and 36.5% did not grow, as in figure (1)**.**

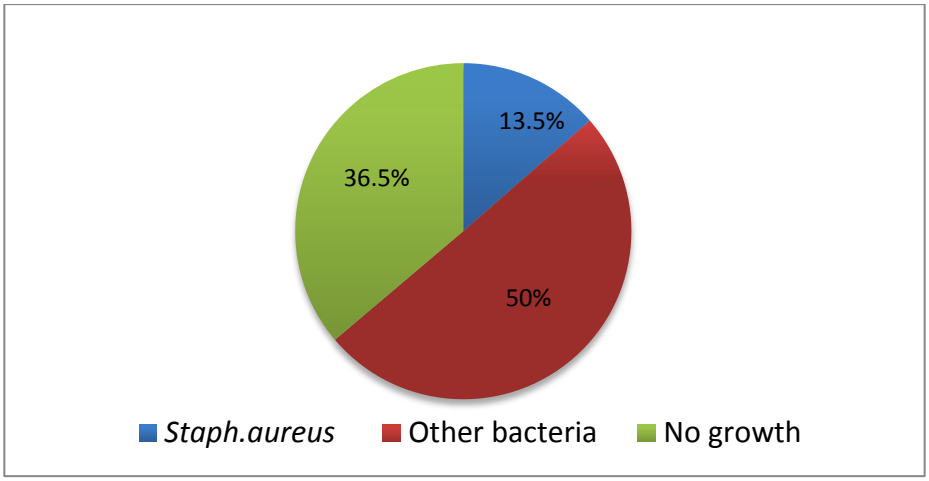


Figure (1): The percentage of *Staph. aureus* and other bacteria isolated from 600 UTI patients.

3.2 Macroscopic & Microscopic examination

Morphology characteristics of *Staph. aureus* grow on different media, such as Blood agar, Mannitol salt agar and Staph 110 meduim, then microscopic examination was applied to all 50 isolates after being stained by gram stain to detect their reaction. The cells appeared as gram-positive cocci, mostly arranged in grapes like irregular clusters.

3.3 Conventional Biochemical tests & Api-20 system identification

In the initial stage of identification, all isolates were identified by biochemical tests such as Catalase, Coagulase**,** Oxidase and Novobiocin. All these tests positive for *Staph. aureus*. Then, used the API 20 *Staph* to

confirm the diagnosis of isolates from *Staph. aureus* and to complete important biochemical tests. The result of API-20 *Staph* test has reveals that only 50 isolates identified as *Staph. aureus.*

3.4 Serological diagnosis

All of *Staph. aureus* isolates were diagnosed by Staphaurex Plus which is a rapid latex agglutination test for the identification of Staphylococci which possess clumping factor, protein A and surface antigens characteristic of *Staph. aureus* . When a drop of the reagent is mixed on a card with *Staph. aureus* organisms, rapid agglutination occurs, indicates a positive result. As illustrated in figure (3).

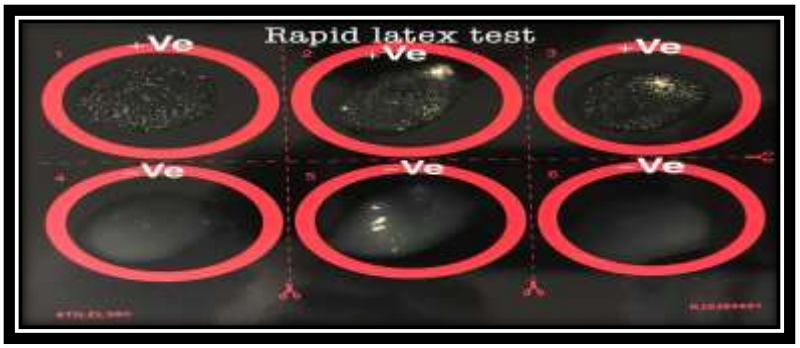


Figure (3): Latex agglutination test for *Staph. aureus*

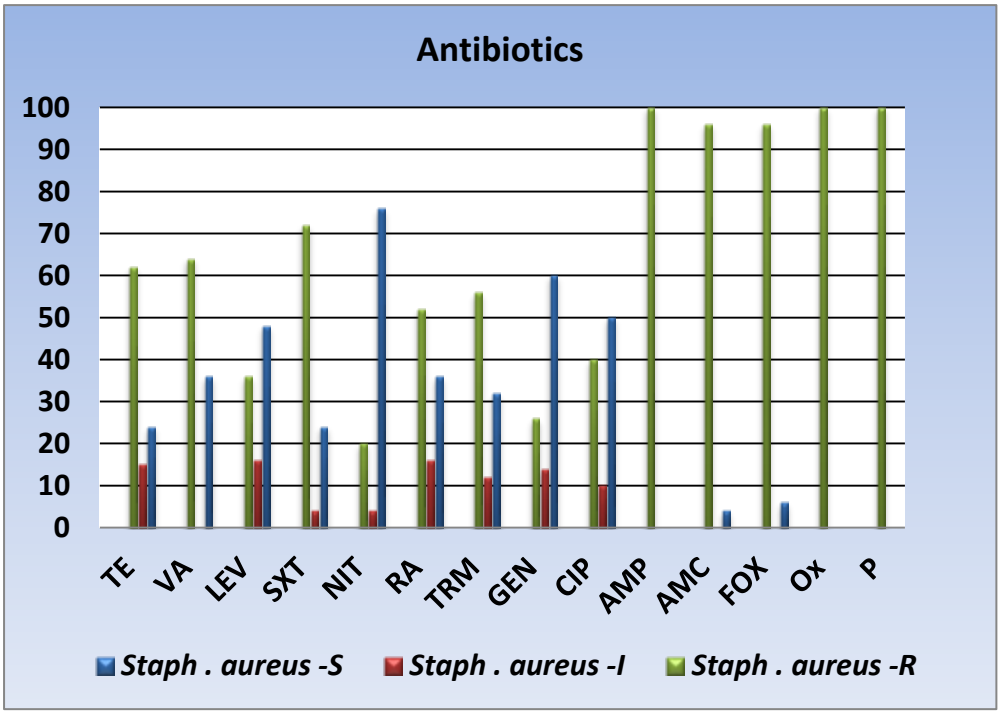
3.5 Antibiotic susceptibility profile of *Staph. aureus*

All *Staph. aureus* (n=50) was assayed for antibiotic susceptibility test against 14 type of antibiotics. The results showed that there was a variation in resistance rates of the tested bacteria. All *Staph. aureus* isolates were completely resistant to penicillin (P), Oxacillin (Ox), and Ampicillin (Amp) with (100%) for the three antibiotics and highly resistant to Trimethoprim-Sulfamethoxazole, Tetracycline (TE) and Rifampicin (RA) with a percentages of (75.0%),(62.0%), and (52.0%), respectively. While the most effective antibiotics for *Staph. aureus* were Nitrofurantoin, Gentamycin where isolates were susceptible to these antibiotics in 76%, and 60%, respectively. As shown in figure (2).

3.6 Molecular diagnosis of *Staphylococcus aureus* by 16S rRNA

Fifty isolates of *Staph. aureus* which were identified by biochemical test, API 20, Vitek 2 system and serological diagnosis were subjected to DNA extraction, PCR assay for sequencing of 16S rRNA gene approximately size (353bp). All the isolates were positive for that gene (100%), as in figure (4). The DNA sequences and phylogenetic tree analysis are accurate and highly sensitive diagnostic methods and can be used in many aspects of life and practice.

Figure (2): The percentage of antimicrobial susceptibility of *Staph. aureus* against isolates 14 types of antibiotic.



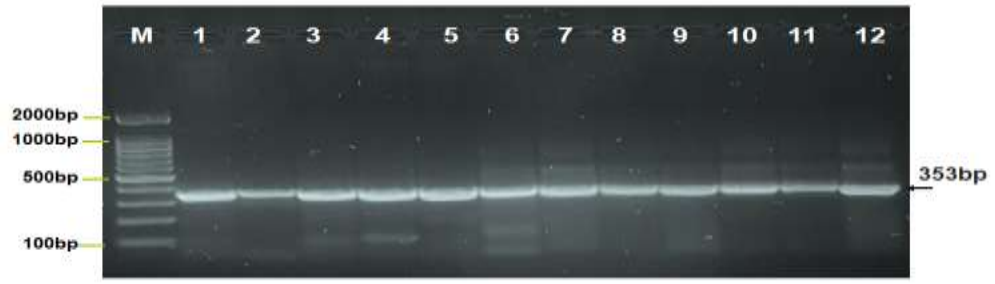
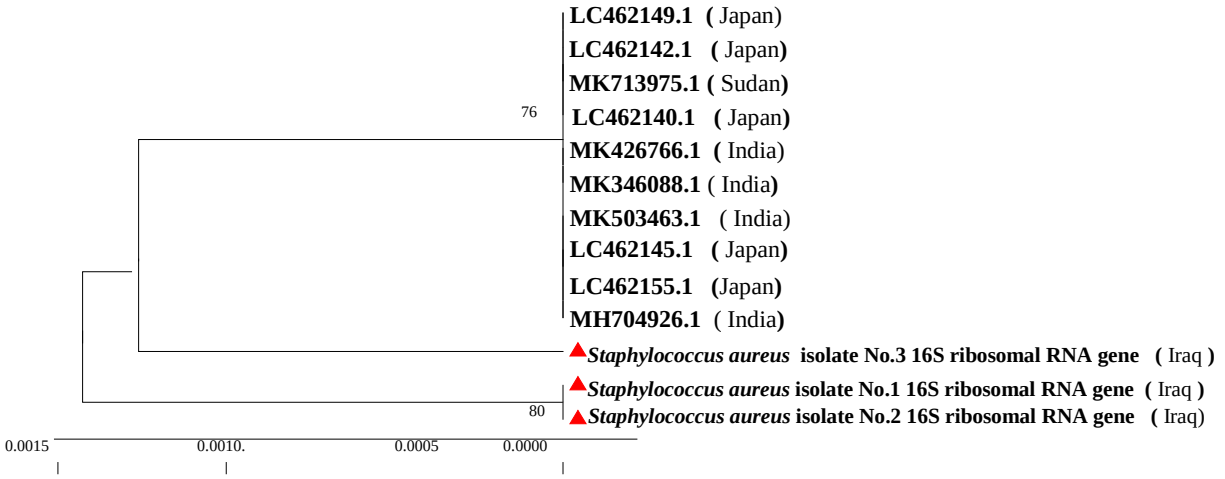


Figure (4): A garose gel electrophoresis presented the analysis of PCR product in *Staph. aureus* 16S ribosomal RNA gene. M represents the Marker ladder with (100-2000bp) while lane (1-12) showed positive *Staph. aureus* isolates at approximately (353bp) product size**.**

Figure (5): Phylogenetic tree analysis based on 16 ribosomal RNA gene



partial sequence in local *Staph. aureus* human urine isolates that used for genetic *Staph. aureus* genetic analysis. The phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (MEGA version 6.0).

4. Discussion

4.1 Isolation of *Staph. aureus*

Problems in managing urinary tract infections are attributed to factors related to the host and those related to the causative agents of urinary tract infections. UTIs can occur due to the pathogenicity of the organism, susceptibility of the host, or a combination of both factors[15]. The results of the present study showed that *Staph. aureus* was the most predominant gram-positive species isolated from all UTI cases with a total number of 50/380 (13.15%). Inside Iraq, the current results agreed with [16,17] who recorded both of them (11%) and [18] whom recorded (14.29%) for *Staph. aureus*. Outside Iraq, the present results agreed with results of study [19] who document that (13.71%), of isolates identified as *Staph. aureus* that isolated from UTI patients. On the other hand, the results of the present study were incompatible with [20,21] where *Staph. aureus* accounted for only 0.5%, and 1.5% of isolates respectively, also the results of this study are disagree to other studies, like the results of [22,23], that described a *Staph. aureus* incidence in UTI with a percentages of 28.9%, and 33.3%, respectively. Prior studies suggest that isolation of *Staph. aureus* from the urine is often secondary to Staphylococcal bacteremia originating at another site (e.g., in cases of endocarditis) [24]. This is probably due to the fact that *Staph. aureus* is a member of the normal flora of both asymptomatic carriers and sick persons thus takes advantage of the weak immune system. This organism can be spread by the hands, expelled from the respiratory tract or transmitted by animate or inanimate objects [25].

4.2 Antibiotic susceptibility of *Staph. aureus*

Antimicrobial resistance represents a serious problem in the treatment of infectious diseases including UTI. Antibiotics are the main treatment for all UTIs. A variety of antibiotics are available, and choices depend on many factors, including whether the infection is complicated or uncomplicated or primary or recurrent [26]. Where worldwide data show that there is increasing resistance among urinary tract pathogens to conventional drugs. Antibiotic resistance by Staphylococci is on the increase [27]. The results of antibiotics susceptibility test for *Staph. aureus* isolates showed that there were a high rate of resistance to most

of Penicillin drugs. Inside Iraq, the result of the current study seems to be in agreement with [16,28], who recorded that the same resistance to this antibiotics. Outside Iraq, the results of the present study were compatible with[29], whom recorded high a resistance rate to Oxacillin (100%), Amoxicillin (91.7%), followed by Penicillin (83.3%). Penicillin resistance may be due to the structural modification of enzymatic action (β-lactame action) or the prevention of access to target by altering the outer membrane permeability and may be due to the alternation of the antibiotic target site and sometimes the resistance is due to efflux pump which pumps out the antibiotic [30]. *Staph. aureus* develops resistance very quickly and successfully to different antimicrobials over some time. The present study showed that *Staph. aureus* isolates were highly susceptible to Nitrofurantoin and Gentamycin, a finding that was consistent to that reported by [31,32] where they recorded a sensitive rate of (79.5% ,52.3%), and (85%, 80%), respectively. On the other hand, these results were incompatible with similar studies performed by [33] who founded completely sensitivity of *Staphylococcus* spp. to gentamycin with a percentage of (100%). Also, do not agree with the [18] where the isolates were (75.6%) resistant to gentamycin.

4.3 Serological diagnosis

Protein A was tested for all isolates of *Staph. aureus* under study. The method used in this research is easy to apply as well as sensitive. *Staph. aureus* isolates showed significant variation in their containment of protein A associated with the cell wall. Some isolates of *Staph. aureus* were found to possess this protein. It is the result of having high protein A content that is part of its wall, but the lack of possession of some isolates of *Staph. aureus* of this protein these are due to many reasons, the most important reasons, where the type of culture medium and its components is of great importance in the production of protein A. Research on the effect of carbon source in the production of protein A indicates that the presence of mannitol in the culture medium leads to inhibition of protein production. The presence of high concentration salt affects the production of protein A inhibits its production and affects the correlation efficiency between protein A and the fraction of FC of IgG [34].

4.4 Molecular detection of *Staph. aureus*

Using of Polymerase Chain Reaction (PCR) technique increases the accuracy and speed of *Staph. aureus* identification and validation [35]. The DNA sequences and phylogenetic tree analysis are accurate and highly sensitive diagnostic methods and can be used in many aspects of life and practice [36]. All isolates gave a positive reaction to the 16S rRNA gene. This finding agrees with other local studies [37,38,39] who revealed the complete percentage of the 16SrRNA gene. Outside Iraq, some results agreed to go for from what was recorded [40,41] who recorded that all isolates had 16S rRNA. Also, the results of this study disagree with[42]. These differences may be due to many factors; such as the sources and number of the clinical samples used, geographical dependency, and the sensitivity of different techniques used. This gene was used for diagnosis and through this tree we found that the isolates of the study were genetically identical by 99% with those found in the genebank. The isolates of the three studies are genetically far from the genes taken from the genebank because they appeared in the out group, especially isolates No.1 and No.2 that appeared genetically identical while isolate No. 3 has a genetic relationship with the genes taken from the genebank this is confirmed by the value of the boot strap, which amounted to 76%. The value of the boot strap that appeared among the study isolates was good. The constructed phylogenetic tree showed that *Staph. aureus* MK910079.1 and MK910080.1 were highly relative to each other in comparison with *Staph. aureus* MK910081.1 that revealed a close relatedness to *Staph. aureus* from India, Sudan and Japan, at total genetic changes (0.0005-0.0015%) (Fig. 5).

Conclusions

Staphylococcus aureus consider as one of important Gram positive causative agents of UTI infections and has become one of the most successful adaptable human pathogens.

References:

[1] Negussie, A.; Worku, G., and Beyene, E. (2018). Bacterial identification and drug susceptibility pattern of urinary tract infection in pregnant women at Karamar Hospital Jigjiga, Eastern Ethiopia. African Journal of Bacteriology Research, 10(2): 15-22.

[2] Hassan, H. E. M.; Altayb, H. N.; El Hassan, M. M., and Elmekki, M. A. (2018). Genotypic detection of the virulence factors of uropathogenic *Escherichia coli* isolated from diarrheic and urinary tract infected patients in

Khartoumn State, Sudan.African Journal of Microbiology Research,12(9): 230-236.

[3] Griebling, T. L. (2018). How Often do Clinically Diagnosed Catheter-Associated Urinary Tract Infections in Nursing Homes Meet Standardized Criteria?. The Journal of urology, 200(6): 1141-1144.

[4] Flores-Mireles, A. L., Walker, J. N., Caparon, M., and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5): 269.284.

[5] Bradley, C. W.; Flavell, H.; Raybould, L.; McCoy, H.; Dempster, L.; Holden, E., and Garvey, M. I. (2018). Reducing *Escherichia coli* bacteraemia associated with catheter-associated urinary tract infections in the secondary care setting. Journal of Hospital Infection, 98(3): 236-237.

[6] Muder, R. R.; Brennen, C.; Rihs, J. D.; Wagener, M. M.; Obman, A.; Obman, A., and Yu, V. L. (2006). Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent Staphylococcal bacteremia. Clinical infectious diseases, 42(1): 46-50.

[7] Brooks, G. F.; Carroll, K. C.; Butel, J. S.; Morse, S. A., and Mietzner, T.A(2013). Medical Microbiology, (26th ed.). New York. USA: McGraw-Hill Education, p.880.

[8] Kumosani, T. (2014). Nosocomial Infections in Saudi Arabia Caused by Methicillin Resistance *Staphylococcus aureus* (MRSA). Clinical Microbiology: Open Access, 03(03): 2–5.

[9] Smith, D. S.; Siggins, M. K.; Gierula, M.; Pichon, B.; Turner, C. E.; Lynskey, N. N., and Sriskandan, S. (2016). Identification of commonly expressed exoproteins and proteolytic cleavage events by proteomic mining of clinically relevant UK isolates of *Staphylococcus aureus*. Microbial genomics, 2(2).

[10] Benson, J. H. (2001). Microbiological Applications: Laboratory Manual in General Microbiology. (8th ed). McGraw-Hill Higher Education, New York, PP: 125-130.

[11] MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria, (3rd ed.). USA: Lippinocott Williams and Wilkins, p. 912.

[12] Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45(4): 493-496.

[13] Clinical and Laboratory Standards Institute (2018). Performance standards for antimicrobial susceptibility testing of anaerobic bacteria: informational supplement. CLSI document M100, (28th ed.) Wayne, PA USA.: 258p.

[14] Untergasser, A.; Nijveen, H.; Rao, X.; Bisseling, T.; Geurts, R., and Leunissen, J. A. (2007). Primer3Plus, an enhanced web interface to Primer3. Nucleic acids research, 35(2): W71-74.

[15] Flores-Mireles, A. L.; Walker, J. N.; Caparon, M., and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5): 269-284.

[16] Al-Jebouri, M. M., and Mdish, S. A. (2013). Antibiotic resistance pattern of bacteria isolated from patients of urinary tract infections in Iraq. Open Journal of Urology, 3(02): 124-131.

[17] Abdulrahman, I. S. (2018). Antimicrobial Susceptibility Pattern of Pathogenic Bacteria Causing Urinary Tract Infections at Azadi Hospital In Duhok City\Kurdistan Region of Iraq. Science Journal of University of Zakho, 6(2): 46-50.

[18] Alsamarai, A. G. M.; Khorshed, S. A., and Ali, H. (2016). Urinary tract infection in female in Kirkuk, Iraq: impact of younger aged women compared to diabetic and pregnant women. World Journal Of Pharmacy and Pharmaceutical Sciences, 5(11), 73-86.

[19] Mollick, S.; Dasgupta, T.; Hasnain, M. J., and Ahmed, M. (2016). Isolation and characterization of pathogens responsible for urinary tract infection in Bangladesh and determination of their antibiotic susceptibility pattern. Journal of Applied Pharmaceutical Science, 6(04): 072-076.

[20] Barrett, S. P.; Savage, M. A.; Rebec, M. P.; Guyot, A.; Andrews, N., and Shrimpton, S. B. (1999). Antibiotic sensitivity of bacteria associated with community-acquired urinary tract infection in Britain. Journal of Antimicrobial Chemotherapy, 44(3): 359-365.

[21] Mane, M. S.; Sandhya, B. K.; Priya, R .L., and Magdalene, J. (2015). Prevalence and antibiotic susceptibility pattern of bacterial isolates from urinary tract infection in a tertiary care hospital in Tamilnadu. Journal of medical and dental sciences, 14 (7): 59-65.

[22] Okonko, I. O.; Ijandipe, L. A.; Ilusanya, A. O.; Donbraye-Emmanuel, O. B.; Ejembi, J., Udeze, A. O., and Nkang, A. O. (2010). Detection of urinary tract infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria. Malaysian journal of Microbiology, 6(1): 16-24.

[23] Alwash, S. J., and Saleh, D. S. (2013). Comparison between Cefoxitin disk diffusion, Crome agar and EPI-M Screening Kit for Detection of Methicillin-Resistant *Staphylococcus aureus*. Iraqi Journal of Science, 54(4): 847-850.

[24] Muder, R. R.; Brennen, C.; Rihs, J. D.; Wagener, M. M.; Obman, A.; Obman, A., and Yu, V. L. (2006). Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent Staphylococcal bacteremia. Clinical infectious diseases, 42(1): 46-50.

[25] DeLeo, F. R.; Diep, B. A., and Otto, M. (2009). Host defense and pathogenesis in *Staphylococcus aureus* infections. Infectious disease clinics of North America, 23(1): 17-34.

[26] Zahera, M.; Rastogi, C.; Singh, P.; Iram, S.; Khalid, S., and Kushwaha, A. (2011). Isolation, identification and characterization of *Escherichia coli* from urine samples and their antibiotic sensitivity pattern. European Journal of Experimental Biology, 1(2): 118-124.

[27] Khan, R. A.; Khan, F. A.; Iqbal, M., and Nigar, H. (2019). Prevalence and antibiotic susceptibility of multi-drug resistant *Staphylococcus aureus* and *Acinetobacter baumannii* in clinical samples from intensive care unit patients in a tertiary care hospital at Peshawar (Pakistan). Anaesthesia, Pain and Intensive Care, 231-236.

[28] Hussein, N. R.; Daniel, S.; Salim, K., and Assafi, M. S. (2018). Urinary Tract Infections and Antibiotic Sensitivity Patterns Among Women Referred to Azadi Teaching Hospital, Duhok, Iraq. Avicenna Journal of Clinical Microbiology and Infection, 5(2): 27–30.

[29] Chandrasekaran, D.; Venkatesan, P.; Tirumurugaan, K. G.; Nambi, A. P.; Thirunavukkarasu, P. S.; Kumanan, K., and Ramesh, S. (2014). Pattern of antibiotic resistant mastitis in dairy cows. Veterinary World, 7(6): 389-394.

[30] Chakraborty, S. P.; KarMahapatra, S.; Bal, M., and Roy, S. (2011). Isolation and identification of vancomycin resistant *Staphylococcus aureus* from postoperative pus sample. Al Ameen Journal of Medical Sciences, 4(2): 152-168.

[31] Hammoudi, A. A. (2013). Urinary tract infection of adults in Baghdad City. International Journal of Current Microbiology and Applied Sciences, 2: 1-6.

[32] Sujatha, R., and Pal, N. (2015). Antibiotic Resistance Pattern Of The Hospital And Community Acquire Isolates Of Uropathogens In A Teritiary Care Centre at Kanpur" Rama Univ. Journal of Medical Sciences, 1(1): 10-17.

[33] Bendahou, A.; Lebbadi, M.; Ennanei, L.; Essadqui, F. Z., and Abid, M. (2008). Characterization of *Staphylococcus* species isolated from raw milk and milk products (lben and jben) in North Morocco. The Journal of Infection in Developing Countries, 2(03): 218-225.

[34] Cheung, A. L.; Nishina, K. A.; Trotonda, M. P., and Tamber, S. (2008). The SarA protein family of *Staphylococcus aureus*. The international journal of biochemistry and cell biology, 40(3): 355-361.

[35] Rallapalli, S.; Verghese, S., and Verma, R. S. (2008). Validation of multiplex PCR strategy for simultaneous detection and identification of methicillin resistant *Staphylococcus aureus*. Indian Journal of Medical Microbiology, 26(4): 361-364.

[36] Didelot, X.; Bowden, R.; Wilson, D. J.; Peto, T. E., and Crook, D. W. (2012). Transforming clinical microbiology with bacterial genome sequencing. Nature Reviews Genetics, 13(9): 601-612.

[37] Ibed, A. N., and Hamim, S. S. (2014). Molecular detection of methicillin resistant *Staphylococcus aureus* isolated from burns infection in Al-Nasiriyah City. World Journal of Pharmaceutical Sciences, 2: 950-954.

[38] Degaim, Z. D.; Shani, W. S., and Hamim, S. S. (2015). Virulence factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from burn patients. International Journal of Current Microbiology and Applied Sciences, 4: 898-906.

[39] Hashim, I. A.; Wdaah, Q. H., and Atya, A. A. (2019). Potential effect of antimicrobial agents against *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains from patients with skin infections. University of Thi-Qar Journal of Science, 7(1): 7-14.

[40] Makgotlho, P. E.; Kock, M. M.; Hoosen, A.; Lekalakala, R.; Omar, S.; Dove, M., and Ehlers, M. M. (2009). Molecular identification and genotyping of MRSA isolates. Federation of European Microbiological Societies Immunology and Medical Microbiology, 57(2): 104-115.

[41] Al-Talib, H.; Yean, C. Y.; Al-Khateeb, A.; Hassan, H.; Singh, K. K. B.; Al-Jashamy, K., and Ravichandran, M. (2009). A pentaplex PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* and Panton-Valentine Leucocidin. BMC microbiology, 9(1): 113-119.

[42] Zhang, K.; Sparling, J.; Chow, B. L.; Elsayed, S.; Hussain, Z.; Church, D. L., and Conly, J. M. (2004). New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from Coagulase-Negative Staphylococci. Journal of clinical microbiology, 42(11), 4947-4955

الكشف المصلي والجزيئي للمكورات العنقودية الذهبية المعزولة من مرضى التهاب المسالك البولية

احمد عبدالرزاق عباس * سعد سلمان هميم **

hamim_pa@sci.utq.edu.iq

*** مكتب وزارة الصحة ذي قار**

**** قسم التحليلات المرضية ، كلية العلوم ، جامعة ذي قار ، العراق**

المستخلص:

تعتبر التهابات المسالك البولية واحدة من أكثر الإصابات شيوعًا التي يواجهها الأطباء على الرغم من توفر الأدوية المضادة للميكروبات على نطاق واسع. الالتهابات ناجمة عن مسببات بكتيرية مختلفة تنتشر بصورة واسعه ويمكن الحصول على العدوى بهذا المرض من خلال كل من المستشفى والمجتمع. هدفت الدراسة الحالية التحري عن بكتيريا المكورات العنقودية الذهبية المعزولة من مرضى التهاب المسالك البولية بحالاته المختلفة البسيطة والمعقدة، والكشف عن مضادات الميكروبات الأمثل لعلاج هذه الالتهابات. حيث تم جمع 600 عينة ادرار من منتصف مجرى البول خلال الفترة من أغسطس إلى ديسمبر 2018 من المرضى الذين يشتكون من التهاب المسالك البولية في مستشفى الحسين التعليمي في مدينة الناصرية, جنوب العراق. الدراسة شملت عزل وتشخيص المكورات العنقودية الذهبية على أساس الوصف المظهري والمجهري والأختبارات الكيميائية الحيوية وتأكيدها بواسطة أنظمة API-20 و Vitek2. اضافة إلى ذلك ، تعرضت جميع عزلات المكورات العنقودية الذهبية للتشخيص المصلي للبروتين A باستخدام اختبار تراص اللاتكس واستخدمت تقنية انزيم تفاعل البلمرة التقليدية للكشف عن وجود جين 16s RNA(353 زوج قاعدي). حيث تم تسجيل (380) عزلة موجبة ، وتم تحديد المكورات العنقودية الذهبية ب 50 عزلة (13.5%). ايضا خضعت جميع عزلات المكورات العنقودية لفحص الحساسية الدوائية ضد 14 نوع من المضادات الحيوية المختارة بأستخدام طريقة الأنتشار عبر الأقراص. حيث كانت جميع عزلات المكورات العنقودية الذهبية مقاومة تمامًا لمضادات البنسلين (P) والأوكساسيلين (Ox) والأمبيسيلين (Amp). بينما المضادات الحيوية الأكثر فعالية للمكورات العنقودية الذهبية كانت هي نيتروفورانتوين (NIT)و جنتاميسين (GN) ، حيث أظهرت العزلات حساسية لهذه المضادات بنسبة 76 ٪ و 60% تواليا .اما فيما يخص تحليل تسلسل النيوكليوتيدات لجين 16s RNA ، أظهرت النتائج أن هذه البكتيريا مرتبطة فعليًا بالبكتريا العنقودية الذهبية، ووفقًا للنتيجة الحالية ، ثلاثة من هذه العزلات سجلت عالميًا في بنك الجينات،وأرقام الانضمام إلى هذه العزلات هي (MK910079.1و MK910080.1 و MK910081.1).

الكلمات المفتاحية: المكورات العنقودية الذهبية ، التهابات المسالك البولية ، التشخيص ، الكشف المصلي والجزيئي.