Original Article

BACTERIOLOGICAL STUDY OF COAGULASE-NEGATIVE Staphylococcus spp ISOLATED FROM URINARY TRACT INFECTION AND THEIR RESISTANCE TO ANTIBIOTICS

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Abstract

From the rare species that cause urinary tract infections, seven different types of coagulation-negative Staphylococcus bacteria were isolated (9.82%) Staphylococcus epidermidis (8.9%) Staphelococcus haemolyticus, (3%) Staphylococcus xylosus (0.89%) for each bacteria Staphylococcus auricularis, Staphylococcus simulans, Staphylococcus hominis, and Staphylococcus lugduensis, as all isolates were 100% producers of biofilms by tube method and (40%) of Congo Red by Staphelococcus. haemolyticus, while the rest of the species were not producers by the Congo Red method and all isolates were fully resistant to the antibiotics. Amoxicillin/Clavulanic Acid, Oxacillin, Cefotaxim, Cefepime, Levofloxacin, and in a high percentage of cases highly sensitivite to the antibiotics Rifampcin and Amikacin.

Keywords: Biofilms, urinary tract infections, Staphylococcus spp.

INTRODUCTION

The urinary system is one of the body's most important organs. If it doesn't work right, it can affect the other organs because it helps control the amount of cellular fluid and its content . (Goldstein,2022) The urinary system works to get rid of harmful substances in the blood that the body doesn't need. These substances are then thrown away as urine (Wilson and Wilson,2021). As the urinary system works with other body systems to keep the acid-base balance and the water-salt balance in the blood, the urine and what's in it can be used to tell a lot about the body's normal pathological or physiological state (Ridley, 2018). We also know that the kidneys, ureters, bladder, and urethra are all parts of the urinary system. Because the ureters go into the bladder and there is a valve there, urine can't go back into the kidneys. These things keep the kidneys from catching diseases of the upper urinary tract (Harris and Bivalacqua, 2020). We also talk about the kidney, which is the most important part of the urinary system. The kidney filters out toxins, unwanted waste products, and extra ions while keeping the important parts of the blood. It works to make erythropoietin, which increases the production of red blood cells (RBCs) and renin, which helps control blood pressure (Ogobuiro and Tuma, 2020).

Urinary tract infections(UTIs) are caused by bacteria getting into a normally clean urinary tract. These are some of the most common bacterial infections in the world. Infections of the urethra, bladder, ureters, and kidneys are a major cause of disease in babies, older men and and women of all ages (Seifu and Gebissa, 2018) . They can lead to serious problems like frequent infections, pelvic and kidney infections that cause sepsis, and kidney damage in young children (Lombel et al., 2022) . When a pregnant woman has a urinary tract infection, it can cause the baby to come early (Ghouri et al.,2019) . This increases the chance that the mother or baby will get sick or die, even if the infection has no symptoms. We know that there are many types of bacteria that can cause urinary tract infections, but Klebsiella spp. is one of the most important ones. Staphylococcus spp , Proteus spp., Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa (Azab, 2021).

MATERIALS AND METHODS

Materials

Collection of samples

From December 1, 2021, to March 30, 2022, urine samples were collected from people with urinary tract infections at Ibn Sina

Hospital and Mosul General Hospital. A total of 170 mid urine stream samples, 100 from women and 70 from men, were taken. from different ages, using plastic containers, and advising all patients to take a sample during the middle of urination. After taking the sample and writing information on it and on the questionnaire form, it is sent to the lab within half an hour, where it is implanted by a sterile agricultural carrier (loop) using the flame method on the medium Agar Makonki and the medium of blood Agar. The rest of the urine is put in sterile test tubes and spun in a centrifuge at 2000 cycles per minute for 5 minutes. A drop of the precipitate is then put on a glass slide and looked at with a photomicroscope to look for bacterial cells, pus cells, red blood cells, particles of crystals, and epithelial cells (Karah et al., 2020; Price et al., 2016).

Identification Methods:

First: it depends on the Gram stain, the catalase test, the coagulase test, and the mannitol fermentation test on Mannitol Salt Agar (Granato et al., 2019; Wanger et al., 2017).

Second: using the VITEK 2 System from the French company Biomerieux

Sensitivity Test:

The hypersensitivity test was done using a modified version of the Kirby-Bauer method, as described in (Chess, 2019; Granato et al., 2019), and antibiotics made in Turkey by Bio-analyse were used in a concentration that is standard around the world. Pure bacterial colonies (18-24 hours old) were placed in a test tube with 50 ML of physiological brine to make a bacterial suspension, and the turbidity of the suspension was adjusted with a cotton swap to the turbidity of the McFarland solution (0.5), the bacterial suspension was spread on the centre of the Mueller-Hinton agar, and the discs were placed on the center and incubated at 37 °C for 24 hours. At the end of the incubation period, the inhibition area around the inhibition disc is measured with a measuring ruler, and the resistance or sensitivity of the bacterial cell to the antibiotics used in Table (1) is monitored.

Table 1. Antibiotics for sensitivity testing for Staphylococcus spp.

Antibiotics	code	Concentration(µg)	Sensitive mm	Medium sensitivity mm	Resistant mm
Levofloxacin	LEV	5	>28	22-28	< 22
Cefepime	CEP	10	>32	26-32	<26
Rifampin	RA	5	>34	26-34	<26
Vancomycin	VA	30	>21	17-21	<17
Cefotaxime	CTX	30	>22	18-22	<18
Oxacillin	OX	5	>24	18-24	<18
Ceftriaxone	CRO	10	>28	22-28	<22
Methicillin	ME	10	>34	30-34	<30
Amikacin	AK	10	>26	20-26	<20
Amoxicillin/clavulanic acid	AMC	20/10	>27	27-35	<35

(CLSI, 2022).

Methods and media used to detect the ability of bacteria to form biofilms

First : Tryptic soy broth medium added 1 % glucose (Cappuccino and Welsh,2018)

The bacteria that had been found and identified were turned on by taking a drop of the ....-..sample that had been kept and putting it on the blood media. The media were then put in an incubator at 37°C for 24 hours, and a piece of the growing colony was used to vaccinate each species in a test tube with a medium of Tryptic soy broth that had been made ahead of time and contained 1% glucose. then the medium that contain the bacteria there washes with phosphate buffer solution The tubes were then put in an incubator at 37°C for 24 hours. Then, (1%) Violet Crystal Dye is added to each tube, and the tubes are left for (15) minutes. The tubes are then washed with deionized water and turned upside down to dry, and biofilms are seen in each tube (Sultan and Nabiel, 2019).

Second : Congo Red Agar medium (Cappuccino and Welsh, 2018)

After activating the samples and planting them on rich mediums, the cultivated carrier ... took a seed from the sample and planted it in the center of the Congo Red Agar that had been prepared in advance. After 24 hours of incubation at 37 °C, the bacteria that make up the biofilm were detected by the color of the medium changing from red to black. This shows that the bacteria are producing biofilm , when the medium do not change it is color this indicate that the bacteria not produce biofilm (Shrestha et al., 2018)

RESULTS AND DISCUSSION

The result showed that among 170 specimens of people with urinary tract infection we had rare species of the bacterial genus staphylococcus spp as shown in)fig1(.it was S.hemolyticus in 10 of the isolate at rate of 8.9% this type of bacteria is pathological when it found in places of her than their natural environment they have virulence factor as a biofilm producer and antibiotic resistance like aminoglycoside and their role in blood hemolysis .the large use of antibiotic can had to appearance of resistance (pain et al.,2019). This number was about the same as what (Luty et al., 2020) said about the infection rate, which was (11.5%). Also, our results were different from (Kabduova et al., 2022). This type of bacteria caused (21.17%) of infections. With a rate of (9.82%), the S.hemolyticus infection affect specifically the patient with low immunity because they are largely resist to antibiotic from all (CONS) (Barros et al.,2012) there were also (11) S. epidermidis bacteria isolates with rate of 9.82% . Our results were similar to those of (Seifu and Gebissa, 2018) because (12.3%) of the bacteria were isolated. They were also similar to those of (Shahab and Salih, 2017) because (7.5%) of the bacteria were isolated. However, our results do not match those of (Luty et al., 2020) because only (0.90%) of the bacteria were isolated. It was one of the causes of hospital infection because it was form over the skin and mucous membrane and enter the urinary tract through the urethra ,it had a capability to accommodate and can cause infection that relate to catheterization and had genes that is resistant to antibiotic (chabi and momtaz,2019) As for S.xylosus, (3) bacterial isolates were found at a rate of (2.6%). This was the same as what the researcher (Obaid et al., 2020) found, which was that (5%) of this type of bacteria was isolated. They had a virulence factor which made it opportunistic infection for (CONS) that have the least rate in our current study S.auricularis , S.lugdunensis,S.simulans and S. hominis where there one isolated for each species at rate of 0.89% this result agreed whit researcher (Luty et al.,2020) it was at rate of (0.3% and 0.6%) respectively for S. lugdunensis and S. hominis while for S.simulans our result differ with (Szemraj et al.,2020) that was isolated at (22.5%) our current study, S. hominis, S. simulans, S. lugdunensis, and S. auricularis are the species that are the least different from each other where there was one bacterial isolate for each species, which is a rate of (0.89%) S.lugdunensis by (0.6%(and)0.3%), respectively, as for S.auricularis and S.hominis, and our results agreed with the results of the researcher (Chesca et al., 2022) to S. auricularis and S. simulans for the study done at the University of …Transylvania Brasov, Romania, where the percentages were (0.73% (and)2.44%) respectively, as mentioned by the researcher (Szemraj et al., 2020) that these species can't be thought of as a source of contamination because they have virulence factors that make them opportunistic pathogens and can quickly pick up genes that make them resistant to antibiotics. They also cause infections that are similar to those caused by S. aureus. As it gets more resistant to antibiotics, S.aureus (CONS) is becoming a bigger threat to public health around the world. Because it is a pathogen and not just a pollutant, it is often found in hospitals where there is inflammation (Asante et al.,2020).

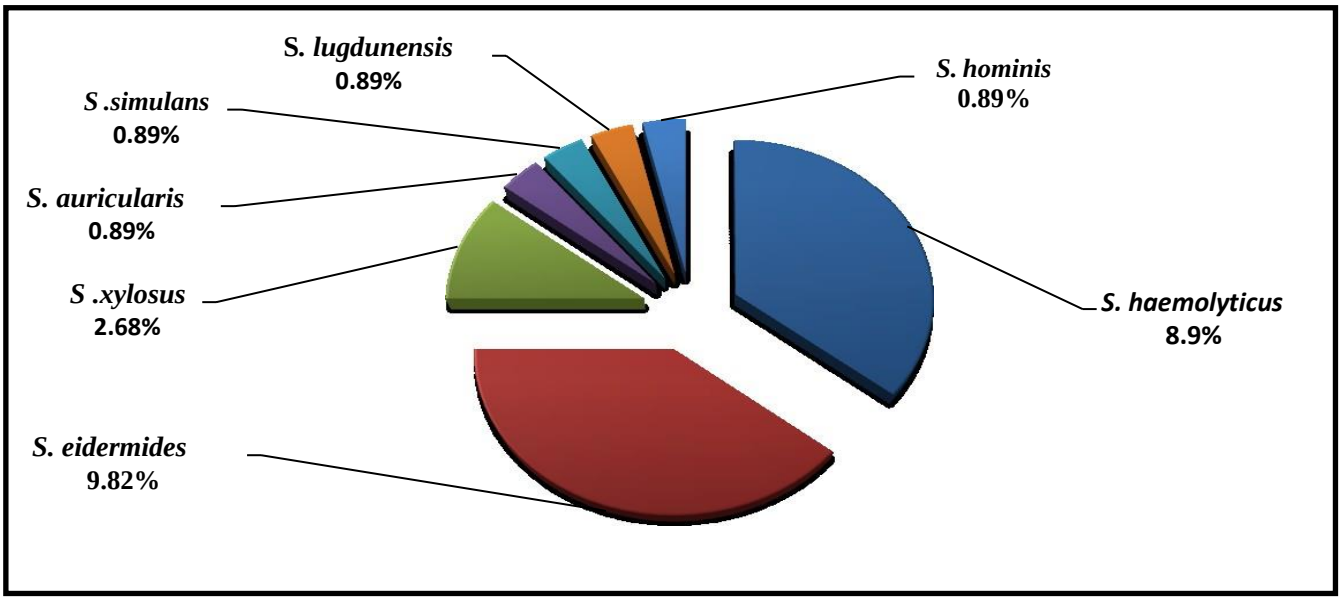

Fig. (1) The Percentage of rare bacterial species found in urinary tract infections

Results of biofilm formation:

Rare bacteria from urinary tract infections have been shown to be able to form biofilms using the tube method. This is shown by the appearance of violet at the bottom or on the side of the tube, which means the result is positive (Figure 2). There was also the Red Congo Method (Figure 3) shows that the bacteria that made biofilms had black colonies with a crystalline shape, while the bacteria that didn't make biofilms had red or pink colonies. The tube method and the Congo Red method are

the best ways to find bacteria that make biofilm. While the Congo red method is used as a general way to find biofilm-producing bacteria in laboratory (Wadher and Rewatkar, 2013) showed that the appearance of a violet layer under the tube is proof that the result is positive. In the case of the Congo Red Agar, the appearance of colonies with a bright black color in the middle is proof that it is the ability causing the formation of biofilms (Kirmusaogu, 2019 ; Shrestha et al., 2018).

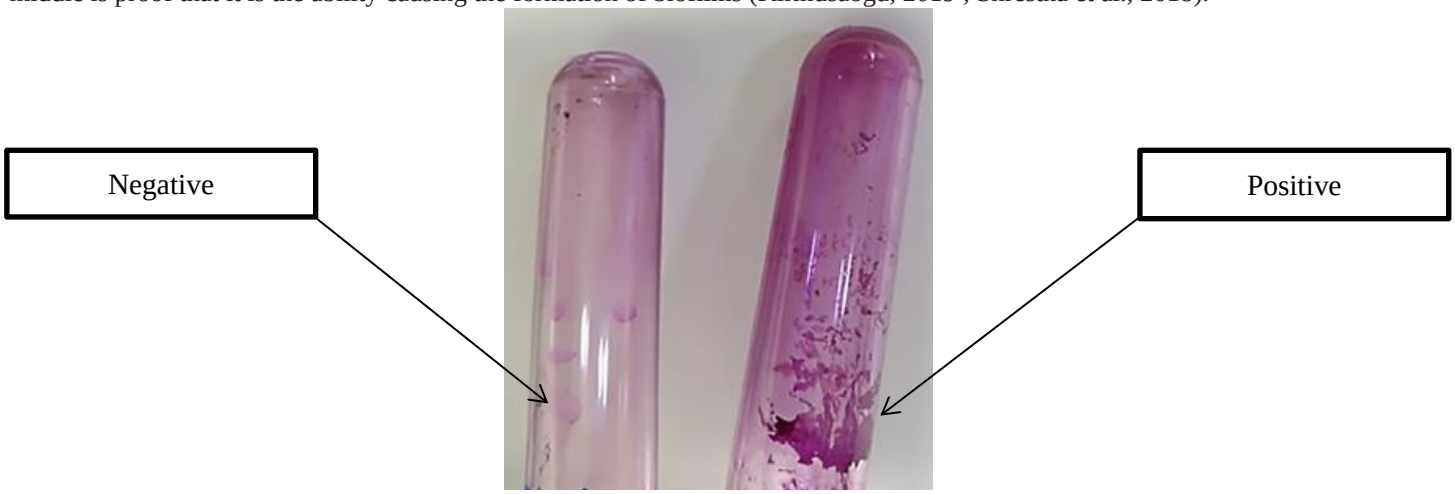

Fig. (2) Positive and negative results of biofilms forming in tube method

Fig. (3) Biofilms forming

Fig. (4) Non Biofilms forming

Staph. haemolyticus. showed that it could form biofilms by being positive by the tube method (40%) of the time and weakly positive (60%) of the time. Biofilms showed up (40%) of the time when the Congo Red method was used. Our results were different from those of the researcher (Deka, 2014), who said that (21%) of the S.haemolyticus bacteria were found in isolation. Our results match those of the researcher (Omari, 2020) in a study done in the city of Mosul at Ibn Sina Teaching Hospital, where (100%) of biofilms were formed using the tube method and (50%) were formed using the Congo Red method.

Both the tube method and the Congo Red method fail to make biofilms from S. epidermidis. Our results are the same as those of the researcher (Jabir, 2022), where none of his isolates were able to make biofilms. They are also the same as those of (Al-Omari, 2013), where none of his isolates were able to make biofilms using the Congo

Red method, but (66.6%) of bacteria were able to do so using the tube method. For the other species S. xylosus, S. auricularis, S. simulans, S. lugdunensis, and S. hominis the rate of forming biofilm for those species were 100% by tube method while no biofilm formation by using Congo red for all isolates .The researcher (Yong et al., 2019) confirmed our results and said that these species are opportunistic pathogens that cause clinical infections because they have virulent factors, such as the ability to form biofilms. This makes them important, even though some strains of Staphylococcus are negative for biofilm formation. Researchers (Szczuka et al., 2016) confirmed that some strains of bacteria that don't make an enzyme for coagulation can still form biofilms, which is one of the most important factors in pathological events.

This study found that S. haemolyticus was (90%) sensitive to antibiotics (Levofloxacin, Amikacin), (80%) sensitive to Rifampin, (40%) sensitive to Methicillin, and vancomycin (20%) but resistant .to. .antibiotics.(Amoxicillin/clavulanic acid, Ceftriaxone,oxacillin,cifepime and Ceftaxime) at rate 100%. Our results were similar to those of the researcher (Sarker.et.al.,.2021).who found that the bacteria were highly resistant to Amoxicillin/clavulanic at (100%), However the bacteria were sensitive to the antibiotics Levofloxacin and Amikacin, with sensitivity rates of (73.08 and 84.61%) respectively. Our results were different when it came to the antibiotic ceftriaxone with sensitivity rate (80.76%)

The S..xylosus bacteria was sensitive to antibiotics Levofloxacin, Rifampin, Amikacin, Methicillin, and Vancomycin, at rate (33.3%). While the antibiotics Cefepime, Cefotaxime, Oxacillin, Ceftriaxone, and Amoxicillin/clavulanic acid Show resistance The researcher (Onyebueke et al., 2019) found that the average level of sensitivity to the antibiotic Levofloxacin was (69.7%)at the University of Nigeria Teaching Hospital in Nigeria, where his study was done. Our result where average with (Maroff,2018) in study done in Tikrit Hospital which was sensitive to Amikacin (39%).

As for the bacteria that didn't have a clotting enzyme and had the lowest rates in our study, they were S.hominis, S.epidermidis, S. simulans, S. lugdunensis, and S. auricularis. S. epidermidis bacteria were completely sensitive to the antibiotic Amikacin, moderate sensitivity to levofloxacin and cefepime this result was average with researcher (Abdulwahhab and Atiyea ,2021) at rate of (69.7%) for levofloxacin and at rate of (88.9%) for Amikacin and it was deferent in Amoxicillin/clavulanic acid at rate(88.9) sensitivity, whereas S. simulans bacteria were completely sensitive to Rifampcin and Vancomycin. And moderately sensitive to Amikacin . S. hominis and S.lugdunensis bacteria were both highly sensitive to the antibiotics Rifampcin, Amikacin, Methicillin and Levofloxacin and moderately sensitive to Vanomycin .These results match those of the researcher (Onyebueke et al., 2019), who found that S. hominis bacteria were resistant to antibiotics containing Amoxicillin / Clavulanic Acid and had different levels of sensitivity to other antibiotics.

For S..lugdunensi bacteria, our results were the same as the researcher's (Luty et al., 2020), where the bacteria was highly sensitive to the Levofloxacin and resistant to the rest of the antibiotics while with the S.simulans the result was agreed with the researcher (Drobeniuc et al.,2021). Even though these bacteria are rare, they can cause endocarditis, bacteremia, and urinary tract infections. This study found that S. haemolyticus was (90%) sensitive to antibiotics (Levofloxacin, Amikacin), (80%) sensitive to Rifampcin, (40%) sensitive to Methicillin, and vancomycin (20%) but resistant to antibiotics (Amoxicillin/clavulanic, Ceftriaxone,oxacillin,cifepime and Ceftaxime) at rate 100%. Our results were similar to those of the researcher (Sarker.et.al.,.2021), who found that the bacteria were highly resistant to Amoxicillin/clavulanic at (100%), However the bacteria were sensitive to the antibiotics Levofloxacin and Amikacin, with sensitivity rates of (73.08 and 84.61%) respectively. Our results were different when it came to the antibiotic ceftriaxone with sensitivity rate (80.76%)

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For S. lugdunensi bacteria, our results were the same as the researcher's (Luty et al., 2020), where the bacteria was highly sensitive to the Levofloxacin and resistant to the rest of the antibiotics while with the S.simulans the result was agreed with the researcher (Drobeniuc et.al.,2021). Even though these bacteria are rare, they can cause endocarditis, bacteremia, and urinary tract infections.

CONCLUSION

It's not right to leave out rare bacteria or call them just "contaminants." They cause infections in people that are similar to those caused by Staphylococcus aureus because they have highly infectious agents like biofilms and can quickly pick up genes that make them resistant to antibiotics. They are opportunistic pathogens because they have traits that make them dangerous.

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ETHICAL STATEMENT

This study was approved by the ethical committee of Ibn Sina Teaching Hospital and Mosul General Hospital

REFERENCES

1. Abdulwahhab, M. A., & Atiyea, Q. M. (2021). Isolation and Diagnosis of Escherichia Coli Causing Urinary Tract Infections from People of Different Ages in Mosul City. Annals of the Romanian Society for Cell Biology, 7017-7025.
2. Al-Omari, A. W. R., (2013). Investigation of the bacteria forming biofilms in patients using urinary catheters and the study of their resistance to some antibiotics using a locally manufactured dish. Master's Thesis, College of Science, Tikrit University, 107 pages
3. Al-Omari, Raad Muhammad Mahmoud (2020) Investigating the pathogenic microbes among dialysis patients in the city of Mosul and their ability to form biofilms. Master's thesis, College of Science, University of Mosul, 109 pages
4. Asante, J., Amoako, D. G., Abia, A. L., Somboro, A. M., Govinden, U., Bester, L. A., & Essack, S. Y. (2020). Review of clinically and epidemiologically relevant coagulase- negative Staphylococci in Africa. Microbial Drug Resistance, 26(8), 951-970.
5. Azab, K. (2021). Prevalence and relation of urinary tract infection bacterial pathogens to sex and ages among patients in three arab countries al-Azhar Journal of Pharmaceutical Sciences, 63(1), 194-206.
6. Barros, E. M., Ceotto, H., Bastos, M. C. F., Dos Santos, K. R. N., and Giambiagi-deMarval, M. (2012). Staphylococcus haemolyticus as an important hospital pathogen and carrier of methicillin resistance genes. J. Clin. Microbiol. 50, 166–168. doi: 10.1128/JCM.05563-5511
7. Cappuccino, J. G. and Welsh, C. (2018). Microbiology A Laboratory Manual. Pearson Education limited, US
8. Chabi, R., & Momtaz, H. (2019). Virulence factors and antibiotic resistance properties of the Staphylococcus epidermidis strains isolated from hospital infections in Ahvaz, Iran. Tropical medicine and health, 47(1), 1-9.
9. Chesca, A. E., Abdulina, G. G., Medetova, A. A., Kabduova, A. K., Beysembayeva, G. G., Moraru, D. D., & Sandle, T. S. (2022). Opportunistic Pathogens in Patients with Urinary Tract Infection.
10. Chess,B.(2019). Laboratory Applications in Microbiology: A Case Study Approach. Fourth Edition. McGraw-Hill Education. New York. USA.
11. CLSI ,Clinical and Laboratory Standards Institute (2022). Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100 (ISBN 978-1-68440-134-5 [Print]; ISBN 978-1-68440-135-2 .
12. Deka, N. (2014). Comparison of Tissue culture plate method, Tube method and Congo Red Agar method for the detection of biofilm formation by Coagulase-negative Staphylococcus isolated from non-clinical isolation. Int. J. Curr. Microbiol. App. Sci., 3(10):810- 815.
13. Drobeniuc, A., Traenkner, J., Rebolledo, P. A., Ghazaryan, V., & Rouphael, N. (2021). Staphylococcus simulans: A rare uropathogen. IDCases, 25, e01202.
14. Ghouri, F., Hollywood, A., & Ryan, K. (2019). Urinary tract infections and antibiotic use in pregnancy-qualitative analysis of online forum content. BMC pregnancy and childbirth, 19(1), 1-8.
15. Goldstein, D. L. (2022). Renal and extrarenal regulation of body fluid composition. In Sturkie's avian physiology (pp. 411-443). Academic Press.
16. Granato, P.a.; Morton, V. and Morello, J. A. (2019). Laboratory manual and workbook in Microbiology Application to patient care. McGrow-Hill Education, New York, USA.
17. Harris, K., & Bivalacqua, T. J. (2020). Regenerative medicine in urology: the future of urinary reconstruction. Trends in Urology & Men's Health, 11(2), 9-12.
18. Jabir, D. M. (2022) .Evaluation of the ability of some bacterial species isolated from UTI to form biofilm.
19. Kabduova, A., Beysembayeva, G., Moraru, D., and Sandle, T. (2022). Opportunistic Pathogens in Patients with Urinary Tract Infection
20. Karah, N.; Rafei, R.; Elamin, W.; Ghazy, A.,; Abbara, A.; Hamze, M. and Uhlin, B. E. (2020). Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens in LowResource Settings. Diagnostics (Basel, Switzerland)., 10(10): 832.
21. Kirmusaogu, S. (2019). The methods for detection biofilm and screening antibiofilms of activity. In Exopolysaccharides method of preparation and Application, DOI: http://dx. doi. org/10. 5772/intechopen. 84411
22. Lombel, R. M., Brakeman, P. R., Sack, B. S., & Butani, L. (2022). Urologic Considerations in Pediatric Chronic Kidney Disease. Advances in Chronic Kidney Disease, 29(3), 308-317.

23. Luty, R. S., Fadil, A. G., Najm, J. M., Abduljabbar, H. H., and Kashmar, S. A. A. (2020). Uropathogens antibiotic susceptibility as an indicator for the empirical therapy used for urinary tract infections: a retrospective observational study. Iranian Journal of Microbiology, 12(5), 395.
24. Maroff, M. N. (2018). Determination of the inhibitory activity of some biological extracts agaiast multi rrsistans antibiotic Staphylococcus specis which and isolated from different sources of infechtion. Tikrit Journal of Pure Science, 22(11), 6-14
25. Obaid, A. A., Lateef, A. S., Oudah, L. A., Harjan, Q. J., & Oudah, Z. A. (2020). Isolation of Staphylococcus xylosus from Urinary Tract Infection in Al-Diwaniya City, Iraq. International Journal of Research in Pharmaceutical Sciences, 11(1), 760-764
26. Ogobuiro, I.and Tuma, F. (2020). Physiology, Renal. StatPearls, Treasure Island (FL): StatPearls Publishing
27. Onyebueke, E. A., Onyemelukwe, N. F., & Oladeji, D. S. (2019). Antibiotic susceptibility pattern of Staphylococcus species implicated in urinary tract infection in Enugu State, Nigeria. PharmacologyOnline, 1, 166-176.
28. Pain, M., Hjerde, E., Klingenberg, C., & Cavanagh, J. P. (2019). Comparative genomic analysis of Staphylococcus haemolyticus reveals key to hospital adaptation and pathogenicity. Frontiers in microbiology, 10, 2096.
29. Price, T. K., Dune, T., Hilt, E. E., Thomas-White, K. J., Kliethermes, S., Brincat, C., ... & Schreckenberger, P. C. (2016). The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. Journal of clinical microbiology, 54(5), 1216-1222.
30. Rewatkar, A. R., & Wadher, B. J. (2013). Staphylococcus aureus and Pseudomonas aeruginosa Biofilm formation Methods. J Pharm Biol Sci, 8(5), 36-40
31. Ridley, J. W. (2018). Diseases of the urinary system. In Fundamentals of the Study of Urine and Body Fluids (pp. 99-142). Springer, Cham
32. Sarker, S. (2021). Molecular Characterization and Antibiogram Profiling of Multidrug Resistant Staphylococcus haemolyticus Isolated from Patients with Urinary Tract Infection in Bangladesh.
33. Seifu, W. D., & Gebissa, A. D. (2018). Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. BMC infectious diseases, 18(1), 1-9.
34. Shahab , N. W.; Ali, C. I.; Salih S. M.(2017) Isolation and Identification of bacteria causing urinary tract infections in children in Kirkuk city. Tikrit Journal of Pure Science., 22(2): 8-12
35. Shrestha, L. B.; Bhattaria, N. R. and Khanal, B. (2018). Comparative evaluation of methods for the detection of biofilm formation in coagulase-negative staphylococci and correlation with antibiogram. J. Infec. Drug Risest., 11(1):607 – 613 .
36. Sultan, A. M. and Nabiel, Y. (2019). Tube Method and Congo Red Agar Versus Tissue Culture Plate Method for Detection of Biofilm pruduction by pathogen Isolated from Midstream urine. African J. Clin. Exper. Microbiol., 20(1):60 – 66 .
37. Szczuka, E., Jabłońska, L., & Kaznowski, A. (2016). Coagulase-negative staphylococci: pathogenesis, occurrence of antibiotic resistance genes and in vitro effects of antimicrobial agents on biofilm-growing bacteria. Journal of Medical Microbiology, 65(12), 1405-1413
38. Szemraj, M., Grazul, M., Balcerczak, E., & Szewczyk, E. M. (2020). Staphylococcal species less frequently isolated from human clinical samples-are they a threat for hospital patients?. BMC Infectious Diseases, 20(1), 1-10.
39. Wanger, A.; Chavez, V.; Huang, R. S. P.; Wahed, A.; Actor, J. K. and Dasgupta, A. (2017). Microbiology and Molecular Diagnosis in Pathology. Elsevier Inc. All Rights Reserved.
40. Wilson, M., & Wilson, P. J. (2021). Cystitis. In Close Encounters of the Microbial Kind (pp. 347-360). Springer, Cham.
41. Yong, Y. Y., Dykes, G. A., and Choo, W. S. (2019). Biofilm formation by Staphylococci in health-related environments and recent reports on their control using natural compounds. Critical reviews in microbiology, 45(2), 201-222