Chelonian Conservation And Biology

Vol. 18 No. 2 (2023) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.11(2).1103.1113

THE PREVALENCE OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM URINARY TRACT INFECTIONS IN SHEEP AND SHEEP BREEDERS

Areean Ibrahim Hassan Bak(1), Ali Ibrahim Ali Al-Ezzy(2), Al-Zubaidi Raad Mahmood Hussein(3)

[1, 3]Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq.

[2],Departments of Pathology, College of Veterinary Medicine, University of Diyala, Iraq.

Email: dr.areeanibrahim@gmail.com.

Abstract

The objective of this research was to examine the prevalence of bacterial urinary tract infections in sheep and sheep breeders, as well as their antibiotic susceptibility. In Sulaymaniyah province (Kalar district and neighboring villages) and Diyala province (Khanaqin district, villages of Qorato district), **120** urine specimens from sheep and sheep breeders with urinary tract infections (UTIs) were collected from the first of October 2022 to March 2023. Current study includes **(49)** females sheep breeders and **(71)** ewes suffering from clinical signs of urinary tract infections. A total of **27/ 49, (55.10%)** of urine specimens give positive urine culture among female sheep breeders versus 22 out of **49, (44.89%)** give negative urine culture. A total of **35/71, (49.29%)** of urine specimens gives positive urine culture among ewes versus 36 out of **71, (50.70%)** give negative urine culture. Results showed that *Staphylococcus aureus* represent **3(4.83%),** in Human, while in sheep *S.aureus* represent **7(11.29%)**, then their susceptibility to wards selected antibiotics were detected. Results appeared the *S.aureus* shown **(100%)** resistance for the following Class of antibiotics, Penicillines and Cephalosporins. Methicillin resistance was detected by Cefoxitin Screen test which indicate that **3/3, (100%)** of S. *aureus* have resistance which confirmed early by detection of MecA gene. Resistance of *S.aureus* to Polypeptides antibiotics was detected in **3/3, (100%)** for Vancomycin and **2/3, (66%)** for Teicoplanin in female sheep breeders, while in sheep *S.aureus* shown **(100%)** resistance for the following Class of antibiotics, Penicillines and Cephalosporins. Methicillin resistance was detected by Cefoxitin Screen test which indicate that **7/7, (100%)** of *S. aureus* have resistance which confirmed early by detection of MecA gene. Resistance of *S.aureus* to polypeptides antibiotics was detected in **7/7,**

(100%) for vancomycin and **6/7, (85.72%)** for Teicoplanin. Resistance of *S.aureus* to macrolides antibiotics was detected in **1/7, (14.28%)** for Azithromycin. Resistance of *S.aureus* to Lincosamides antibiotics, Clindamycin was detected in **1/7, (14.28%)**

Introduction

One of the most vital systems in both animal and human anatomy is the urinary system, which has the primary job of eliminating harmful waste from the body. As well as the control of the body's fluid components (Mohammed et al., 2020). Byron (2019) defines the urinary tract infection (UTI) as the urothelium's inflammatory reaction to bacterial invasion and the second most prevalent kind of infection in the body. An estimated 150 million of the urinary tract infections occur annually worldwide (Sahu et al., 2019). The bacteria that cause UTIs originate in the gastrointestinal system and colonize the external genitalia, invading the bladder and urethra to obstruct the flow of urine (Abdullah and Mustafa, 2019).In addition, study done by Mohammed, et al. (2020) found that urinary tract infections (UTIs) harm the vascular system of the bladder, which lowers kidney function and disrupts the excretion of metabolic end products.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) was identified as the primary cause of hospital-associated infections in the last ten years and as one of the most nosocomial bacteria globally in the 1980s (Alsolami et al., 2023). Ali, et al. (2021) it has been demonstrated that the versatile bacteria S. aureus may infect humans as well as animals. It is related to animal mucous membranes and skin. TWAFIK (2023) when Comparing sheep to other ruminant species, infections of the urinary tract have not been as common and most frequent outcome in sheep is inflammation of the kidneys. This ultimately causes urinary tract infections, which result in significant economic losses, and lowers animal productivity, both quantitatively and qualitatively.

Hospital-associated MRSA isolates are a commonly seen phenomenon worldwide. The mecA gene, which is in charge of encoding resistance to all β-lactam antibiotics other than fifth-generation cephalosporins, typically encodes this resistance. PBP2a, or Penicillin-Binding Protein 2a, has a modest affinity for β-lactam antibiotics and is encoded by the mecA gene. In S. aureus, a mecA homolog gene called mecC has been identified. It likewise encodes a mutated PBP (Lakhundi and Zhang, 2018). These bacteria can infect fish and birds in addition to humans, and they can survive in the environment (water, air, and dung) and behave as commensal and opportunistic pathogens.(Heaton et al., 2020)

Materials and Methods

Samples: This study included the collection of One hundred and twenty urine samples were collected from Patients with clinical signs of urinary tract infection sheep (71 samples) and their breeders (49 samples), in Diyala and Sulaymaniyah province from the first of October 2022 to March 2023; all enrolled breeders were subjected to questionnaire before samples were collected from breeders and sheep, also questioner related to risk factor for sheep infection was filled by breeders.

Figure (1) sample collection in

Laboratory investigations: In order to prevent contamination, the urine specimens were first cultured instantly on mannitol salt agar and incubated for 24 hours at 37 oC. (Mafisa, 2022).The morphological characteristics (colony size, shape, color, hemolysis, translucency, edge, elevation, and texture) on culture media and biochemical tests, Antibiotics susceptibility test were used to identify S. aureus. Gram stain was applied to the isolates in order to observe how they responded to the stain and how they were arranged (Pervin et al., 2019).

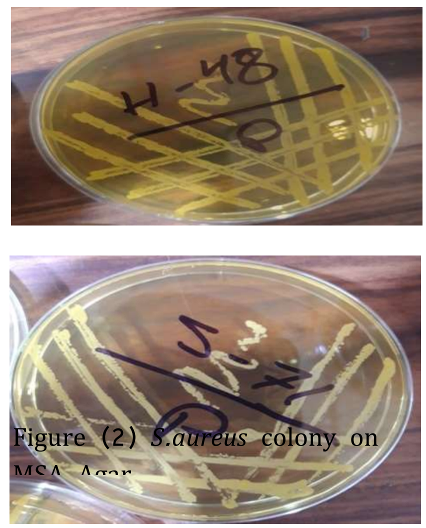

Figure (2) *S.aureus* colony on MSA Agar

Confirmatory Diagnosis of S. aureus: Testing for antibiotic susceptibility and bacterial identification is done by the fully automated VITEK® 2 System. Based on the manufacturer's instructions, which called for planting urine samples—isolated from sheep and humans—on Mannitol Salt Agar (MSA) and incubating them for 24 hours at 37 °C, the diagnosis was made. Once bacterial colonies started to form on the medium, a pure colony was transferred into a sterile inoculating loop and combined with physiological normal saline in a manufacturer-approved tube(EL-Marakby et al., 2018)

Results

A- Bacterial Isolates from Female Sheep Breeders and Ewes with Clinical Manifestation of Urinary Tract Infections

The investigation used the Vitek 2 system and conventional PCR using specific gene S. aureus 23srRNA primers (Staur 4, Staur 6) to identify S. aureus in 3/62 (4.83%) of female sheep breeders with clinical manifestations of UTI versus 7/62 (11.29%) among ewes with clinical manifestations of urinary tract infections. The study was conducted on 62 bacterial isolates, as shown in Table 1.

These isolates were initially identified as Staphylococcus aureus by traditional culture on mannitol salt agar and standard biochemical tests.

Table 1: Bacterial Isolates from Female Sheep Breeders and Ewes with Clinical Manifestation of Urinary Tract Infections

Bacterial isolates from infected individuals	Female sheep breeders with clinical manifestation of UTI	Ewes with clinical manifestation of UTI	Total
Staphylococcus epidermidis	10(16.12%)	10(16.12%)	20(32.25%)
Staphylococcus aureus	3(4.83%)	7(11.29%)	10(16.12%)
Staphylococcus equorum	1(1.61%)	7(11.29%)	8(12.90%)
Staphylococcus haemolyticus	6(9.67%)	2(3.22%)	8(12.90%)
Staphylococcus lentus	2(3.22%)	2(3.22%)	4(6.45%)
Unknown	1(1.61%)	1(1.61%)	2(3.22%)
Staphylococcus xylosus	0(0%)	2(3.22%)	2(3.22%)
Staphylococcus warneri	1(1.61%)	1(1.61%)	2(3.22%)
Staphylococcus gallinarum	1(1.61%)	0(0%)	1(1.61%)
staphylococcus cohnii ssp. urealyticus	0(0%)	1(1.61%)	1(1.61%)
Staphylococcus arlettae	1(1.61%)	0(0%)	1(1.61%)
Enterococcus faecalis	1(1.61%)	0(0%)	1(1.61%)
Alloiococcus otitis	0(0%)	1(1.61%)	1(1.61%)
Aerococcus viridans	0(0%)	1(1.61%)	1(1.61%)
Total	27(43.55%)	35(56.45%)	62(100%)

B- Antibiotic Sensitivity Pattern for S. aureus Isolated from Female sheep Breeders and Ewes

Penicillins and cephalosporins are the antibiotic classes to which 3/3 (100%) of S. aureus isolated from female sheep breeders are resistant, as Table 2 illustrates. The cefoxitin screen test revealed methicillin resistance, indicating that 3/3 (100%) of S. aureus have resistance. The early discovery of the MecA gene further supported this finding. S. aureus was shown to be resistant to 2/3, 66% of teicoplanin and 3/3,100% of vancomycin when it came to polypeptide antibiotics.

Table 3 illustrates that 7/7 (100%) of the S. aureus isolates from ewes are resistant to cephalosporins and penicillins, two types of medicines. The cefoxitin screen test revealed methicillin resistance, indicating that 7/7 (100%) of S. aureus have resistance, which was verified early by finding the MecA gene. 7/7,100% of S. aureus samples showed resistance to vancomycin, and 6/7, 85.72% to teicoplanin, among other polypeptide antibiotics. 1/7 cases (14.28%) of S. aureus resistance to macrolide antibiotics was found to be azithromycin-resistant. Clindamycin, a lincosamides antibiotic, was shown to be resistant to S. aureus in 1/7 (14.28%) cases.

Table 2: Antibiotic Sensitivity Pattern for S. aureus Isolated from Female sheep Breeders

Class of antimicrobial agents	**Antimicrobial**	**MIC**	**Interpretation**	**No. (%) of S.aureus isolates**	**Class of antimicrobial agents**	**Antimicrobial**	**MIC**	**Interpretation**	**No. (%) of s. aureus isolates**
Penicillines	**Benzylpenicillin**	>= 0.5	R	3/3,(100%)	**Macrolides**	**Azithromycin**		S	3/3,(100%)
	Amoxicillin Clavulanic acid		R	3/3,(100%)		**Erythromycin**	<= 0.25	S	3/3,(100%)
	Oxacillin	>=4	R	3/3,(100%)	**Lincosamides**	**Clindamycin**	<= 0.25	S	3/3,(100%)
Cephalosporins	**Cefoxitin Screen**	Positive	Methicillin resistance	3/3,(100%)	**oxazolidinone**	**Linezolid**	2	S	3/3,(100%)
	Cefalexin		R	3/3,(100%)	**Polypeptides**	**Teicoplanin**	<=0.5	S	1/3, (33.33%)
								R	2/3, (66.67%)

	Cefazolin		R	3/3,(100%)		**Vancomycin**	*=32	R	3/3, (100%)
	Cefapime		R	3/3,(100%)					
Aminoglycosides	**Gentamicin**	<=0.5	S	3/3,(100%)	**Tetracycline**	**Doxycycline**		S	3/3,(100%)
	Tobramycin	<= 1	S	3/3,(100%)		**Tetracycline**	<=l	S	3/3,(100%)
Quinolones Fluoroquinolones	**Ciprofloxacin**		S	3/3,(100%)		**Tigecycline**	<= 0.12	S	3/3,(100%)
	Gatifloxacin		S	3/3,(100%)	**nitrofuran antibiotic**	**Nitrofurantoin**	<— 16	S	3/3,(100%)
	Levofloxacin	<= 0.12	S	3/3,(100%)	**Fusidane**	**Fusidic Acid**	°= 0.5	S	3/3,(100%)
	Moxifloxacin	<=0.25	S	3/3,(100%)	**Ansamycins**	**Rifampicin**	<= 0.5	S	3/3,(100%)
	Norfloxacin		S	3/3,(100%)	**Sulfonamides**	**Trimethoprim/ Sulfamethoxazole**	<= 10	s	3/3,(100%)

Table 3: Antibiotic Sensitivity Pattern for S. aureus Isolated from Ewes

Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of S.aureus isolates	Class of antimicrobial agents	Antimicrobial	MIC	Interpretation	No. (%) of S.aureus isolates
Penicillines	Benzylpenicillin	>= 0.5	R	7/7,(100%)	Macrolides	Azithromycin		R	1/7, (14.28%)
	Amoxicillin Clavulanic acid		R	7/7,(100%)		Erythromycin	<= 0.25	S	7/7, (100%)
	Oxacillin	>=4	R	7/7,(100%)	Lincosamides	Clindamycin	<= 0.25	S	6/7, (85.72%)
								R	1/7, (14.28%)
Cephalosporins	Cefoxitin Screen	POS	Methicillin resistance	7/7,(100%)	oxazolidinone	Linezolid	2	S	7/7,(100%)
	Cefalexin		R	7/7,(100%)	Polypeptides	Teicoplanin	<=0.5	S	1/7, (14.28%)
								R	6/7, (85.72%)
	Cefazolin		R	7/7,(100%)		Vancomycin	*=32	R	7/7, (100%)
	Cefapime		R	7/7,(100%)					
Aminoglycosides	Gentamicin	<=0.5	S	7/7,(100%)	Tetracycline	Doxycycline		S	7/7,(100%)
	Tobramycin	<= 1	S	7/7,(100%)		Tetracycline	<=1	S	7/7,(100%)

Quinolones Fluoroquinolones	**Ciprofloxacin**		**S**	**7/7,(100%)**		**Tigecycline**	**<= 0.12**	**S**	**7/7,(100%)**
	Gatifloxacin		**S**	**7/7,(100%)**	**nitrofuran antibiotic**	**Nitrofurantoin**	**<— 16**	**S**	**7/7,(100%)**
	Levofloxacin	**<= 0.12**	**S**	**7/7,(100%)**	**Fusidane**	**Fusidic Acid**	**°= 0.5**	**S**	**7/7,(100%)**
	Moxifloxacin	**<=0.25**	**S**	**7/7,(100%)**	**Ansamycins**	**Rifampicin**	**<= 0.5**	**S**	**7/7,(100%)**
	Norfloxacin		**S**	**7/7,(100%)**	**Sulfonamides**	**Trimethoprim/ Sulfamethoxazole**	**<= 10**	**s**	**7/7,(100%)**

Discussion

Results showed that in sheep *S.aureus* represent (11.29%) this rate disagree with (Abdul-Ratha and Mohammad, 2013), who found in study in Baghdad city that *S.aureus* represent (5.6%), with (FARAJZADEH et al., 2011), who found in study in Iran that *S.aureus* represent (3.4%), with (Ali et al., 2017) who found in study in Nigeria that *S.aureus* represent (90%), with (TWAFIK, 2023) who found in study in Egypt that *S.aureus* represent (25%), with (Mahouz et al., 2015) who found in study in Algeria that *S.aureus* represent (30.4%), with (Sarhan and Mohammed, 2019) who found in study in Wasit city that *S.aureus* represent (20%).

In sheep breeders Specimens results of microbial positive growth *S.aureus* represent (4.83%), and this rate disagree with finding of (Mhana and Aljanaby, 2023) who found in study in Kufa city that *S.aureus* represent (8.5%), with (Belete and Saravanan, 2020) who found in study in Countries in Africa and Asia that *S.aureus* represent(8.3%), this rate disagree with (Al-Awkally et al., 2022) who found in study in Libya that *S.aureus* represent (0.5%), with (Goudarzi et al., 2019) who found in study in Iran that *S.aureus* represent (74.7%), with (Singh et al., 2019) who found in study in India that *S.aureus* represent (68.18%). The improper use of antibiotics and the development of bacterial resistance in the microorganisms that cause urinary tract infections have been extensively documented in the scientific literature (Pal et al., 2020). Results showed the resistance of *S.aureus* in sheep (100%) to Penicillines, Cephalosporins, Cefoxitin, polypeptides antibiotics and vancomycin, while for Teicoplanin was (85.72%), and for macrolides antibiotics, Azithromycin, Lincosamides antibiotics, Clindamycin was (14.28%).When comparing these results with the study he conducted (Abed et al., 2021), in Egypt we notice that the results are consistent with resistance to Penicillines (96%), Lincosamides and Clindamycin (28%) and not consistent with the resistance results to Cefoxitin (56 %), vancomycin (16 %). in other study done by (Salgueiro et al., 2020), in Portugal the results are not consistent with resistance to cefoxitin (0.0 %), vancomycin(0.0 %), ciprofloxacin (16.7%,), Teicoplanin (0.0 %). in other study done by (Pérez-Sancho et al., 2020), in Spain the results are not consistent with resistance to Cephalosporins, Cefoxitin ((21.05%), Vancomycin (0.0 %), Teicoplanin (0.0 %), but results are consistent with resistance to Penicillines (89.47%), Lincosamides and Clindamycin (15.79%). While in female sheep breeders resistance of *S.aureus* shown (100%) resistance for Penicillines, Cephalosporins, Cefoxitin, Vancomycin and, and (66%) for Teicoplanin, when comparing these result with study done by (Mustafa et al., 2023) in Kirkuk city agreed with resistance to Penicillines (92%), and disagreed with resistance to Cefoxitin (48%), Teicoplanin (12%), while the study he conducted (Okoye et al., 2022) in Nigeria show incompatibility in resistance to Vancomycin (0.0 %). In study done by (Ali and Aljanaby, 2023) in Babylon City Show compatibility in resistance to Penicillines (100%) and incompatibility in resistance to Vancomycin (60%), the result also show compatibility with study done by (Amin et al., 2021) in Saudi Arabia in resistance to Vancomycin(100%) and incompatibility in resistance to Penicillines (0.0 %)

References

1. Mohammed, Y., J. Mustafa, and A. Abdullah, Isolation and molecular study of some bacterial urinary tract infections of sheep in Basrah province. The Iraqi Journal of Agricultural Science, 2020. 51(3): p. 885-893.

2. Byron, J.K., Urinary tract infection. Veterinary Clinics: Small Animal Practice, 2019. 49(2): p. 211-221.

3. Sahu, R., et al., Urinary Tract Infection and its management. Systematic Reviews in Pharmacy, 2019. 10(1): p. 42-48.

4. Abdullah, A.R. and J.Y. Mustafa, Isolation and molecular detection study of bacterial causes pyelonephritis of cattle in Basrah province. Biochemical and cellular archives, 2019. 19(2): p. 9-56.

5. Alsolami, A., et al., Community-Acquired Methicillin-Resistant Staphylococcus aureus in Hospitals: Age-Specificity and Potential Zoonotic–Zooanthroponotic Transmission Dynamics. Diagnostics, 2023. 13(12): p. 2089.

6. Ali, H., et al., Phenotypic and genotypic profiling of Methicillin-resistant Staphylococcus aureus isolates from human and bovine milk. Egyptian Journal of Agricultural Research, 2021. 99(2): p. 190-196.

7. TWAFIK, J.H., STUDIES ON RENAL BACTERIAL AFFECTIONS IN SHEEP IN MATROUH GOVERNORATE. Assiut Veterinary Medical Journal, 2023. 69(179): p. 160-171.

8. Lakhundi, S. and K. Zhang, Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology. Clinical microbiology reviews, 2018. 31(4): p. 10.1128/cmr. 00020-18.

9. Heaton, C.J., et al., Staphylococcus aureus epidemiology in wildlife: A systematic review. Antibiotics, 2020. 9(2): p. 89.

10. Mafisa, E.M., Molecular characterization of selected pathogens in milk. 2022, North-West University (South Africa).

11. Pervin, R., et al., Isolation, identification and characterization of Staphylococcus aureus from raw milk in different places of savar, Bangladesh. International Journal of Sciences: Basic and Applied Research, 2019. 48(2): p. 1-25.

12. EL-Marakby, H.-A.F., et al., Automated Vitek-2 System versus D Test in Detection of Inducible Clindamycin Resistance Staphylococcus aureus. Egyptian Journal of Medical Microbiology, 2018. 27(2): p. 81-86.

13. Abdul-Ratha, H.A. and A.J. Mohammad, The occurrence of urinary tract infection caused bacteria in human and animals in Baghdad city and it's susceptibility to antibiotics. Journal of Genetic and Environmental Resources Conservation, 2013. 1(3): p. 204-208.

14. FARAJZADEH, S.A., et al., Prevalence of asymptomatic bacteriuria in elderly referred to outpatient clinics in Talegani hospital, Abadan, Iran. 2011.

15. Ali, M., et al., Characterization and Determination of Antimicrobial Sensitivity Pattern of Staphylococcus aureus Associated with Urinary Tract Infection. Journal of Advances in Biology & Biotechnology, 2017. 12(4): p. 1-6.

16. Mahouz, F., F.B. Khoudja, and M. Chikhaoui, Bacteriological and Pathological Investigations on Ovine Renal Diseases. World Applied Sciences ournal, 2015. 33(1): p. 142-145.

17. Sarhan, S.R. and H.A. Mohammed, Isolation and Identification of mecA gene from MRSA isolated from Local Sheep and Evaluate the Inhibitory effect of Cranberry Leaves extract In-Vitro. Research Journal of Pharmacy and Technology, 2019. 12(5): p. 2131-2136.

18. Mhana, S.M.Y. and A.A.J. Aljanaby. Bacteriological Investigation of Pathogenic Bacteria Causing Urinary Tract Infections: A cross-Sectional Study. in IOP Conference Series: Earth and Environmental Science. 2023. IOP Publishing.

19. Belete, M.A. and M. Saravanan, A systematic review on drug resistant urinary tract infection among pregnant women in developing countries in Africa and Asia; 2005–2016. Infection and drug resistance, 2020: p. 1465-1477.

20. Al-Awkally, N.A.M., et al., Study of antibiotic sensitivity pattern in urinary tract infection. International Journal of Health Sciences, 2022. 6: p. 8896-8913.

21. Goudarzi, M., et al., Genetic diversity and biofilm formation analysis of Staphylococcus aureus causing urinary tract infections in Tehran, Iran. The Journal of Infection in Developing Countries, 2019. 13(09): p. 777-785.

22. Singh, D., A. Chand, and S. Goel, Prevalence of MRSA among Staphylococcus aureus isolated from patients of urinary tract infection along with its antibiogram. Int J, 2019. 2(4): p. 364.

23. Pal, M., et al., Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of staphylococcus aureus: a comprehensive review. American Journal of Public Health Research, 2020. 8(1): p. 14-21.

24. Abed, A.H., et al., Subclinical mastitis in selected bovine dairy herds in North Upper Egypt: Assessment of prevalence, causative bacterial pathogens, antimicrobial resistance and virulence-associated genes. Microorganisms, 2021. 9(6): p. 1175.

25. Salgueiro, V., et al., Genetic relatedness and diversity of Staphylococcus aureus from different reservoirs: humans and animals of livestock, poultry, zoo, and aquaculture. Microorganisms, 2020. 8(9): p. 1345.

26. Pérez-Sancho, M., et al., Antimicrobial resistance of coagulase-positive Staphylococcus isolates recovered in a veterinary university hospital. Antibiotics, 2020. 9(11): p. 752.

27. Mustafa, N.R., I.T. Abdullah, and S. Jabbar, Phenotypic detection of virulence determinants and antibiotics resistance in Staphylococcus aureus from different clinical isolates in Kirkuk city. HIV Nursing, 2023. 23(1): p. 891-899.

28. Okoye, E.L., M.J. Omeje, and E.T. Ugwuoji, Detection and prevalence of methicillin and vancomycin resistant staphylococcus aureus among clinical isolates in ESUTH, Enugu State. Journal of Current Biomedical Research, 2022. 2(2, Mar-April): p. 170-186.

29. Ali, M.A. and A.A.J. Aljanaby. An Investigation of Bacterial Infections in the Urinary Tract of Babylon City Women in Iraq, a Cross-Sectional Study. in IOP Conference Series: Earth and Environmental Science. 2023. IOP Publishing.

30. Amin, S.S.A., et al., Evaluation of resistant urinary tract infections by Gram-positive bacteria in Medina, Saudi Arabia. Am J Microbiol Res, 2021. 9(1): p. 14-24.