Kufa Medical Journal | Vol. 21, No. 2, 2025

Original article | DOI: https://doi.org/10.36330/kmj.v21i1.19696

p-ISSN: 1993-517X
e-ISSN: 2709-4464

# Identification of Bacterial Isolates from Urine Samples of Patients with Ascending Pyelonephritis at Al-Sadder Teaching Hospital

**Mariam Ahmed Abdulsahib Al-Najafi[1] and Khawlah Abdallah Salman*[2]**

[1,2]*University of Kufa, College of Medicine, Najaf, Iraq.*

***Corresponding Authors Email:** mariama.alnajafy@student.uokufa.edu.iq, * Khawla.alzurfi@uokufa.edu.iq

## ABSTRACT

***Background:*** *Pyelonephritis is a sever type of upper urinary tract infection (UTI) that impact the renal parenchyma and pelvis. The common causative factor is migration of the bacterial infections from the lower urinary tract. Gram-negative bacteria are involved in higher incidences of infection in acute and chronic pyelonephritis cases than are Gram-positive bacteria. Therefore, the entire study was aimed at determining the type of bacterial pathogens responsible for causing the incidence of pyelonephritis. The urine samples were collected from patients clinically diagnosed to have pyelonephritis at Al-Sadder Teaching Hospital. Of the 60 cases, urine samples showing significant bacterial growth were taken and cultured using standard microbiological procedures. Identification of bacterial isolates was done by physical performance, biochemical tests, and the VitecK2 system. It was shown that Gram-negative bacteria, most commonly Escherichia coli (20.0%), cause pyelonephritis. Other Gram-negative bacteria include Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, and Enterobacter cloacae. On the other side, Staphylococcus saprophyticus is the ultimate Gram-positive pathogen (11.7%), which is then followed by Staphylococcus aureus, Streptococcus agalactiae, Enterococcus faecalis, and finally Staphylococcus epidermidis.* ***Conclusion****: This study demonstrated that pyelonephritis can be caused by both Gram-positive and Gram-negative bacteria; however, Gram-negative organisms, particularly E. coli, were found to be more prevalent than Gram-positive bacteria such as S. saprophyticus.*

**Keywords: Pyelonephritis, Urinary Tract Infections, Bacteria.**

**Article Information**
**Received: May 16, 2025; Revised: June 28, 2025; Online December, 2025**

## INTRODUCTION

Urinary Tract Infections(UTIs) are among the most common bacterial infections, affecting individuals of all ages and genders, but showed they are more prevalent in females[1]. Pyelonephritis, a type of upper UTI, occurs when the bacterial infection ascends to thekidneys. It is typically caused by *E. coli*, it is a Gram-negative bacteria also by Gram-positive bacteria but at less incidence than Gram-negative bacteria.

May have systemic symptoms like fever, flank pain, chills, and nausea. If left untreated, pyelonephritis may lead to kidney damage and sepsis[2]. Common bacteria cause UTIs and pyelonephritis are: *Escherichia coli* bacteria belong to the family *Enterobacteriaceae*, which includes optional anaerobic Gram-negative bacteria[3]. *Klebsiella pneumonia* which is Gram-negative bacteria and member of the *Enterobacteriaceae* family[4]. *Pseudomonas aeruginosa* are non-spore forming can be found in water, plants, soil, and on the skin of humans and animals. They can also be found in the form of single bacilli[5]. *Enterobacter cloacae* is facultatively anaerobic Gram-negative, rod-shaped, catalase-positive, lactose fermenting, oxidase-negative[6]. *Proteus mirabilis* is a Gram-negative bacterium, it is a frequent pathogen of the human urinary system where it causes UTIs and catheter-associated urinary tract infections(CAUTIs)[7].

*Staphylococcus aureus* is one of the opportunistic pathogens and these bacteria colonize the skin and mucous tissues asymptomatically[8].

*Staphylococcus saprophyticus* is common cause of UTIs, this non-hemolytic, coagulase-negative bacterium can be distinguished from other coagulase-negative *staphylococci* by its resistance to novobiocin[9]. *Staphylococcus epidermidis* is a coagulase negative that is frequently found in the normal flora of skin and mucous membrane[10]. *Strptococcus agalactiae* also referred to as group B *streptococcus* (GBS), is a Gram-positive cocci-shaped bacterium that typically occurs in pairs or long chains[11]. *Enterococcus Faecalis* is a Gram-positive bacterium of the commensals it inhabits as an intestinal bacterium from humans and several mammals. It was classified as a member of the Group D *Streptococcus* system in the past[12].

## METHODS

### 1. *Clinical Diagnosis and samples collection:*

A total of 60 urine samples would be collected from patients presenting with signs and symptoms of ascending pyelonephritis during the period running from September 2024 to January 2025 at Al-Sadder Teaching Hospital. The clinical diagnosis was based on symptoms such as fever, flank pain, dysuria, and urinary frequency, in addition to supportive laboratory findings such as elevated white blood cell (WBC) count, positive leukocyte esterase, and nitrite tests in urinalysis. In selected cases, imaging techniques (e.g., Ultrasound or CT scan) were used to confirm renal involvement. However, imaging was not available for all patients as the diagnosis was made solely by the attending physician, and we could not obtain imaging results for those cases.

The patients' age ranged from 13 to 70 years. An equal distribution by sex was observed, with 30 females (50%) and 30 males (50%) suffering from urinary tract infections complicated by pyelonephritis.

### 2. *Bacterial isolation:*

All laboratory procedures involving pathogenic bacteria were conducted under Biosafety Level 2 (BSL-2) conditions. Standard precautions were followed, including the use of personal protective equipment (PPE), work inside biological safety cabinets, sterilization of materials, and proper disposal of biohazardous waste.

Isolation and identification of bacteria were achieved by inoculated on Blood agar, MacConkey agar and HiCrome UTI agar at incubation temperature (35–37°C) and duration (24–48 hours). After showed bacterial growth on culture media were described based on their physical characteristics, a clear threshold for significant bacterial growth should be defined, such as $\geq 10^4$ colony-forming units per milliliter (CFU/ml), to standardize the criteria for sample positivity and ensure consistency across the study, size, margin, color, and odor of the colonies can provide additional information about the type bacteria present.

### 3. *Bacterial Identification:*

After isolation, bacteria were identified using Gram staining and biochemical tests (e.g., coagulase, catalase, oxidase, indole, and urease tests). Additional identification was performed using the Vitek2 system (BioMérieux), a widely accepted automated technique for microbial identification and antibiotic susceptibility testing.

The procedure of Vitek2 system begins by preparing a standardized bacterial suspension from isolated colonies, adjusted to a McFarland turbidity of approximately 0.5.

The suspension is then transferred into a VITEK2 identification (ID) card, which contains multiple biochemical substrates. The card is sealed and automatically loaded into the the VITEK2 instrument, where the organism's metabolic reactions are monitored over time (typically 6–18 hours) through colorimetric and fluorometric changes.

The resulting data are analyzed by the system's database, and the organism is identified based on its biochemical profile. Results are displayed as percentage probability and confidence levels for accurate identification. The system follows manufacturer protocols and complies with Clinical and Laboratory Standards Institute (CLSI) guidelines. Selective and differential media were used as necessary to support accurate classification. Reference bacterial strains like, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used to ensure the accuracy of laboratory tests.

All procedures were performed according to the manufacturer's instructions and in compliance with the Clinical and Laboratory Standards Institute (CLSI) guidelines to guarantee the quality of the results.

## RESULTS

There were 60 pyelonephritis patients, 40 (66.7%) exhibited the Gram-negative bacteria were more common 41 (68.3%) in pyelonephritis patients than the Gram-positive 19 (31.7%) cases. The Gram-negative was more common in chronic pyelonephritis 24 (80%) than acute cases 17 (56.7%). Whereas, the Gram-positive was more common in acute pyelonephritis 13 (43.3%) cases than chronic 6 (20%) cases.

**Table (1): Type of bacterial gram stain according to type of pyelonephritis.**

| Bacteria gram stain | Acute pyelonephritis no. (%) | Chronic pyelonephritis no. (%) | Total | P-value |
|---|---|---|---|---|
| **Gram-Positive** | 13 (21.7%) | 6 (10%) | 19 (31.7%) | 0.095 |
| **Gram-Negative** | 17 (28.3%) | 24 (40%) | 41 (68.3%) | |

A total of 60 bacterial isolates were classified based on Gram stain characteristics. Of these, Gram-negative bacteria predominated, accounting for 41 isolates (68.3%), while Gram-positive bacteria were identified in 19 cases (31.7%). In cases of acute pyelonephritis, Gram-negative bacteria were more frequently isolated (17 cases; 28.3%), compared to 13 cases (21.7%) of Gram-positive bacteria. Conversely, in chronic pyelonephritis, Gram-negative bacteria were identified in 24 cases (40%), whereas only 6 cases (10%) involved Gram-positive bacteria. Although the overall distribution showed a higher prevalence of Gram-negative organisms in both acute and chronic forms, the statistical comparison between Gram stain type and disease chronicity yielded a p-value of 0.095, which does not reach statistical significance ($p > 0.05$), but may suggest a trend worth investigating in larger samples.

**Table (2): Distribution the types of bacteria that caused pyelonephritis.**

| Type of Bacteria | Acute pyelonephritis n.(%) | Chronic pyelonephritis n.(%) | Total |
|---|---|---|---|
| *E. coli* | 5 (8.3%) | 7 (11.7%) | 12 (20.0%) |
| *P. mirabilis* | 2 (3.3%) | 4 (6.6%) | 6 (10.0%) |
| *P. aeruginosa* | 3 (5.0%) | 6 (10.0%) | 9 (15.0%) |
| *K. pneumonia* | 2 (3.3%) | 8 (13.4%) | 10 (16.7%) |
| *E. cloacae* | 1 (1.7%) | 3 (5.0%) | 4 (6.7%) |
| *E. faecalis* | 2 (3.3%) | 1 (1.7%) | 3 (5.0%) |
| *S. saprophyticus* | 4 (6.7%) | 3 (5.0%) | 7 (11.7%) |
| *S. aureus* | 2 (3.3%) | 3 (5.0%) | 5 (8.3%) |

| Type of Bacteria | Acute pyelonephritis n.(%) | Chronic pyelonephritis n.(%) | Total |
|---|---|---|---|
| *S. epidermidis* | 0 (0.0%) | 1 (1.7%) | 1 (1.7%) |
| *S. agalactiae* | 1 (1.7%) | 2 (3.3%) | 3 (5.0%) |

***Chi-Square test used at significant level ≤0.05.***

A total of 60 urine samples were collected from patients with clinical signs of pyelonephritis. Bacterial culture and identification revealed 10 distinct species. The findings showed a total of 10 bacterial species involved in pyelonephritis. Gram-negative bacteria accounted for 68.3% of the total isolates (41/60). *E. coli* was the most prevalent, with 12 isolates (20.0%), including 5(8.3%) from acute and 7(11.7%) from chronic infections. *P. mirabilis* was identified in 6 cases (10.0%) with 2(3.3%) from acute and 4(6.6%) from chronic infections. *P. aeruginosa* in 9(15.0%), including 3(5.0%) from acute and 6(10.0%) from chronic infections. *K. pneumoniae* in 10(16.7%) with 2(3.3%) from acute and 8(13.4%) from chronic infections , and *E. cloacae* in 4(6.7%) with 1(1.7%) from acute and 3(5.0%) from chronic infections. *E. faecalis* had 3 isolates (5.0%) with 2(3.3%) from acute and 1(1.7%) from chronic infections. *S. aureus* represented 5 isolates (8.3%) ,split into 2(3.3%) for acute and 3(5.0%) for chronic infections. *S. saprophyticus* had 7 isolates (11.7%), 4(6.7%) from acute and 3(5.0%) from chronic infections. *S. epidermidis* had 1(1.7%) isolated from chronic infection only while no bacteria isolate were recorded in the case of acute infection. *S. agalactiae* had 3 isolates (5.0%) , with 2(3.3%) from acute and 1(1.7%) from chronic infections.

**Table (3): Distribution the type of Gram stain according to sex.**

| Sex | Gram-positive bacteria no.(%) | Gram-negative bacteria no.(%) | Total | P-value |
|---|---|---|---|---|
| **Male** | 9 (15%) | 21(35%) | 30(50%) | 1.00 |
| **Female** | 10(16.7%) | 20(33.3%) | 30(50%) | |
| **Total** | 19(31.7%) | 41(68.3%) | 60(100%) | |

***Chi-Square test used at significant level ≤0.05.***

In this study showed that the number of Gram-negative bacteria in male 21(35%) was more than female 20(33.3%) at a simple percentage also that the Gram-positive in female 10(16.7%) was more than male 9 (15%) at a simple percentage. It was showed that the Gram-negative bacteria more common in both gender 41(68.3%) that suffering from ascending pyelonephritis than Gram-positive bacteria 19(31.7%). But, there is no statistically significant difference between Gram-positive and Gram-negative bacterial infections by sex (p = 1.0). The lack of significant difference may be related to the balanced sample size between both sexes.

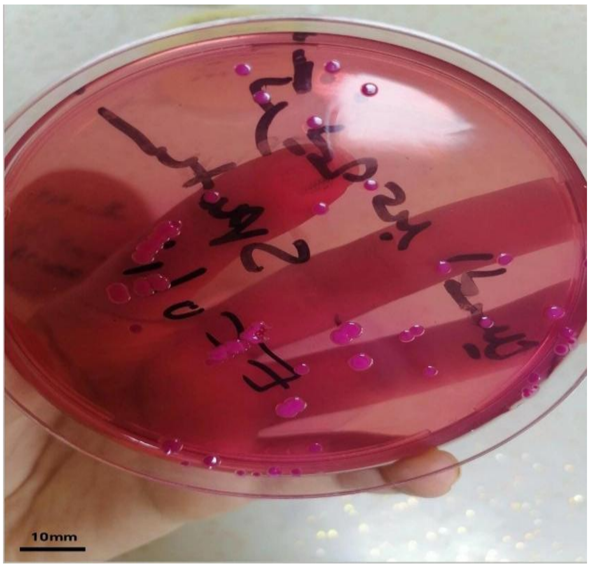


**Figure (1): The growth of *E. coli* on MacConkey agar. Scale bar=10mm.**

The growth showed moist, smooth, rounded colonies and it is pink to red due to lactose fermentation.


**Figure (2): The growth of *K. pneumonia* on MacConkey agar. Scale bar=10mm.**

The colonies are large, dome-shaped, and very mucoid. It appear very sticky and shiny due to capsule production.


**Figure (3) The growth of *P.aeruginosa* on blood agar. Scale bar=10mm.**

The growth showed grayish, flat colonies with a metallic sheen, it has fruity- like odor.


**Figure (4): The growth of *S. saprophyticus* on blood agar. Scale bar=10mm.**

The colonies showed non-hemolytic ,white to slightly yellow, circular and convex.

## DISCUSSION

Studies suggest that bacterial isolates are more frequently associated with chronic pyelonephritis than with acute cases. However, the p-value indicated a non-significant difference, which may be attributed to the relatively small sample size (n = 60). A larger sample size (e.g., ≥200) may yield more statistically robust and accurate results.

The high prevalence of Gram-negative bacteria in pyelonephritis can be attributed to several factors. Firstly, Gram-negative bacteria have a unique cell wall structure that makes them more resistant to antibiotics and able to survive in hard environments[13]. When these bacteria enter the urinary tract, they can cause infections, particularly in cases of UTIs, where they can ascend from the bladder or vagina into the kidney[14].

The most prevalent microorganisms is *E. coli* (20%) in patients with pyelonephritis. According to the study done in 2019, the most common cause of UTI and pyelonephritis is E. coli[15].

This study revealed that *K. pneumoniae* was the second most common bacterial which represent (16.7%). Its ability to form biofilms and resist antibiotics suggests a possible role in persistent or hospital-acquired infections. Biofilm formation is important in persistence and antibiotic resistance, allowing the pathogen to evade host immune responses and establish long-term infections. *K. pneumoniae* further has virulence factors including capsule polysaccharides and siderophores, which enhance its survival and establishment of chronic inflammation in the urinary tract[16]. *P.aeruginosa* total of 9 isolates (15.0%). *P. aeruginosa* is a Gram-negative bacterium commonly associated with hospital-acquired infections. This microorganism has high efficiency in forming biofilms on medical devices; for example, urinary catheters and implicated in the persistent infections and chronic inflammation. In the urinary tract, *P. aeruginosa* can ascend from the lower to the upper tract, leading to pyelonephritis[17]. The study identified *P.mirabilis* with 6 isolates from urine samples of patients (10.0%), in urinary tract this bacteria adherence to uroepithelium to begin infection and colonization[18].

The *E. cloacae* (6.7%) were found in urine samples collected from patients suffering of pyelonephritis; a study agreeing to such a claim has shown that it can cause pyelonephritis[19]. *S. saprophyticus* turns out to be the most common Gram-positive bacterium accounting for 11.7% found in the urine of pyelonephritis patients. The virulent factor of this bacteria and biofilm formation capability supports its pathogenicity and persistence in the urinary tract[20].

The study found that *S. aureus* isolates were present in urine samples (8.3%), it is highly prevalent in hospital environments and the high antibiotic resistance found in some strains[21]. *S. epidermidis* was found in urine sample

(1.7%) from chronic pyelonephritis cases, it is an infrequent cause of catheter-associated urinary tract infections and is rarely associated with pyelonephritis[22]. In this study, *E. faecalis* was found in a total of 3 isolates (5.0%). It also has enterococcal polysaccharide antigen for immune evasion and also enzymes that facilitate the infection[23].

Our study found that *S. agalactiae* resulted in 3 isolates (5.0%) it have various virulence factors, including CovR, HvgA, and the bacterial capsule. These factors contribute to the bacterium's ability to cause infection and evade the host's immune system[24]. This study also showed It was showed that the Gram-negative bacteria more common in both gender 41(68.3%) that suffering from ascending pyelonephritis than Gram-positive bacteria 19(31.7%). This agreed with the study done in Medina, Saudi Arabia showed the bacterial pathogens of Gram-negative bacteria were the most common, while Gram-positive bacteria accounted for a smaller proportion[25]. However, in this study there is no statistically significant difference between Gram-positive and Gram-negative bacterial infections by sex (p = 1.0) may be related to the equal distribution of male and female participants in the study.

## CONCLUSION

The study demonstrated that pyelonephritis can be caused by both Gram-positive and Gram-negative bacteria; however, Gram-negative bacteria were found to be more prevalent than Gram-positive ones.

It is recommended to investigate the potential vaccine (like; targeting UPEC vaccine) to prevent pyelonephritis, and isolation of bacteria by bacterial DNA directly from urine or kidney tissue without need for using culture, like next generation sequencing (NGS) techniques.

## ACKNOWLEDGMENT

I would like to express my sincere appreciation to the staff of Al-Sadder Hospital Laboratory for their support and cooperation during the course of this study. I am also deeply thankful to the patients who contributed by providing samples, making this research possible.

### Ethical approval

The present study Which is conducted by Mariam Ahmed was approved by the local Department of Medical committee. The Kidney disease Department of Al-Sadder Medical City also granted permission, and the patient’s consent was obtained to conduct a questionnaire and collect a urine sample.

### Study limitation

First, the sample size (n=60) was relatively small, which may limit the generalizability of the findings to the broader population. However, this was due to the low incidence of pyelonephritis cases during the study period, as it took four months to collect these 60 sample.

Second, the study did not include antimicrobial susceptibility testing, as the primary focus was on identifying the bacterial species responsible for pyelonephritis rather than evaluating their resistance profiles.

Third, bacterial identification relied primarily on the Vitek2 system without molecular confirmation methods such as PCR or sequencing, which could have enhanced diagnostic accuracy, especially for less common or closely related species. Additionally, identification was supported by Gram staining and standard biochemical tests—such as catalase, coagulase, and oxidase—along with the use of reference strains like *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 to ensure quality control and method validation.

## REFERENCES


1. Vazquez-Montes, M. D., Fanshawe, T. R., Stoesser, N., Walker, A. S., Butler, C., & Hayward, G. (2024). Epidemiology and microbiology of recurrent UTI in women in the community in Oxfordshire, UK. JAC-Antimicrobial Resistance, 6(1), 156. https://doi.org/10.1093/jacamr/dlad156.
2. Al-janabi, D. R. A., & Aljanaby, A. A. J. (2024). RETRACTED: Bacteriological investigation of pyelonephritis in AL-Najaf Governorate, Iraq: a cross-Sectional study. In BIO Web of Conferences (Vol. 84, p. 03014). EDP Sciences. https://doi.org/10.1051/bioconf/20248403014.
3. Zalewska-Piątek, B., & Piątek, R. (2020). Phage therapy as a novel strategy in the treatment of urinary tract infections caused by E. coli. Antibiotics, 9(6), 304. https://doi.org/10.3390/antibiotics9060304.
4. Tomulescu, C., MOSCOVICI, M., Lupescu, I., Stoica, R. M., & Vamanu, A. (2021). A review: Klebsiella pneumoniae, Klebsiella oxytoca. Rom. Biotechnol. Lett, 26, 2567-2586. Doi: 10.25083/rbl/26.3/2567.2586.
5. Urgancı, N. N., Yılmaz, N., Alaşalvar, G. K., & Yıldırım, Z. (2022). Pseudomonas aeruginosa and its pathogenicity. Turkish Journal of Agriculture-Food Science and Technology, 10(4), 726-738. https://doi.org/10.24925/turjaf.v10i4.726-738.4986.
6. Ioannou, P., Vamvoukaki, R., & Kofteridis, D. P. (2022). Infective endocarditis by Enterobacter cloacae: a systematic review and meta-analysis. Journal of Chemotherapy, 34(1), 1-8. https://doi.org/10.1080/1120009X.2021.1959786.
7. Chakkour, M., Hammoud, Z., Farhat, S., El Roz, A., Ezzeddine, Z., & Ghssein, G. (2024). Overview of Proteus mirabilis pathogenicity and virulence. Insights into the role of metals. Frontiers in Microbiology, 15, 1383618. https://doi.org/10.3389/fmicb.2024.1383618.
8. Abban, M. K., Ayerakwa, E. A., Mosi, L., & Isawumi, A. (2023). The burden of hospital acquired infections and antimicrobial resistance. Heliyon, 9(10).8 .. Abban, M. K., Ayerakwa, E. A., Mosi, L., & Isawumi, A. (2023). The burden of hospital acquired infections and antimicrobial resistance. Heliyon, 9(10)..
9. Argemi, X., Hansmann, Y., Prola, K., & Prévost, G. (2019). Coagulase-negative staphylococci pathogenomics. International journal of molecular sciences, 20(5), 1215. https://doi.

10. Brown, M. M., & Horswill, A. R. (2020). Staphylococcus epidermidis—Skin friend or foe?. PLoS pathogens, 16(11), e1009026. https://doi.org/10.1371/journal.ppat.1009026.
11. Dozie-Nwakile, O. C., Nwakile, C. D., Uchendu, I. K., Okoroiwu, H. U., & Onyemelukwe, N. F. (2022). Prevalence, characterization, antimicrobial susceptibility pattern and factors associated with group B streptococci (Streptococcus agalactiae) from clinical and non-clinical sources in South-East Nigeria. Journal of Biological Research-Bollettino della Società Italiana di Biologia Sperimentale, 95(2). https://doi.org/10.4081/jbr.2022.9922.
12. Mullally, C. A., Fahriani, M., Mowlaboccus, S., & Coombs, G. W. (2024). Non-faecium non-faecalis enterococci: a review of clinical manifestations, virulence factors, and antimicrobial resistance. Clinical Microbiology Reviews, 37(2), e00121-23. https://doi.org/10.1128/cmr.00121-23.
13. Fisher, J. F., & Mobashery, S. (2020). Constructing and deconstructing the bacterial cell wall. Protein science, 29(3), 629-646. https://doi.org/10.1002/pro.3737.
14. Wang, S., Mu, L., Yu, C., He, Y., Hu, X., Jiao, Y., ... & Bao, H. (2024). Microbial collaborations and conflicts: unraveling interactions in the gut ecosystem. Gut Microbes, 16(1), 2296603. https://doi.org/10.1080/19490976.2023.2296603.
15. Hyun, Hyun, M., Lee, J. Y., ah Kim, H., Ryu, S. Y. (2019) _Comparison of Escherichia coli and Klebsiella pneumoniae acute pyelonephritis in Korean patients', Infection & chemotherapy, 51(2), pp. 130–141 https://doi.org/10.3947/ic.2019.51.2.130.
16. Vachvanichsanong, P., McNeil, E. B., & Dissaneewate, P. (2021). Extended-spectrum beta-lactamase Escherichia coli and Klebsiella pneumoniae urinary tract infections. Epidemiology & Infection, 149, e12. https://doi.org/10.1017/S0950268820003015.
17. Reynolds, D., & Kollef, M. (2021). The epidemiology and pathogenesis and treatment of Pseudomonas aeruginosa infections: an update. Drugs, 81(18), 2117-2131. https://doi.org/10.1007/s40265-021-01635-6
18. Hayat, F., Khan, M., Umair, M., Akbar, S., Javed, R., & Shah, S. H. (2023). Phenotypic and Genotypic Detection of Virulence Factors Affecting Proteus mirabilis Clinical Isolates. Current Trends in OMICS, 3(1), 73-85. https://doi.org/10.32350/cto.31.05.
19. Chou, A., Welch, E., Hunter, A., & Trautner, B. W. (2022). Antimicrobial treatment options for difficult-to-treat resistant gram-negative bacteria causing cystitis, pyelonephritis, and prostatitis: a narrative review. Drugs, 82(4), 407-438. https://doi.org/10.1007/s40265-022-01676-5.
20. Lawal, O. U., Barata, M., Fraqueza, M. J., Worning, P., Bartels, M. D., Goncalves, L., ... & Miragaia, M. (2021). Staphylococcus saprophyticus from clinical and environmental origins have distinct biofilm composition. Frontiers in microbiology, 12, 663768. https://doi.org/10.3389/fmicb.2021.663768.
21. Tigabu, A., & Getaneh, A. L. E. M. (2021). Staphylococcus aureus, ESKAPE Bacteria Challenging Current Health Care and Community Settings: a

Literature Review. Clinical Laboratory, (7).
DOI: 10.7754/Clin.Lab.2020.200930).

22. Yogo, A., Yamamoto, S., Sumiyoshi, S., Iwamoto, N., Aoki, K., Motobayashi, H., ... & Segawa, T. (2022). Two cases of pyelonephritis with bacteremia by Staphylococcus epidermidis in male patients with nephrolithiasis: Case reports and a literature review. Journal of Infection and Chemotherapy, 28(8), 1189-1192.
https://doi.org/10.1016/j.jiac.2022.04.030.

23. Archambaud, C., Nunez, N., da Silva, R. A., Kline, K. A., & Serror, P. (2024). Enterococcus faecalis: an overlooked cell invader. Microbiology and Molecular Biology Reviews, 88(3), e00069-24..

24. Pena, J. M. S., Lannes-Costa, P. S., & Nagao, P. E. (2024). Vaccines for Streptococcus agalactiae: current status and future perspectives. Frontiers in Immunology, 15, 1430901.
https://doi.org/10.3389/fimmu.2024.1430901.

25. Amin, S. S. A., Abdel-Aziz, N. A., Eltahlawi, R. A., El-Sayed, W. S., Mahmoud, M. I. H., Elsayed, E. M. S., & Eltahlawi, R. A. (2021). Evaluation of resistant urinary tract infections by Gram-positive bacteria in Medina, Saudi Arabia. Am J Microbiol Res, 9(1), 14-24. DOI: 10.12691/ajmr-9-1-3.
https://doi.org/10.1128/mmbr.00069-24