Original Article

Estimation of Some Biomarkers among Patients Suffering from Pyelonephritis and Detection of Uropathogenic *Escherichia Coli* in Hilla City

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ABSTRACT

The current study include (75) samples of patients with pyelonephritis who were admitted clinically private for three months between March and May 2020 for this investigation, Alpha-2-Macroglobulin was estimated in patients suffering from pyelonephritis and positive culture of UPEC by using Alpha-2-Macroglobulin Enzyme-linked Immunosorbent Assay Kit, the results showed that, there were significant difference between study groups (patient's positive culture of UPEC and control group). The mean differences of Alpha-2-Macroglobulin was significant increase and between patient's positive culture of UPEC compared with control group. However, Procalcitonin was estimated in patients suffering from pyelonephritis and positive culture of UPEC, the results showed that, there were significant difference between study groups (patient's positive culture of UPEC and control group). The mean differences of Procalcitonin were significant increase and between patient's positive culture of UPEC compared with control group. All samples were culture in various aerobic media and the result show out of seventy-five samples only 62(82.7%) was positive growth while 13(17.3%) no growth observed. 25 (40.33 %) of the positive cultures were cultured on selective media (EMB) were diagnosed as *E. coli*, while 37 (59.67 %) isolates were related to other species of bacteria. Molecular detection of specific uropathogenic *E. coli* genes were done in all *E. coli* isolates from urine samples, when compared to an allelic ladder, the results showed that all 25 (100%) *E. coli* isolates had positive results for the chuA gene due to the presence of (221) bp bands. In addition, a molecular investigation of the uidA gene was performed for all 25 isolates previously identified as *E. coli*. This gene can be identified using the (PCR) polymerase chain reaction, which is more sensitive and specific Tanique. When compared to an allelic ladder, the results showed that all 25 (100%) *E. coli* isolates had positive results for the uidA gene due to the presence of (259) bp bands.

Aim to study: The study aims to estimation of some biomarkers such as α-macroglobulin and procalcitonin among patients suffering from pyelonephritis and detection of uropathogenic *Escherichia coli* in Hilla City.

Keywords: α2-macroglobulin, procalcitonin, pyelonephritis0, UPEC.

Introduction

Pyelonephritis is a form of infection of the urinary tract that affects either one or both kidneys. A bacterium or a virus will infect them. It can make people feel really ill, and it requires medical attention (Linsenmeyer, 2018). Even though the genitourinary mechanism is designed to keep bacteria out, problems might arise. Through the canal, microorganisms, bacteria from the intestines, such as *E. coli*, can enter the

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urine system.. (Pulipati *et al*., 2017). these bacteria have the ability to move into the bladder. It is possible that this will result in a urinary tract infection (inflammation of the bladder). A urinary tract infection (UTI) is also a possibility (Klein & Hultgren, 2020).

Urinary tract infection happens in one-3 percent of adult patients per year. If the infection spreads to the excretory organs, kidney infection will result. This drawback is rare however it is severe (Zwaans *et al*., 2018). Regarding 1 in each thirty cases of UTI results in a kidney infection, The signs and symptoms of pyelonephritis usually appear over a few hours or days. Symptoms include a high fever, pain when passing urine, and stomach pain that extends from the flank to the back. Usually, it's accompanied by a projection (Johnson & Russo, 2018). Consistent flank or stomach discomfort, infection signals (fever, unexpected weight loss, weariness, and ablated appetite), lower tract symptoms, and blood in the urine are all symptoms of chronic pyelonephritis (Gonchar *et al*., 2019). Pyelonephritis can sometimes result in a fever of unclear origin. In addition, inflammation-related proteins can accumulation in organs, resulting in AA disease. (Karki & Leslie, 2021). During a physical examination, a fever and discomfort at the cost vertebral angle on the affected side may be observed. Urine testing can determine whether or not you have a urinary tract infection (Dubbs & Sommerkamp, 2019).

The presence of group and white blood cells on a urine test strip is enough to diagnose pyelonephritis in persons with typical symptoms, and the presence of these cells is a clue that empirical treatment is required. In blood tests, a whole blood count may detect neutrophilia (Fukami *et al*., 2017). Urine culture with or without blood cultures, as well as antibiotic sensitivity testing, are both required in determining an accurate diagnosis (Rothe *et al*., 2020). The most common cause of urinary tract infections (UTIs) in humans is Uropathogenic Escherichia coli (UPEC) (Frick-Cheng 2020). Clinical detection tests can take anything from hours to days (dipsticks) to complete, limiting how quickly patients can be treated. Molecular approaches, on the other hand, may improve speed and accuracy, but their applicability is hampered by UPEC strain genetic variability (Frick-Cheng *et al*., 2020).

Commensal Despite the fact that Escherichia coli strains are a widespread element of the gut microbiota, they seldom cause health concerns in the gut or in the UT (Kjelstrup *et al*., 2017). Specific *E. coli* strains, have developed into pathogens that cause a variety of illnesses, including UTIs, presumably by horizontal sequence transfer (HGT). *E. coli* strains are divided into three types based on their ecology: commensal (useful colonizers of the digestive system), intestinal problem (enteric illness or diarrheagenic),and strains that are harmful to the intestines (ExPEC, including UPEC)(Rossi *et al*., 2018). Genes concerned within the settlement by Escherichia of the UT, and resultant pathogenesis, might give the key to discover UPECs (Li *et al*., 2017). The pathogenic process is assumed to start with urethral colonization, which is followed by bacterial ascent into the bladder and urine proliferation. After that, UPEC cells attach to the bladder surface and interact with the animal tissue munition (Klein & Hultgren, 2020). (α2M) Alpha-2-macroglobulin is a PI and one of the most important plasma proteins. It transports hormones and enzymes, performs effector and substance tasks within the humor system's development, and suppresses complement and stop system elements (Gianazza *et al*., 2021).

The α2M could be a 720-kilodalton protein discovered in the blood. It's mostly produced by the liver, but macrophages, fibroblasts, and endocrine gland cells also produce it at home. The α2M sequence encodes it in humans (Malik *et al*., 2020). It acts as an antiprotease and is ready to inactivate a vast style of proteinases. It works as a dissolving inhibitor by inhibiting enzyme and kallikrein and works as a clotting inhibitor by inhibiting coagulase (Yaron *et al*., 2021). Because it binds to a variety of growth hormones and cytokines, including as platelet-derived growth factor, basic embryonic cell growth factor, TGF-, insulin, and IL-1, α2M may operate as a carrier macromolecule (Garcia-Ferrer *et al*., 2017). When albumin levels are low, as they are in nephritic syndrome and pyelonephritis (a condition in which the kidneys begin to break down several the smaller blood proteins), α2M levels was a rised. (Šunderić *et al*., 2019). because of its size, it is is able to stay in the bloodstream. When all proteins are produced at a higher rate, alpha-2 macroglobulin concentration rises. This increase has very minimal negative health consequences, yet it is used as a diagnostic clue and it is causes amyloid in long-term chronic failure (Cater *et al*., 2019).

Materials and Methods

Patients and Collection of Samples

The Cross-Sectional study was carried out for a period of (3) month from March (2020) to May (2020). 75 samples were collected from subjects attending to clinical private in AL-Hilla city, both blood and urine samples were obtained from pyelonephritis patients, and 25 blood samples were acquired from healthy patients as a control group. These samples were collected from males between the ages of 18 and 65. (20-55 years).

Samples of Urine

In most cases, pyelonephritis patients were given 10 mL of urine to collect. Urine samples were taken in the middle of the flow in sterile screw-cap containers, Culture media inoculation, then cultivated aerobically for 24 hours at (37)°C (Vandepitte *et al*, 1991).

Blood Sample

Five milliliters of blood were drawn, two milliliters were placed in EDTA tubes to obtain plasma for estimation of -macroglobulin, and three milliliters were placed in gel tubes and allowed to clot at room temperature for thirty minutes before centrifugation at 3000g for three minutes, and the sera were drawn and stored at (-20 C) until procalcitonin analysis (Vandepitte *et al*, 1991).

Ethical Approval

Each patient gave their informed consent before being included in the study.

Identify of Uropathogenic *E. coli* Isolates by Gram Stain, Biochemical Tests

Based on its morphological features, a single colony from each primary positive culture on blood, eosin methylene blue (EMB), and nutrient agar is categorised and inspected by light microscopy after being stained with Gram's stain (colony form, size, color, boundaries, and texture). Following the examination, biochemical assays were performed on each isolate to complete the UPEC diagnosis (Baron *et al*, 1994; MacFadden, 2000).

DNA Extraction of UPEC

Geneaid's DNA purification kit (UK) is a supplement to the DNA extraction .

Identification of Uropathogenic *E. coli* Genes

Table 1 lists the primer sequences for uropathogenic *E. coli* genes with amplicon sizes of base pair (bp) and their conditions (1).

Human Alpha-2-Macroglobulin (α2M) Quantification Using an (ELISA) kit

This kit includes a pre-coated microplate with an anti- 2M antibody. A biotin-conjugated antibody specific for 2M is applied to the relevant microplate wells with standards or samples. Following that, Avidin-Horseradish Peroxidase is incubated in each microplate well (HRP). Only the wells containing 2M, biotin-conjugated antibody, and enzyme-conjugated Avidin will change color after adding the TMB substrate solution. After the injection of sulphuric acid solution finishes the enzyme-substrate reaction, the color change is detected spectrophotometrically at a wavelength of 450nm 10nm. By comparing the O.D. of the samples to the standard curve, the concentration of a2M in the samples can be calculated.

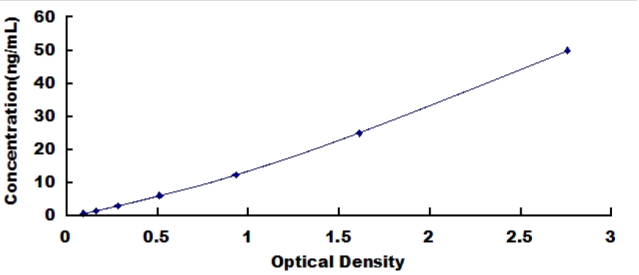

Figure 1: Typical Standard Curve for a2M, Human ELISA

Test for Procalcitonin

The level of procalcitonin in the blood is measured by a procalcitonin test. A high level may indicate a dangerous bacterial illness like sepsis. The body's extreme response to infection is known as sepsis. When an infection in one part of the body, such as the skin or the urinary system, travels to the bloodstream, sepsis develops. This sets out a powerful immunological response. It can induce symptoms such as a racing heart, shortness of breath, low blood pressure, and others. Sepsis can cause organ failure or even death if not

Table 1: Primer sequences for uropathogenic *E. coli* with amplicon size (bp) and their condition

Genes	*Primer sequence*	*Size (bp)*	*Polymerase chain reaction condition*	*Reference*
chuA	**F:** GCTACCGCGATAACTGTCAT **R:** TGGAGAACCGTTCCACTCTA	221	Denaturation 95 ºC for 2 min. Annealing 57.2ºC for 30 sec, Extension 72ºC, 30.0 sec. (29) cycle .	Brons *et al*., (2020)
uidA	**F:** CGCCGATGCAGATATTCGTA **R:** CTGCCAGTTCAGTTCTTGT	259	Denaturation 95 ºC for 2 min. Annealing 57.6ºC for 30 sec, Extension 72ºC, 30.0 sec. (29) cycle. Step 5: Repeat steps 2-4 14 more times Denaturation 95ºC, 30 sec. Annealing 50.6ºC, 30 sec. Extension 72ºC, 30.0 sec. Step 9: Repeat steps 6-8 19 more times	Brons *et al*., (2020)

treated quickly. In the early stages of sepsis or another major bacterial infection, a procalcitonin test can help the health care practitioner decide if patients have sepsis or another serious bacterial infection. This may aid in quick treatment and the avoidance of life-threatening consequences (Flores & Quirós, 2001).

Results and Discussion

In current study (75) blood sample from patient with pyelonephritis were collected and clinical private during a period of three months from march 2020 to May 2020, In this study, Alpha-2-Macroglobulin was estimated in patients suffering from pyelonephritis and positive culture of UPEC by using Alpha-2-Macroglobulin Enzyme-linked Immunosorbent Assay Kit, the results showed that, there were significant difference between study groups (patient's positive culture of UPEC and control group). As demonstrated in Table 2, the mean differences in Alpha-2-Macroglobulin were significantly higher in patients with positive UPEC cultures compared to the control group.

These findings matched Masajes-findings. Zagajewska *et al*., (2017); Kuusela *et al*., (2017) who observed that the biomarker Alpha-2-Macroglobulin was elevated in pyelonephritis patients. Biological indicators (biomarkers) may be used to detect urinary tract infections (UTIs) and to determine the severity of infection (Wu *et al*., 2018). " According to the definition of a biomarker, "a trait that is reliably examined and assessed as an indicator of normal biological processes, pathogenic processes, or pharmacologic reactions to a therapeutic intervention." (Salardini, 2019). Alpha 2M may also cause a rise in viscosity, which could lead to more UTIs (Masajtis-Zagajewska *et al*., 2017). Increased amounts of α2M, on the other hand, may have a favorable effect by acting as a thrombin inhibitor (Hulshof *et al*., 2021). Although anti-thrombin III (AT III) plasma levels have been found to be lowered in pyelonephritis, because AT III, which has a molecular weight like albumin, is lost in the urine, overall anti thrombin activity is enhanced in individuals with pyelonephritis. Although in vitro studies have indicated that the action of a2M as a thrombin inhibitor appears to be of limited consequence in AT (III), this may not be the case for pathophysiological circumstances such as pyelonephritis (Manook *et al*., 2017).

In this study, Procalcitonin was estimated in patients suffering from pyelonephritis and positive culture of UPEC, the results showed that, there were significant difference between study groups (patient's positive culture of UPEC and control group). As demonstrated in Table3, the mean differences in Procalcitonin were significantly higher in patients with a positive UPEC culture compared to the control group.

Procalcitonin (PCT) is a calcitonin propertied, it was a bacterial invasion indicator. It's made by the C-cells of the thyroid gland in healthy people, and the monocyte-macrophage system makes it follow a severe bacterial infection. A typical plasma PCT level in humans after the third day of life is less than 0.5 g/l. (Yu *et al*., 2021). The presence of PCT in the blood and a urinary tract infection were discovered. One study was of high quality, five were of average level, and the rest were of low quality (Hanai *et al*., 2021). The majority of investigations were undertaken in children, with the goal of distinguishing between acute pyelonephritis and lower UTI (Al Rushood & Amal, 2020; Krzemień *et al*., 2021). PCT can be used as a marker of the severity of acute pyelonephritis, according to Vijayan *et al*., (2017). According to Yunus *et al*., (2018), mean PCT rose in direct proportion to the severity of renal involvement (P 0.001). Patients with reversible pyelonephritis had lower PCT levels at entry (mean 3.25 ng/ml). PCT was proven to be a good biomarker for diagnosing acute pyelonephritis at a rate of 93.7 percent by Uwaezuoke (2018). All samples were submitted to aerobic culturing on various media, and 62 (or 82.7 percent) of the total 75 samples exhibited positive bacterial culture. Other 13 (17.3%) samples showed no growth, indicating the presence of microorganisms that are difficult to culture, for instance

Table 2: Mean differences Alpha-2-Macroglobulin biomarker between study groups

Biomarker	*Patients group* *No. = 25* *Mean ± S.D*	*Control group* *No. = 25* *Mean ± S.D*	*P value*
Alpha-2-Macroglobulin (mg\ml)	316.62 ± 79.67	229.26 ± 56.59	<0.001*

*: significant difference ≤ 0.005.

Table.3: Procalcitonin biomarker mean differences between study groups

Biomarker	*Patients group* *No. = 25* *Mean ± S.D*	*Control group* *No. = 25* *Mean ± S.D*	*P value*
Procalcitonin (ng/ml)	4.06 ± 1.11	2.01 ± 0.41	<0.001*

*: significant difference ≤ 0.005.

Tabl.4: Shows the prevalence of *E. coli* and other etiological agents in samples isolated

No. of samples	*Positive result of other bacteria*	*Negative result of bacteria culture*	*Positive culture of E. coli*	*Other types of bacteria*
75	62(82.7%)	13(17.3%)	25(40.33%)	37(59.67%)
Total	75		62	

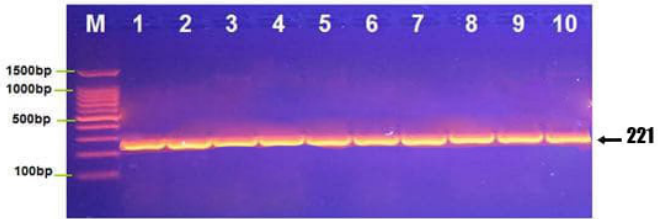

Figure 2 : Electrophoresis pattern of PCR amplified of chuA gene 221 bp of *E. coli* ,Agarose (1.5%) time 55 min ,70 volt, M: ladder, 1-10 amplify of chuA gene in *E. coli* isolates.

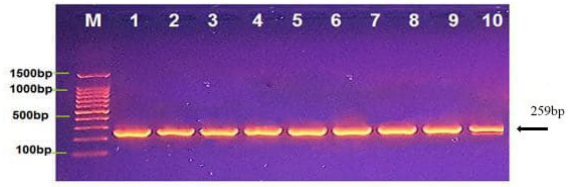

Figure 3: PCR amplification of the uidA gene 259 bp of (*E. coli*) for (55 min) at (70 volt), M: ladder (DNA marker), samples (1- 10) amplify the uidA gene in *E. coli* isolates on an agarose gel electrophoresis (1.5 percent).

virus, fungus, and other agents, or It could be because the samples have different sizes and types. As shown in Table (2) 1. 25 (40.33 %) of the positive cultures cultured on EMB (selective media) were recognized as *E. coli*, whereas 37 (59.67%) were associated to other species of bacteria.

These findings matched those of Principe *et al*., (2018), who discovered that *E. coli* accounted for about 45 percent of all clinically significant urinary isolates, and Elamary *et al*., (2020), who discovered that *E. coli* was the most common pathogen isolated from patients with UTIs in 61 percent of cases. *E. coli* was the most common uropathogen isolated from pyelonephritis patients, according to a study by Sheikh *et al*., (2019). UTIs, or urinary tract infections, are the most frequent bacterial infection today (Kaufman *et al*., 2019), *E. coli* is the most frequent bacteria that causes these infections, accounting for more than 85 percent of all urinary tract infections (Jena *et al*., 2017). Uropathogenic Escherichia coli (UPEC) is the causal agent in most urinary tract infections (UTIs), including cystitis and pyelonephritis, as well as infectious complications that can lead to acute renal failure in both healthy people and people who have had a kidney transplant. To penetrate the mucosal barrier's inertia, UPEC expresses a slew of virulence factors (Karam Alla, 2018). When UPEC infects the typically sterile urinary tract, the host's inflammatory response is triggered, resulting in the generation of cytokines, neutrophil influx, and exfoliation of infected bladder epithelial cells (Kudinha, 2017).

In this study, all *E. coli* isolates from urine samples were subjected to molecular detection of specific uropathogenic *E. coli* genes. The results revealed that all 25 (100%) *E. coli* isolates tested positive for the chuA gene due to the presence of (221) bp bands when compared to an allelic ladder, as shown in Figure 2 . In addition, all 25 isolates previously identified as *E. coli* were subjected to a molecular analysis of the uidA gene. This gene can be identified using the polymerase chain reaction, which is a sensitive and specific approach. When tested to an allelic ladder, all 25 (100%) *E. coli* isolates had positive results for the uidA gene due to the presence of (259) bp bands, as shown in Figure 3.

yrbH, chuA, and uidA were uropathogenic *E. coli* specific genes, according to Brons *et al*., (2020), who discovered that three genes (perhaps implicated in the ecology of virulence) satisfied the criteria to operate as targets in the detection system. The chuA gene produces an outer membrane receptor protein that may aid in the absorption of heme and other chemicals. This gene is found in the heme transport (perhaps importing iron) genetic locus, which appears to be broadly distributed among pathogenic *E. coli* strains. (Richard *et al*., 2019). The ChuA gene, which has numerous biofilm-like features, appears to be critical during the creation of intracellular bacterial communities by UPECs (Matinfar *et al*., 2021). These intracellular biofilms allow a reservoir of dormant pathogen cells to grow inside bladder epithelial cells, allowing them to withstand the immunological response of the host. Given its projected role in the bladder during urinary tract infections, the use of ChuA in a detection system is thus reasonable (Brons *et al*., 2020). The *E. coli* unique beta-D-glucuronidase-encoding gene iUdA was used as an internal amplification control. This gene, which produces an *E. coli*-specific enzyme, is commonly employed in *E. coli* identification tests and as a particular marker (Kiel *et al*., 2018). It was detected in 97.7% of clinical samples, with the gene uidA being found in all of them (Brons *et al*., 2020). UidA is used as an *E. coli* marker all over the world, according to this study. The presence of a UPEC may be determined by detecting any one of the three marker genes, according to our multi-gene identification method.

Conclusion

Alpha-2-Macroglobulin and procalcitonin were a potential biomarker that can help in diagnosis in pyelonephritis. Uropathogenic *E. coli* was predominant bacterial infection

responsible for pyelonephritis. *chuA* and *uid*A marker genes were specific genes for identification of UPEC.

Ethical Approval

The manuscript is written in original and all the data, results pertaining to this manuscript are original according to the research performed. The authors followed academic integrity and have not copied any content/results from another source.

Informed Consent

The authors of the manuscript agrees to publish this research in the journal if it's considerable by the editors of the journal. The authors provide full consent for reviewing and publishing this manuscript.

All the authors of this study contributed equally in terms of performing the research as well as in preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author. Any query/suggestion related to the manuscript can be reached to the corresponding author

References

1. Al Rushood, M., & Amal, A. E. (2020). Serum and Urine Interleukin-6 and Interleukin-8 Levels Do Not Differentiate Acute Pyelonephritis from Lower Urinary Tract Infections in Children. *Journal of Inflammation Research*, *13*, 789.
2. Baron, E. J., Peterson, L. R. and Finegoldens, S. M. (1994). Bailey and Scott's diagnostic microbiology. 9th Ed. Mosology. Co. USA.
3. Brons, J. K., Vink, S. N., de Vos, M. G., Reuter, S., Dobrindt, U., & van Elsas, J. D. (2020). Fast identification of Escherichia coli in urinary tract infections using a virulence gene based PCR approach in a novel thermal cycler. *Journal of microbiological methods*, *169*, 105799.
4. Cater, J. H., Wilson, M. R., & Wyatt, A. R. (2019). Alpha-2-macroglobulin, a hypochlorite-regulated chaperone and immune system modulator. *Oxidative medicine and cellular longevity*, *2019*.
5. Dubbs, S. B., & Sommerkamp, S. K. (2019). Evaluation and management of urinary tract infection in the emergency department. *Emergency Medicine Clinics*, *37*(4), 707-723.
6. Elamary, R. B., Albarakaty, F. M., & Salem, W. M. (2020). Efficacy of Acacia nilotica aqueous extract in treating biofilm-forming and multidrug resistant uropathogens isolated from patients with UTI syndrome. *Scientific Reports*, *10*(1), 1-14.
7. Flores, J. C., & Quirós, A. B. (2001, January). Procalcitonina: un nuevo marcador de infección bacteriana. In *Anales de Pediatría* (Vol. 54, No. 1, pp. 69-73). Elsevier Doyma.
8. Frick-Cheng, A. E., Sintsova, A., Smith, S. N., Krauthammer, M., Eaton, K. A., & Mobley, H. L. (2020). The gene expression profile of uropathogenic Escherichia coli in women with uncomplicated urinary tract infections is recapitulated in the mouse model. *Mbio*, *11*(4), e01412-20.
9. Fukami, H., Takeuchi, Y., Kagaya, S., Ojima, Y., Saito, A., Sato, H., ... & Nagasawa, T. (2017). Perirenal fat stranding is not a powerful diagnostic tool for acute pyelonephritis. *International journal of general medicine*, *10*, 137.
10. Garcia-Ferrer, I., Marrero, A., Gomis-Rüth, F. X., & Goulas, T. (2017). α 2-Macroglobulins: Structure and function. *Macromolecular Protein Complexes*, 149-183.
11. Gianazza, E., Eberini, I., Palazzolo, L., & Miller, I. (2021). Hemolymph proteins: An overview across marine arthropods and molluscs. *Journal of Proteomics*, 104294.
12. Gonchar, M. O., Muratov, G. R., Alenina, I. S., & Omelchenko, O. V. (2019). Differentiated diagnosis of inflammatory kidney diseases in children.
13. Hanai, Y., Takahashi, Y., Niwa, T., Mayumi, T., Hamada, Y., Kimura, T., ... & Takesue, Y. (2021). Optimal trough concentration of teicoplanin for the treatment of methicillin-resistant Staphylococcus aureus infection: A systematic review and meta-analysis. *Journal of Clinical Pharmacy and Therapeutics*.
14. Hulshof, A. M., Hemker, H. C., Spronk, H. M., Henskens, Y., & Ten Cate, H. (2021). Thrombin–Fibrin (ogen) Interactions, Host Defense and Risk of Thrombosis. *International Journal of Molecular Sciences*, *22*(5), 2590.
15. Jena, J., Sahoo, R. K., Debata, N. K., & Subudhi, E. (2017). Prevalence of TEM, SHV, and CTX-M genes of extended-spectrum β-lactamase-producing Escherichia coli strains isolated from urinary tract infections in adults. *3 Biotech*, *7*(4), 1-7.
16. Johnson, J. R., & Russo, T. A. (2018). Acute pyelonephritis in adults. *New England Journal of Medicine*, *378*(1), 48-59.
17. Karam Alla, T. (2018). EL Sheikh and Al Fadhil A. Omer. Phenotypic and Genotypic Characterization of Escherichia coli carrying Chromosomal-mediated Quinolone Resistance Determinants isolated from Hemodialysis Patients. *African Journal of Medical Sciences*, *3*(1).
18. Karki, N., & Leslie, S. W. (2021). Struvite And Triple Phosphate Renal Calculi.
19. Kaufman, J., Temple-Smith, M., & Sanci, L. (2019). Urinary tract infections in children: an overview of diagnosis and management. *BMJ paediatrics open*, *3*(1).
20. Kiel, M., Sagory-Zalkind, P., Miganeh, C., Stork, C., Leimbach, A., Sekse, C., ... & Dobrindt, U. (2018). Identification of novel biomarkers for priority serotypes of Shiga toxin-producing Escherichia coli and the development of multiplex PCR for their detection. *Frontiers in microbiology*, *9*, 1321.
21. Kjelstrup, C. K., Barber, A. E., Norton, J. P., Mulvey, M. A., & L'Abée-Lund, T. M. (2017). Escherichia coli O78 isolated from septicemic lambs shows high pathogenicity in a zebrafish model. *Veterinary research*, *48*(1), 1-8.
22. Klein, R. D., & Hultgren, S. J. (2020). Urinary tract infections: microbial pathogenesis, host–pathogen interactions and new treatment strategies. *Nature Reviews Microbiology*, *18*(4), 211-226.
23. Krzemień, G., Pańczyk-Tomaszewska, M., Górska, E., & Szmigielska, A. (2021). Urinary vanin-1 for predicting acute pyelonephritis in young children with urinary tract infection: a pilot study. *Biomarkers*, *26*(4), 318-324.

24. Kudinha, T. (2017). The pathogenesis of *Escherichia coli* urinary tract infection. *Escherichia coli—Recent Advances on Physiology, Pathogenesis and Biotechnological Applications. InTech*, 45-61.

25. Kuusela, P., Saraswat, M., Joenväärä, S., Kaartinen, J., Järvinen, A., & Renkonen, R. (2017). Changes in plasma protein levels as an early indication of a bloodstream infection. *PloS one*, *12*(2), e0172987.

26. Li, K., Wu, K. Y., Wu, W., Wang, N., Zhang, T., Choudhry, N., ... & Zhou, W. (2017). C5aR1 promotes acute pyelonephritis induced by uropathogenic *E. coli*. *JCI insight*, *2*(24).

27. Linsenmeyer, T. A. (2018). Catheter-associated urinary tract infections in persons with neurogenic bladders. *The journal of spinal cord medicine*, *41*(2), 132-141.

28. MacFadden, J. F., (2000). Biochemical tests for identification of medical bacteria. 3rd ed. ed. Baltimore Md: Lippincott Williams and Wilkins.

29. Malik, H. I., Mir, A. R., Abidi, M., Habib, S., Khan, F. H., & Moinuddin. (2020). Preferential recognition of epitopes on peroxynitrite-modified alpha-2-macroglobulin by circulating autoantibodies in rheumatoid arthritis patients. *Journal of Biomolecular Structure and Dynamics*, *38*(7), 1984-1994.

30. Manook, M., Kwun, J., Burghuber, C., Samy, K., Mulvihill, M., Yoon, J., ... & Knechtle, S. J. (2017). Thrombalexin: use of a cytotopic anticoagulant to reduce thrombotic microangiopathy in a highly sensitized model of kidney transplantation. *American journal of transplantation: official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *17*(8), 2055-2064.

31. Masajtis-Zagajewska, A., & Nowicki, M. (2017). New markers of urinary tract infection. *Clinica chimica acta*, *471*, 286-291.

32. Matinfar, S., Ahmadi, M., Sisakht, A. M., Sadeghi, J., & Javedansirat, S. (2021). Phylogenetic and antibiotics resistance in extended-spectrum B-lactamase (ESBL) Uropathogenic Escherichia coli: An update review. *Gene Reports*, 101168.

33. Principe, L., Piazza, A., Mauri, C., Anesi, A., Bracco, S., Brigante, G., ... & Luzzaro, F. (2018). Multicenter prospective study on the prevalence of colistin resistance in Escherichia coli: relevance of mcr-1-positive clinical isolates in Lombardy, Northern Italy. *Infection and drug resistance*, *11*, 377.

34. Pulipati, S., Babu, P. S., Narasu, M. L., & Anusha, N. (2017). An overview on urinary tract infections and effective natural remedies. *J. Med. Plants*, *5*, 50-56.

35. Richard, K. L., Kelley, B. R., & Johnson, J. G. (2019). Heme uptake and utilization by gram-negative bacterial pathogens. *Frontiers in cellular and infection microbiology*, *9*, 81.

36. Rossi, E., Cimdins, A., Lüthje, P., Brauner, A., Sjöling, Å., Landini, P., & Römling, U. (2018). "It'sa gut feeling"–Escherichia coli biofilm formation in the gastrointestinal tract environment. *Critical reviews in microbiology*, *44*(1), 1-30.

37. Rothe, K., Spinner, C. D., Waschulzik, B., Janke, C., Schneider, J., Schneider, H., ... & Katchanov, J. (2020). A diagnostic algorithm for detection of urinary tract infections in hospitalized patients with bacteriuria: The "Triple F" approach supported by Procalcitonin and paired blood and urine cultures. *PloS one*, *15*(10), e0240981.

38. Salardini, A. (2019, April). Interpretation of Biomarker Data in Diagnosis of Primary Dementias. In *Seminars in neurology* (Vol. 39, No. 02, pp. 200-212). Thieme Medical Publishers.

39. Sheikh, A. F., Goodarzi, H., Yadyad, M. J., Aslani, S., Amin, M., Jomehzadeh, N., ... & Hashemzadeh, M. (2019). Virulence-associated genes and drug susceptibility patterns of uropathogenic Escherichia coli isolated from patients with urinary tract infection. *Infection and drug resistance*, *12*, 2039.

40. Šunderić, M., Križáková, M., Malenković, V., Ćujić, D., Katrlík, J., & Nedić, O. (2019). Changes due to ageing in the glycan structure of alpha-2-macroglobulin and its reactivity with ligands. *The protein journal*, *38*(1), 23-29.

41. Uwaezuoke, S. N. (2018). Biomarkers of Common Childhood Renal Diseases. *Biomarker: Indicator of Abnormal Physiological Process*, 125.

42. Vandepitte, J., Engbaek, P., Piot, P. and Heuck, C. (1991). Basic laboratory procedures in clinical bacteriology.Geneva, World Health Organization: 31-35.

43. Vijayan, A. L., Ravindran, S., Saikant, R., Lakshmi, S., & Kartik, R. (2017). Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy. *Journal of intensive care*, *5*(1), 1-7.

44. Wu, D. F., Behringer, V., Wittig, R. M., Leendertz, F. H., & Deschner, T. (2018). Urinary neopterin levels increase and predict survival during a respiratory outbreak in wild chimpanzees (Taï National Park, Côte d'Ivoire). *Scientific reports*, *8*(1), 1-9.

45. Yaron, J. R., Zhang, L., Guo, Q., Haydel, S. E., & Lucas, A. R. (2021). Fibrinolytic Serine Proteases, Therapeutic Serpins and Inflammation: Fire Dancers and Firestorms. *Frontiers in Cardiovascular Medicine*, *8*, 153.

46. Yu, H., Li, Y., & Huang, A. (2021). Detection of sialic acid using boronic-acid-functionalized metal organic framework UiO-66-NH2@ B (OH) 2. *Talanta*, *232*, 122434.

47. Yunus, I., Fasih, A., & Wang, Y. (2018). The use of procalcitonin in the determination of severity of sepsis, patient outcomes and infection characteristics. *PloS one*, *13*(11), e0206527.

48. Zwaans, B. M., Lamb, L. E., Bartolone, S., Nicolai, H. E., Chancellor, M. B., & Klaudia, S. W. (2018). Cancer survivorship issues with radiation and hemorrhagic cystitis in gynecological malignancies. *International urology and nephrology*, *50*(10), 1745-1751.