Detection of Escherichia coli From Urinary Tract by Using by VITK 2 System

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Abstract

The present study focused on the importance of Escherichia coli which carries the genetics of the virulence of highly pathogenic UTI in human population. This study dealt with patient at wasit the period extending October 2021 , a total 100 samples of urine . The specimens were immediately transported to the laboratory by a cool box. The isolation and description of Escherichia coli was done by using selective media, gram stain, biochemical test and VITEK 2 assay. The results showed one positive one samples was isolated from the human urine with urine tract infection which means Escherichia coli is pathogenic. Also, the present study found that all most ages that susceptible for a infections by Escherichia coli.

Keywords: *Escherichia coli, UTI , VITEK 2, urine.*

INTRODUCTION

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and or need to urinate, pain during urination, and cloudy urine (Urinary Tract Infections 2010).The main causal agent is Escherichia coli. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it . When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI. The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Although they cause discomfort, urinary tract infections can usually be easily treated with a short course of antibiotics with no significant difference between the classes of antibiotics commonly used (Trestioreanu et al., 2010). The most common organism implicated in UTIs (80–85%) is E.coli, (Nicolle . 2008) while Staphylococcus saprophyticus is the cause in 5–10%. The bladder wall, in common with most epithelia is coated with a variety of cationic antimicrobial peptides such as the defensins and cathelicidin which disrupt the integrity of bacterial cell walls. (Ali et al: 2009). In addition, there are also mannosylated proteins present, such as Tamm-Horsfall proteins (THP), which interfere with the binding of bacteria to the uroepithelium. As binding is an important factor in establishing pathogenicity for these organisms, its disruption results in reduced capacity for invasion of the tissues. Moreover, the unbound bacteria are more easily removed when voiding. The use of urinary catheters (or other physical trauma) may physically disturb this protective lining, thereby allowing bacteria to invade the exposed epithelium. During cystitis, Uropathogenic Escherichia coli (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial

communities (IBCs) (Justice et al., 2006). By working together, bacteria in biofilms build themselves into structures that are more firmly anchored in infected cells and are more resistant to immune-system assaults and antibiotic treatments. This is often the cause of chronic urinary tract infections. E.coli are not always confined to the intestine ,and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination (Feng et al., 2002) and is now classified as part of the Enterobacteriaeae family of gamma-probacteria.

Objectives of the study

1 - Isolation of E. coli bacteria from urine of patients with urinary tract infections by culture, biochemical .

2- Identification of E. coli using methods VITIK 2 System .

Material and methods

1. Sample Collection

Overall 100 samples of urine . Were collected from humans the samples were obtained from Al- Zahra Teaching Hospital in wasit . The specimens were directly culture onto MaCconkey , Eosin methylene blue agar and blood agar sand were incubated at 37ºC for 24 hours.

2. Identification of the Isolates

Isolates were identified depending on morphological and biochemical tests as compared with identification scheme described by (Becton Dickinson, 2013), and by using Vitek 2 system to confirmatory isolated .

Results and Discussion

1. Isolation and Characterization of Proteus spp.

One hundred samples of urine were collected from patients in wasit city hospitals. As display in table (1). Thirty seven local isolates were characterized depending on cultural and microscopic characteristic . Genus and species were characterized by using biochemical tests and Vitek 2 system.

Table 1: Types of sample, number and Percentage of Proteus mirabilis isolated from human and cats' samples

Types of sample	Number of samples	Number of isolates	Percentage%
Human	100	36	36 %

2. Cultural Characteristics

The isolates displayed various colonies on selective and differential culture media that have grown in 24 hours at 37ºC. The colonies of the isolates showed colorless colonies, non-mucoid ,dry and donut-shaped, on MacConkey agar . They do not produce any hydrogen sulfide, one isolate was β hemolysis on blood agar . on EMB the colonies are appears may have a green metallic sheen .

3. Microscopically Characteristics

The bacteria appeared in light microscope as gram negative, short rods, single cells and pair after 24 hrs incubation at 37˚C

4. Biochemical tests .

Conventional biochemical tests were done to initial identification on the genus level. All the isolates gave positive results for the Catalase test. While gave negative results for the Oxidase . The results revealed these isolates were suspected E.coli . For confirmation of the isolate , using VITEK2 compact system (Biomerieux - France). This system technology has improved the field of bacterial examination

by providing more reliable technology, high speed and high sensitivity for bacterial identification, the results as high as 97% accuracy. The result of this study was 96% probability E. coli as in Table (2).

Table (2): VITEK ®2 technique, used for identification of E. Coli

Test	Human sample
APPA	_
ADO	_
PyrA	_
IARL	_
Dcel	_
BGAL	+
H2S	_
BNAG	_
AGLTp	_
dGLU	+
GGT	_
OFF	+
BGLU	_
dMAL	+
dMAN	+
dMNE	+
BXYL	_
BAlap	_
ProA	_
LIP	_
PLE	_
TyrA	+
URE	_
dSOR	+
SAC	+
dTAG	–
dTRE	+
CIT	_
MNT	_
5KG	+
ILATK	+
AGLU	_
SUCT	+
NAGA	_
AGAL	+
PHOS	_
GIy A	_
ODC	_
LDC	+
IHISa	_
CMT	+
BGUR	+
O129R	+
GGAA	_
IML Ta	_
ELLM	_
ILATa	_
identification	97 %

Cultural characteristics and biochemical tests are in agreement with (Leininger et al., 2001 , Islam et al., 2014). The first stage to recover E. coli on EMB agar after the enrichment step was done E. coli isolates on the EMB media are recovered in order that take place the distinction between E. coli and other intestinal

organisms present in human urine that may be pathogenic or natural. All isolates of E. coli had given green minerals sheen in EMB media, because these media consist of eosin and methylene blue dyes. It has the property of discoloration, so the fast fermentation of lactose E. coli produced the acid which this lead to a decrease in the pH, which lead to stimulation of the colonies to absorb the pigments and give them the purple-black color will give a distinct metallic sheen when exposed to light (Leininger et al., 2001), the method of pre-isolation utilized in the current study to help in identified and differentiate of bacteria E. coli from other bacteria the step agreeing (Islam et al., 2014). More confirmation of E. coli O157:H7 was performed via the VITEK 2 automated systems was use up a gram negative (GN ID) card according to the Manufacturer's instructions (Guran et al., 2017; Al-Saadi et al.,2018) confirmed E.coli O157:H7 by using Vitek 2

Conclusions

1. Isolation E. coli recovery from urine samples and in conformation of these bacteria with high specificity and sensitivity.

2. The current study showed dispersal from E. coli into UTI more .

3. The results showed that infection in humans was in the ages of (15-30 year).

Recommendations

1. It is highly recommended to reduce the unethical use of antibiotics to reduce the development of antibiotic resistance.

2. Future work should involve more parameters like real time PCR.

3. Risks of microbial diseases such as E. coli in human effect on public health and safety.

Reference

Anis S Mokhtar*, Nurhayo Asib, A. R. R. . R. M. A. . (2022). Development of Saponin based Nano emulsion formulations from Phaleria macrocarpa to Control Aphis gossypii. Journal Of Advanced Zoology, 43(1), 43–55. Retrieved from http://jazindia.com/index.php/jaz/article/view/113

E. Soydan, S. Bıyık, C.T. Supuran, D. Ekinci, et al. "Carbonic anhydrase from Apis mellifera: purification and inhibition by pesticides", Journal of enzyme inhibition and medicinal chemistry., 32 (1), 47–50 (2017).

Faisal, H. T. ., Abid, M. K. ., & Abed, A. . (2022). Study Of Some Biochemical Parameters in Dose During Pregnancy in Goats. Journal Of Advanced Zoology, 43(1), 01–06. https://doi.org/10.17762/jaz.v43i1.109

Mokhtar, A. R. R. A. S. . (2022). Development Of Saponin Based Wettable Powder Formulation from Phaleria macrocarpa To Control Pomacea maculate. Journal Of Advanced Zoology, 43(1), 17–31. Retrieved from http://jazindia.com/index.php/jaz/article/view/111

Ali, A. S., Townes, C. L., Hall, J., & Pickard, R. S. (2009). Maintaining a sterile urinary tract: the role of antimicrobial peptides. The Journal of urology, 182(1), 21-28.

Al-Saadi, Z. H. ; Tarish, A. H. and Saeed, E. A. (2018). Phenotypic detection and antibiotics resistance pattern of local serotype of E. coli O157:H7 from children with acute diarrhea in hilla city/Iraq. Journal of Pharmaceutical Sciences and Research, 10(3): 604–609.

Becton Dickinson (2013). Bula BD MacConkey Agar with Sorbitol. 4.

Feng, P., Weagant, S. D., Grant, M. A., Burkhardt, W., Shellfish, M., & Water, B. (2002). BAM: Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological analytical manual, 13(9), 1-13.

Guran, H. S ; Vural, A. ; Erkan, M. E. and Durmusoglu, H. (2017). Prevalence and some virulence genes of Escherichia coli O157 isolated from chicken meats and giblets . Annals of Animal Science, 17(2), 555–563. https://doi.org/10.1515/aoas-2016-0061

Islam, M. Z. ; Musekiwa, A. ; Islam, K. ; Ahmed, S. ; Chowdhury, S. ; Ahad, A. and Biswas, P. K. (2014). Regional variation in the prevalence of E. coli O157 in cattle: a meta-analysis and meta-regression. PloS One, 9(4).

Justice, S. S., Hunstad, D. A., Seed, P. C., & Hultgren, S. J. (2006). Filamentation by Escherichia coli subverts innate defenses during urinary tract infection . Proceedings of the National Academy of Sciences, 103(52), 19884-19889.

Leininger, D. J. ; Roberson, J. R. and Elvinger, F. (2001). Use of eosin methylene blue agar to differentiate Escherichia coli from other gram-negative mastitis pathogens. Journal of Veterinary Diagnostic Investigation, 13(3): 273-275.

Nicolle, L. E. (2008). Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urologic Clinics of North America, 35(1), 1-12.

Trestioreanu, A. Z., Green, H., Paul, M., Yaphe, J., & Leibovici, L. (2010). Antimicrobial agents for treating uncomplicated urinary tract infection in women. Cochrane database of systematic reviews, (10).

Urinary Tract Infections. Retrieved, 2010.