Study of some virulence factors of some urepathogenic bacteria

Jwan Ahmed AL-Hamawandi,
College of Medicine, University of Babylon..

Abstract:-

In this study, sixty urine samples (60 samples) were obtained from patients suffering from urinary tract infections (UTIs), who have attending to Hilla Teaching Hospital for Surgery Department of Urology for the period from April to August 2012. These samples were identification by biochemical test to diagnosed types of bacteria, it was found that 20(33.3%) of the isolates were *E. coli* while other gram negatives were found in small in number, 2(3.3%) isolates for *Pseudomonas aeruginosa*, 7(11.6%) isolates for *Klebseilla pneumonia* and one(1.6%) isolates for *Proteus mirabilis*. On the other hand the isolated Gram positive bacteria were 18(30%) for *Staphylococcus aureus* and 5 (8.3%) *Staphylococcus fecalis*, 3(5%) isolates for *Streptococcus agallagtia* and 4(6.6%) isolates from *streptococcus pyogenes.* Mannose resistance hemoglutinin type IV was studied, it was found that all isolated have this variance factor. Also, hemolysin production by gram- positive and gram-negative bacteria isolated from urinary tract infection was studied, and it was found that *E. coli, Pseudomonas*, and *Staphylococcus aureus, Staphylococcus fecalis, streptococcus pyogenes. Proteus mirabilis* have able to produce hemolysin as show narrow zone of β- hemolysis around colonies on blood agar plate, while *Klebseilla,* were non hemolytic which no color change around the bacterial colonies on blood agar. Extracellular protease production by gram- positive and gram-negative bacteria isolated from urinary tract infection was detected in this study, and it was found that all isolates of this study (*E. coli, Pseudomonas, Staphylococcus aureus, Staphylococcus fecalis streptococcus pyogenes, Proteus mirabilis* and *Klebseilla*) have able to produce protease production in M_9 media (supplemented with 20% glucose and 1% gelatin) after 24 hours of incubation. A clear hallo of transparent area was found around the colony after the addition of 3 ml of 5% trichloroacetic acid. On the other hand, it was detection of lipase production, it was found that *Klebseilla* and *Proteus mirabilis* give appositive results, while *E. coli, Pseudomonas, Staphylococcus aureus, Staphylococcus fecalis, Streptococcus pyogenes* give variable results.

الخلاصة

تم في هذه الدراسة جمع 60 عينه ادرار لمرضى يعانون من التهاب المجاري البولية من المراجعين إلى العيادة الاستشارية في مستشفى الحلة التعليمي وللفترة من شهر نيسان إلى شهر اب 2012 وتم تشخيص انواع مختلفه من البكتريا بواسطه الاختبارات الكيموحيويه، حيث وجدت بكتريا *E. coli* 20(33.3%) و*Pseudomonas aeruginosa* 2(3.3%) و7(11.6%) عزله لبكتريا *Klebseilla pneumonia* وعزله واحده (1.6%) لبكتريا *Proteus mirabilis*، ايضا تم تشخيص بكتريا *Staphylococcus aureus* 18(30%) و 5(8.3%) عائده الى بكتريا *Staphylococcus fecalis* و3 (5%) لبكتريا *Streptococcus agallagtia* بالاضافة الى 4(6.6%) بكتريا *Streptococcus pyogenes*. درس ايضا اختبار مقاومه المانوز للتلازن النوع الرابع، وجد ان جميع العزلات كانت تمتلك هذا النوع من عوامل الضراوة.

كما درست ايضا امكانيه امتلاك عامل التحلل hemolysin اذ وجد ان بكتريا *E. coli, Pseudomonas*, *Staphylococcus aureus*, *Staphylococcus fecalis*, *Streptococcus pyogenes* و*Proteus mirabilis* تمتلك القابليه على انتاج هذا العامل على شكل هاله حول المستعمره البكتيريه، في حين بكتريا *K. pneumonia*. لاتمتلك هذا العامل عند زراعتها على اكار الدم. انتاج انزيم خارج خلوي ايضا درس على البكتريا المعزوله. فوجد ان القابليه على انتاج هذا الأنزيم بعد زراعتها على وسط اكار M9 بعد تغذيته بـ 20% كلوكوز و1%جيلاتين. ايضا أجريت اختبار أنتاج أنزيم اللابيز على العزلات البكتيريه، وجد ان بكتريا *K. pneumonia* و *Proteus mirabilis* لها القابلية على انتاج هذا النوع من الانزيمات بينما الأنواع الاخرى المعزوله في هذه الدراسه اعطت نتائج متغايره.

Introduction:

Among the most common infectious diseases, urinary tract infections (UTIs) are a commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million (Ronald *etal*,2001 ; Baris *etal*, 2003). UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:-bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria]) and also can be asymptomatic or symptomatic, UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated," whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic(Stamm and Hooton, 1993 ; Gonzalez and Schaeffer, 1999). It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually (Wilson and Gaido, 2004). Many different microorganisms can cause UTIs though the most common pathogens causing the simple ones in the community are *Escherichia coli* and other Enterobacteriacae, which accounts approximately 75% of the isolates. In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas spp*. are comparatively more common. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization (Sefton, 2000). Treatment of UTIs cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens (Bonadio *etal*, 2001). However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections (NCCLS, 2000; Grude *etal*, 2001). The prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide. Associated resistance, i.e. the fact that a bacterium resistant to one antibiotic is often much more likely to be resistant to other antibiotics, drastically decreases our chances of getting a second empirical attempt right (Kripke, 2005). Resistance rates to the most common prescribed drugs used in the treatment of UTIs vary considerably in different areas world-wide. The estimation of local etiology and susceptibility profile could support the most effective empirical treatment (Sundqvist and Kahlmeter, 2009). Therefore, investigating epidemiology of UTIs (prevalence, risk factors, bacterial isolates and antibiotic sensitivity) is fundamental for care givers and health planners to guide the expected interventions. Thus, the aim of this study was to determine bacterial etiologic agent of uropathogens and evaluate their in vitro susceptibility pattern to commonly used antimicrobial agents.

Materials and methods:

A-Patients and specimens: 60 urine samples were collected from patients suffering from urinary tract infections; these samples have attending to Hilla Teaching Hospital for Surgery Department of Urology for the period from April 2012 to August 2012.

B-Bacterial diagnosis: Isolation of uropathogens was performed by a surface streak procedure on both blood and MacConkey agar using calibrated loops for semi-quantitative method and incubated aerobically at 37 °C for 24 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/ml. Bacterial identification was made using biochemical tests, namely indole,

citrate, oxidase, H2s production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase and manitol fermentation (CLSI, 2005; Farajnia *etal*, 2009).

C-Detection of mannose resistance hemoglutinin type IV: It was done according to (Sambrook and Rusell, 2001).

D-Detection of hemolysin: It was done according to (De Boy *etal*, 1980).

E-Detection of extracellular protease production test: It was done according to (Benson, 1998).

F-Detection of lipase production: It was done according to (Collee *etal*, 1996).

Results & Discussion:

Out of 60 cultured urine specimens, significant bacteriuria was detected. As it is shown in Tabel (1), 20(33.3%) of the isolates were *E. coli* while other Gram negatives were found in small in number, 2(3.3%) isolates for *Pseudomonas arugenosa*, 7(11.6%) isolates for *Klebseilla pneumonia* and one(1.6%) isolates for *Proteus mirabilis*.

On the other hand the isolated Gram positive bacteria were 18(30%) for *Staph. aureus* and 5 (8.3%) *S. fecalis*, 3(5%) isolates for *Strept. agallagtia* and 4(6.6%) isolates from *strept. pyogenes.*

Table [1] bacterial isolated from urinary tract infection

Urine sample	Bacterial isolates	No. of isolates
60 urine sample	*E. coli*	20(33.3%)
	Pseudomonas aeruginosa	2(3.3%)
	Klebseilla pneumonia	7(11.6%)
	Proteus mirabilis	1(1.6%)
	Staphylococcus aureus	18(30%)
	Staphylococcus fecalis	5 (8.3%)
	Streptococcus agallagtia	3(5%)
	Streptococcus pyogenes	4(6.6%)
		Total: 60 (100%)

Escherichia coli (*E. coli*) is the major etiological agent in causing UTI, In this study, the most frequent uropathogens were Gram negatives which made up (33.3%) of all the isolates. *E. coli* is by far the most common bacteria isolated from urine samples in both outpatients and inpatients of both sexes, and this finding is in agreement with others finding too (Water *etal*, 1996; Tessema *etal*, 2007; Kebira *etal*, 2009). Gram-negative bacilli were responsible for UTI infections in our patients. The most common isolated bacteria from urinary tract infections were *E. coli* (Rakaa *etal*, 2004).

The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in the region or internationally (Ronald, 2002), however different results have been reported. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each country (Dromigny *etal*, 2002).

In this study, mannose resistance hemoglutinin type IV was detected, it was found that all isolated have this variance factor, This means that, the positive group is so called mannose resistant hemagglutination-assay positive and the second is mannose sensitive.

However, the presence of mannose resistant adhesion factor may help in the bacteria in adhesion to the epithelial cells, and then enhance the pathogenicity of bacteria. This results similar result obtained by (Chin *etal*, 2011) was found that *E. coli, Proteus, Klebseilla* was tested for MRHA and the results give positive. The ability to agglutinate human erythrocytes in the presence of the receptor analog D-mannose, and the $MRHA^+$ phenotype were more frequent among symptomatic-UTI isolates than among ABU isolates. The frequencies of P pilus genes among symptomatic-UTI isolates and ABU strains were similar. These results were shown in Table (2).

Table (2) Detection of mannose resistance hemoglutinin type IV in all isolates

Urine sample	Bacterial isolates	Mannose resistance hemoglutinin type IV
60 urine sample	*E. coli*	+
	Pseudomonas aeruginosa	+
	Klebseilla pneumonia	+
	Proteus mirabilis	+
	Staphylococcus aureus	+
	Staphylococcus fecalis	+
	Streptococcus agallagtia	+
	Streptococcus pyogenes	+

In this study, hemolysin production by Gram- positive and Gram-negative bacteria isolated from urinary tract infection was studied, and it was found that *E. coli, Pseudomonas*, and *Staphylococcus aureus S. fecalis*, *strept pyogenes*, *Proteus mirabilis* have able to produce hemolysin as show narrow zone of *β*- hemolysis around colonies on blood agar plate, while *Klebseilla*, were non hemolytic which no color change around the bacterial colonies on blood agar. These results show in Table (3).

Table (3) Detection of hemolysin that produce from bacterial isolation from UTIs

Bacterial isolates	Hemolysin production
E. coli	+
Pseudomonas aeruginosa	+
Klebseilla pneumonia	_
Proteus mirabilis	+
Staphylococcus aureus	+
Staphylococcus fecalis	+
Streptococcus agallagtia	+
Streptococcus pyogenes.	+

Many types of bacteria have able to produce *β*- hemolysin when cultured on blood agar, and produces zones of hemolysis that are only slightly larger than the colonies themselves (Sharma *etal*, 2007). Production of hemolysin confers the ability of the organism to invade the tissues of the host and increase the pathogenic capacity of an organism which determined as virulence factors. This important virulence factor which is cytotoxic due to the formation of trans membranous pores in the host cell membrane (Cruickshank *etal*, 1973). Hemolysin production is another important virulence property

of UTIs. Hemolysins inflict direct cytotoxic effects on renal epithelium resulting in scaring. Alpha-hemolysin is described to be a lethal factor with dermonecrotic effects and is antigenic in nature. Also, hemolysins are toxic to a series of host tissues and cells including RBCs, leucocytes, epithelial and endothelial cells (Griffiths and McClain, 1988).

Extracellular protease production by Gram- positive and Gram-negative bacteria isolated from urinary tract infection was studied, and it was found that all isolates of this study (*E. coli, Pseudomonas, Staphylococcus aureus, S. fecalis, strept. pyogenes, Proteus mirabilis* and *Klebseilla*) have able to produce protease production in M9 media (supplemented with 20% glucose and 1% gelatin) after 24 hours of incubation. A clear hallo of transparent area was found around the colony after the addition of 3 ml of 5% Trichloroacetic acid. These results show in Table (4). This results was in agreement with the results of (Choong *etal*, 2001) who is found that (100%) isolates of uropathogenic bacteria were produce extracellular protease. Also, these results were identical with that obtained by (D¨oring *etal*, 1981) who found that the detection of extracellular protease from microorganism on agar plate was used by gelatin as substrates into agar and varying culture medium compositions. Extracellular protease plays an important role in the cell survival and cell-cell communication (Esposito *etal*, 1980).

Also, the production of protease was induced during the growth of organism in the medium containing amino acids (Caballero *etal*, 2001). The proteases proposed as virulence factors in a variety of diseases caused by this microorganism, it is partly determined by exo-products such as alkaline protease and elastase that are responsible for the damage of tissues by degrading elastin collagen and proteoglycans, these enzymes also shown to degrade proteins that function in host defense in vivo (Amara *etal*, 2009).

In this study, the detection of lipase production was studied, it was found that *Klebseilla* and *Proteus mirabilis* give appositive results, while *E. coli, Pseudomonas, Staphylococcus aureus, S. fecalis, Strept. pyogenes* give variable results as shown Table (4). This result is consistent with (Svendsen, 2000), who found that bacteria *Klebseilla* give positive result in a test for lipase production. Lipase is water-soluble enzyme that catalyzes the hydrolysis of ester chemical bonds in water insoluble lipid substrate, most lipases act as a specific position on the glycerol backbone of lipid substrate (Andrews, 1998). Two distinct lipase enzymes were produced by *Ps. aeruginosa*, PLC-N (non-hemolytic) and PLC-H (hemolytic), both enzymes are phosphate regulated. The two enzymes could work sequentially and synergistically to lyse host cells (Van Dyke *etal*, 1991). The lipase production can be arrested by various compounds such as tetracycline which are effective against lipase production by interfering with protein synthesis by binding to bacterial ribosome (Chamberlain and Brueggmann, 1997).

Table (4) Detection of extracellular protease and lipase production from bacterial isolation from UTIs

Bacterial isolates	lipase production	Extracellular protease
E. coli	+/-	+
Pseudomonas aeruginosa	+/-	+
Klebseilla pneumonia	+	+
Proteus mirabilis	+	+
Staphylococcus aureus	+/-	+
Staphylococcus fecalis	+/-	+
Streptococcus agallagtia	+/-	+
Streptococcus pyogenes.	+/-	+

References

- Amara, A. A.; Salem, S. R. and Shabeb, M. S. A. (2009). Biodetergent: The possibility to use bacterial protease and lipase as detergent, Microbiol 20(3):312-320
- Andrews, S. C. (1998). Iron Storage in bacteria. Adv. in Microbial Physiol, 40: 281 - 351.
-Baris, Z.; Babic´-Erceg, A. and Borzic, E.l. (2003). Urinary tract infections in South Croatia: aetiology and antimicrobial. Intl. *J*. Antimicrob Agents. 22:S61–S64.
-Benson, R. H. (1998). Microbiological application: laboratory Manual in general Microbiology .17th Ed. WB. M.C. Graw. HILL.
-Bonadio, M.; Meini. M.; Spetaleri, P. and Gilgi, C. (2001). Current microbiological and clinical aspects of urinary tract infections. Eur. *J*. Urol. 40:439–445.
-Caballero, A. R.; Moreau, J. M. and ´Callaghan, R. J. (2001). *P. aeruginosa* protease IV enzyme assays and comparison to other *Pseudomonas* proteases. Analytical Biochemistry . 290:330-337.
- Chamberlain, N. R. and Brueggmann, S. A. (1997). Characterization and Expression of Fatty Acids Modifying Enzymes Produce by *Staph. epidermidis .J*. Med. Microb. 46 : 693-697.
-Chin, B. S.; Kim, M.S. and Han, S.H. (2011). Risk factors of all-cause in-hospital mortality among Korean elderly bacteremic urinary tract infection (UTI) patients. Archives of Gerontology and Geriatrics. 52:e50–e55.
- Choong, S. K. S.; Wood, S.; Fry, C. H. and Whitfield, H. N. (2001). Catheter associated urinary tract infection and encrustation. Int. *J*. Antimicrobial Agents. 17: 305-310.
-Clinical and Laboratory Standards Institute, author. (2005). Supplemental tables. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. CLSI Publication M100-S15, M2-A8 and M7-A6. Pennsylvania: CLSI.
-Collee, J. G.; Fraser A. G.; Marmino, B. P. and Simons, A. (1996). Mackie and McCartney Practical Medical Microbiology. 14th Ed. The Churchill Livingstone, Inc. USA.
-Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (1973). Medical Microbiology, 12thed., Vol. I. Churchill Livingstone, Edinburgh

-De Boy, J.; Wachsumth, K. and Davis, B. (1980). Hemolysin activity in enterotoxogenic and non-enterotoxogenic strains of E. coli. J. Clin. Microbiol. 12: 193-198.

-D¨oring, G.; Obernesser, H. J. and Botzenhart, K. (1981). Extracellular toxins of *Pseudomonas aeruginosa*. II. Effect of two proteases on human immunoglobulin IgG, IgA and secretory IgA. Zentralbl. Bakteriol. Parasitenkd. Infektionskr.

-Dromigny, J. A.; Nabeth, P.; Perrier, G. and Claude, J. D. (2002). Distribution and susceptibility of bacterial urinary tract infections in Dakar, Senegal. Int. Antimicrob Agents. 20:339–347.

-Esposito A. L.; Gleckman, S.; Cram, M.; Crowley, F.; McCabe, L. and Drapkin, M.S. (1980). Community-acquired bacteremia in the elderly: analysis of one hundred consecutive episodes, *J.* AM. Geriater. Soc. 28: 315-319.

-Farajnia, S.; Alikhani, M.Y.; Ghotaslou, R. and Naghili, B. (2009). Nakhlband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int. *J.* Infect. Dis. 13:140–144.

-Griffiths, B. B. and McClain, O. (1988). The role of iron in the growth and hemolysin (Streptolysin S) production in *Streptococcus pyogenes*. *J.* Basic. Microbiol. 28 (7): 427–36.

-Grude, N.; Tveten, Y. and Kristiansen, B. E. (2001). Urinary tract infections in Norway: bacterial etiology and susceptibility, a retrospective study of clinical isolates. Clin Microbiol Infect. 7:543–547.

-Gonzalez, C.M. and Schaeffer, A. J. (1999). Treatment of urinary tract infection: what's old, what's new, and what works. World. *J.* Urol. 17: 372–382.

-Kebira, A.N.; Ochola, P. and Khamadi S. A. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections. *J.* Appl. Biosci. 1320–1325.

-Kripke, C. (2005). Duration of therapy for women with uncomplicated UTI. Am Fam Physician. 72:2219.

- National Committee for Clinical Laboratory Standards, author. (2000). Performance standards for antimicrobial disc susceptibility tests. 7th ed. Wayne, Pennsylvania, USA: NCCLS; M2-A7.

-Rakaa, L.; Mulliqi-Osmani, G. and Berisha, L. (2004). Etiology and susceptibility of urinary tract isolates in Kosova. Int. *J.* Antimicrob agents. 23S1S2–23S1S5.

-Ronald, A. R.; Nicolle, L.E. and Stamm, E. (2001).Urinary tract infection in adults: research priorities and strategies. Int. *J.* Antimicrob Agents. 17: 343–348.

-Ronald, A. (2002). The etiology of urinary tract infection: Traditional and Emerging Pathogens. Am. *J.* Med. 113:14S–19S.

-Sambrook, J. and Rusell, D. W. (2001). Molecular cloning. A laboratory manual. Third ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, N.Y.

- Sefton, A. M. (2000). The impact of resistance on the management of urinary tract infections. Int. *J.* Antimicrob Agents. 16:489–491.

-Sharma, S.; Bhat, G. and Shenoy, S. (2007). Virulence factors and drug resistance in *E.coli* isolates from extraintestinal infections. Indian .*J.* Med. Microbiol. 25(4): 369-373.

-Stamm, W.E. and Hooton, T. M. (1993).Management of urinary tract infections in adults. N. Engl. *J.* Med.329:1328–1334.

-Svendsen, A. (2000). Lipase protein enginerring.Acta Biochem. Biophys. 1543(2): 223-228.

-Sundqvist, M. and Kahlmeter, G. (2009). 'Pre-emptive culturing' will improve the chance of getting it right' when empirical therapy of urinary tract infections fails. J. Antimicrob Chemother. 64:227–228.

- Tessema, B.; Kassu, A. and Mulu, A. (2007). Predominant Isolates of Urinary Tract Pathogens and their susceptibility Patterns in Gonder Univesity Teaching Hospital, Northwest Ethiopia. Ethio. Med. *J*. 45:61–67.

-Water, G.; Harrison. B. and Kunin, G. (1996). Urinary tract infection. N. *Eng. Med J*. 248–250.

-Wilson, M.L. and Gaido, L. (2004) Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. Clin. Infect. Dis. 38:1150–1158.

-Van Dyke, M. I.; Lee, H. and Trevors, J. T. (1991). Applications of microbial surfactants. Biotechnol. Adv. 9: 241-252.