

Full Length Research Paper

## Assessment of bacteria as virulence agents for urinary tract infection in Egyptian patients

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This study involved the examination of bacteriuria according to the results of quantitative cultures in over 300 urine samples collected from patients admitted at El-Hussein University Hospital. The infection rate of both *Escherichia coli* and *Klebsiella pneumonia* were found to be 26.92 and 11.54%; respectively. As the glucose and albumin concentration increased, the number of all infectious organisms greatly increased. Similarly, when creatinine concentration elevated up to 3.5 g/l, the infectious organisms (*Enterobacter faecalis*, *Streptococcus* sp. (B) group, *Proteus mirabilis*, *P. aeruginosa*, *Enterobacter* sp. and *Citrobacter freundii*) significantly increased. The effect of sodium chloride (NaCl), calcium oxalate (CaC<sub>2</sub>O<sub>4</sub>), magnesium chloride (MgCl<sub>2</sub>) and uric acid (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O<sub>3</sub>) concentrations fluctuated according to the concentration used and the type of each infectious organism. Noracin was effective against all tested organisms. *Acinetobacter* sp. recorded 50% resistance to ampicillin while it was sensitive to all other tested antibiotics.

**Key words:** Bacteriuria, creatinine, antibiotics.

### INTRODUCTION

Urinary tract infections (UTIs) represent one of the most common diseases encountered in medical practice today and encompass a broad range of clinical entities that are associated with a common finding of a positive urine culture (Chomarat, 2000). UTIs can be caused by exogenous microorganisms such as *P. aeruginosa* or by endogenous faecal or urethral microorganisms (Abou-Dobara et al., 2010). The microorganisms responsible for UTIs are usually the microbial flora found in the gut and are always present as a potential source of reinfection (Hillier et al., 2007). The endogenous microorganisms causing UTIs include *E. coli*, *Staphylococcus epidermidis*, *S. saprophyticus*, *Proteus* spp. and *Klebsiella* spp. (Godfrey and Evans, 2000; Pape et al., 2004; Jacobsen et al., 2008).

Bacteria can cause UTIs when they invade the mucosa

(Pinkerman, 1994), attach mucosal surfaces, grow in the host's tissue, or interfere with host defense causing damage to the host (Smith, 1995).

Bacterial factors play an important role in the pathogenesis of UTI. Certain bacteria possess properties that enable them to attach or adhere to uroepithelial cells or to the surface of catheter materials (Watts et al., 2010). Mannose – specific ligands on the fimbria or pili of *E. coli* bind to mannose receptors on urethral and bladder epithelial cells. Once attachment has occurred, the ability to infect the urinary tract starts, causing local cystitis or renal parenchymal infection. Bacterial attachment depends on the strain of bacteria, the presence of urinary proteins and salts, and the pH of the urine (Stamm, 1991). The accumulation of bacteria, glycocalyx, protein, crystalline salts and amorphous cellular debris eventually

**Table 1.** Age group of males and number of infected samples.

Age group	3-13	14-24	25-35	36-46	47-57
Number of patient surveyed	30	30	30	30	30
Number of infected samples	2	4	8	10	12
Percentage of infected samples	6.6%	13.3%	26.6%	33.3%	40%

causes encrustations that can obstruct the flow of urine and serve as a persistent nidus for infection. This study will focus on the prevalence of bacteria isolated from urine cultures received from hospitalized patients. Analysis of glycocalyx as partial pathogenic factor as well as the effect of urine chemical elements on urinary pathogens will be investigated.

## MATERIALS AND METHODS

### Urine samples

The present study was conducted at Urology Department., El-Hussein University Hospital during the period from September to November 2003. Urine samples were collected from patients (males and females) immediately after their admission to hospital. Two specimens were collected from each patient under proper aseptic condition, one for bacterial culture and sensitivity test and the other for fresh film examination.

### Bacteriological media

Three different types of enrichment media were used for isolating proper aerobic and/or facultative anaerobic bacteria: Nutrient agar medium, and MacConkey's agar medium. All media were readily prepared (Oxoid, England).

### Assessment and purification of bacterial isolates

Plates containing the three types of media were incubated after inoculation at 37°C for 24 and 48 h, respectively. The colonies grown were selected and purified by several consecutive streaking on agar plates. Purity was checked by microscopic examination of the isolates using gram stain. Purified isolates were subjected to a scheme of experimental identification.

### Bacterial identification

Many biochemical reactions were performed for identification. The identification process was carried out according to the methods described in Cowan and Steel (1974), Manual of Methods for General Bacteriology (1981) and Medical Laboratory Manual for Tropical Countries, Vol. II: Microbiology (Cheesbrough, 1984).

### Chemical environmental factors affecting the growth of the isolated bacteria

The natural selection, fitness, pathogenicity and communities of isolated bacteria could be affected by some ecological factors. The environmental changes were mainly different concentrations of

each chemical constituent of urine especially glucose, uric acid, sodium chloride, calcium carbonate, calcium oxalate, albumin, creatinine and magnesium chloride.

Each substrate was prepared in different concentrations (as indicated in the results) with normal urine, which was used as natural medium. The urine and substrate were sterilized by filtration. Each bacterial strain was inoculated in different concentrations of each chemical constituent and incubated at 37°C for 48 h. The results were expressed as optical density (O.D) at 660 nm using UV/Vis spectrophotometer (Unicam, England).

### Effect of different antibiotics on bacterial growth

Nutrient agar medium (Bennet et al., 1966) seeded with the bacterial strains isolated from patients were screened concerning their sensitivity to different antibiotics. The disc diffusion method was applied using commercial paper discs (Oxoid) impregnated with antibiotics. The results were expressed as percentage of resistant strains.

### Separation and analysis of bacterial capsule

#### A- Separation of the bacterial capsule

The method used for separation of the capsule from bacteria was preceded according to study of Humphery et al. (1974). The separation of capsule components was proceeded using TLC plates (cellulose F254, Merck, Germany). A set of plates were used for separation of sugars and another set were used for amino acids separation. After complete separation process whether sugars or amino acids, the appeared spots on plates were identified using authentic maps.

## RESULTS AND DISCUSSION

### Surveillance results regarding UTI

The total numbers of infected urine samples collected from the surveyed patients enrolled in this study were 300 samples. The fresh film examination was considered as a base line to differentiate between infected and noninfected patients.

Since age is considered to be one of the risk factors for UTI (Little et al., 2009), classification of patients in the current study were surveyed according to age group. Tables 1 and 2 show the incidence of UTI for patients surveyed by sex and age distribution. It is obvious that the most infected age group (21/70%) was found between 36-46 years old women, followed by age group 47-57, which give rise to (18/60%) for the same sex.

**Table 2.** Age group of females and number of infected samples.

Age group	3-13	14-24	25-35	36-46	47-57
Number of patient surveyed	30	30	30	30	30
Number of infected samples	3	7	16	21	18
Percentage of infected samples	10.0%	23.3%	53.3%	70%	60%

**Table 3.** Number and percentage of etiological agents of UTI.

Organism	Total isolates	Percentage of isolates
<i>Staphylococcus aureus</i>	6	7.69
<i>Staphylococcus saprophyticus</i>	6	7.69
<i>Enterococcus faecalis</i>	7	8.94
<i>Streptococcus</i> sp. (B)group	8	10.26
<i>Escherichia coli</i>	21	26.92
<i>Klebsiella pneumoniae</i>	9	11.54
<i>Proteus mirabilis</i>	4	5.13
<i>Pseudomonas aeruginosa</i>	3	3.85
<i>Acinetobacter</i> sp.	2	2.56
<i>Enterobacter</i> sp.	7	8.94
<i>Citrobacter freundii</i>	5	6.41

Meanwhile the younger males and females exhibited low UTI rate (2/ 6.6%) and (3/10%) respectively. On these bases; it is clearly established that older people were more susceptible to UTI than younger ones thereby indicating that senility plays an important role in UTI. Indeed; these results are in agreement with those obtained by Herruzo-Cabrera et al. (2001) and Ahmed (2003).

#### Identification and determination of bacteria as an etiological agent of UTI

In this study seventy-eight bacterial isolates were collected from infected samples. These isolates were tentatively identified to species level using international reference keys of Krieg (1984), Sneath (1986) and Holt et al. (1994). The isolates were found to belong to 10 genera: *Staphylococcus* (*aureus*, *saprophyticus*), *Enterococcus faecalis*, *Streptococcus* sp. (B) group, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp. and *Citrobacter freundii*.

Incidence of etiological agents sometimes reflects the sensitivity of UTI. The number and percentage of these etiological agents are recorded in Table 3. *E. coli* was the most predominant organism causing UTI in 21 isolates (26.92%), followed by *Klebsiella pneumonia* in 9 isolates (11.54%), and *Streptococcus* sp. (B) group in 8 isolates (10.26%).

Many investigations had been reported that *E. coli* and

*Klebsiella* spp. were the most frequent organisms isolated from UTIs (Goldman, 2001; Shao et al., 2003). Others reported that, two additional organisms including *Enterobacter* and *Pseudomonas* spp. may cause UTI (Taneja et al., 2004; Sood et al., 2008; Becerra et al., 2010). The results of the present study emphasized that different incidence of positively reported positive culture may be caused 'partly' by differences in procedures and microbiological methods rather than true differences in incidence.

It is generally acceptable that each microbial species has its unique biochemical and morphological properties which permit the development of such an organism in some environments but not in others. The distribution patterns of bacteria in relation to disease and/or patient, or the microenvironment in which they reside are determined by these traits. The prevalence of certain bacterial species and the non detection of others may be attributed to the specific culture media used in the isolation process.

Although *Staphylococcus aureus* were isolated from most cultures in 7.69%, none of the previous report (Das et al., 2006) has mentioned it as an etiological agent for UTI.

It was reported that urine specimens with more than one bacterial spp. were considered to be contaminated with skin, vaginal or periurethral flora. However, Leblebicioglu and Esen (2003) and Çetin et al. (2005) stated that polymicrobial infection is seen in more than 15% of urine samples; thereby indicating that polymicrobial bacteriuria is the rule, rather than the

**Table 4.** Growth of etiological agents of UTI in different concentrations of CaC<sub>2</sub>O<sub>4</sub> and CaCO<sub>3</sub> (results expressed as O.D. at 660 nm).

Microorganism	Control (normal urine)	CaC <sub>2</sub> O <sub>4</sub> (g/l)			CaCO <sub>3</sub> (g/l)		
		0.05	0.1	0.15	0.5	1.0	1.5
<i>Staphylococcus saprophyticus</i>	0.230	0.312	0.457	0.501	0.295	0.476	0.495
<i>Enterococcus faecalis</i>	0.111	0.172	0.376	0.529	0.151	0.217	0.254
<i>Streptococcus</i> sp. (B) group	0.115	0.221	0.416	0.791	0.236	0.382	0.400
<i>Escherichia coli</i>	0.107	0.100	0.076	0.065	0.100	0.094	0.083
<i>Klebsiella pneumoniae</i>	0.085	0.081	0.062	0.041	0.077	0.075	0.025
<i>Proteus mirabilis</i>	0.732	0.537	0.422	0.311	0.068	0.019	0.007
<i>Pseudomonas aeruginosa</i>	0.478	0.311	0.227	0.162	0.385	0.183	0.084
<i>Acinetobacter</i> sp.	0.130	0.118	0.087	0.044	0.087	0.062	0.056
<i>Enterobacter</i> sp.	0.090	0.142	0.356	0.418	0.119	0.476	0.512
<i>Citrobacter freundii</i>	0.250	0.491	0.672	0.692	0.280	0.305	0.370

**Table 5.** Growth of etiological agents of UTI in different concentrations of MgCl<sub>2</sub> and NaCl (results expressed as O.D. at 660 nm).

Microorganism	Control (normal urine)	MgCl <sub>2</sub> (g/l)			NaCl (g/l)		
		0.5	0.5	1.5	20	25	30
<i>Staphylococcus saprophyticus</i>	0.230	0.212	0.201	0.161	0.517	0.688	0.743
<i>Enterococcus faecalis</i>	0.111	0.194	0.277	0.350	0.375	0.421	0.431
<i>Streptococcus</i> sp.(B) group	0.115	0.292	0.334	0.385	0.410	0.474	0.514
<i>Escherichia coli</i>	0.107	0.105	0.081	0.061	0.987	1.175	1.225
<i>Klebsiella pneumoniae</i>	0.085	0.117	0.131	0.152	0.700	0.753	0.889
<i>Proteus mirabilis</i>	0.732	0.325	0.310	0.286	0.342	0.271	0.184
<i>Pseudomonas aeruginosa</i>	0.478	0.515	0.531	0.675	0.431	0.409	0.283
<i>Acinetobacter</i> sp.	0.130	0.082	0.034	0.022	0.120	0.082	0.045
<i>Enterobacter</i> sp.	0.090	0.381	0.575	0.619	0.070	0.050	0.023
<i>Citrobacter freundii</i>	0.250	0.530	0.562	0.570	0.231	0.187	0.144

exception. In this context; two to three different bacterial species are often recovered from aseptically collected urine specimens even when no symptoms of UTI are present (Garibaldi et al., 1982).

Similarly Kohlor – Ockmore and Feneley (1996), found that 177 urine samples contained mixed cultures while 51 samples were pure culture. In this work 218 urine samples were found to contain mixed cultures especially *E. coli* with *Pseudomonas aeruginosa* or *E. coli* with *Streptococcus* sp. (B) group while 82 samples were found to be pure cultures, that is one organism only isolated from each sample.

#### Chemical constituents of urine and their relation to bacterial growth

To live in an environment, bacteria must be able to endure all the abiotic stress characteristics of that environment. These stress conditions include different chemical constituents of urine. If an organism in a culture

is unable to survive and occasionally grows when exposed to these stresses, even under artificial conditions, it is unlikely to be an inhabitant of an environment in which those stresses occur. These stresses are easy to establish and to show the important factors in determining the absence of an organisms (Alexander, 1997). In this regards 10 bacterial genera were tested in order to investigate the effect of different chemical constituents of urine on their growth in order to figure out their adaptability powers.

Tables 4-7 show the effect of urine chemical constituents and their different concentrations (g/l) such as CaC<sub>2</sub>O<sub>4</sub> (0.05, 0.1 and 0.15); CaCO<sub>3</sub> (0.5, 1.0 and 1.5); MgCl<sub>2</sub> (0.5, 1.0 and 1.5); NaCl (20, 25 and 30); Citric acid (1.0, 1.5 and 2.0); glucose (1.0, 2.0 and 3.0); albumin (1.0, 1.5 and 2.0) and creatinine (2.5; 3.0 and 3.5).

Gram-negative bacteria showed a decrease in the OD of its growth as increasing the concentration of Ca-C<sub>2</sub>O<sub>4</sub> (except *Citrobacter* spp.) or CaCO<sub>3</sub> (except *Citrobacter*

**Table 6.** Growth of etiological agents of UTI in different concentrations of uric acid and glucose (results expressed as O.D. at 660 nm).

Microorganism	Control (normal urine)	Uric acid (g/l)			Glucose (g/l)		
		1	1.5	2.0	1	2	3
<i>Staphylococcus saprophyticus</i>	0.230	0.173	0.152	0.077	1.687	1.961	2.157
<i>Enterococcus faecalis</i>	0.111	0.150	0.100	0.073	0.776	1.624	1.865
<i>Streptococcus</i> sp.(B) group	0.115	0.094	0.560	0.000	1.032	1.245	1.725
<i>Escherichia coli</i>	0.107	0.321	0.478	0.891	1.306	1.387	1.422
<i>Klebsiella pneumoniae</i>	0.085	0.272	0.625	0.957	0.984	1.039	1.520
<i>Proteus mirabilis</i>	0.732	0.942	1.256	1.428	0.877	1.580	1.878
<i>Pseudomonas aeruginosa</i>	0.478	0.753	1.450	1.827	1.586	1.652	1.711
<i>Acinetobacter</i> sp.	0.130	0.090	0.042	0.000	0.753	1.720	1.813
<i>Enterobacter</i> sp.	0.090	0.125	0.672	0.811	1.680	2.053	2.417
<i>Citrobacter freundii</i>	0.250	0.725	0.922	1.440	1.391	1.465	1.682

**Table 7.** Growth of etiological agents of UTI in different concentrations of albumin and creatinine (results expressed as O.D. at 660 nm).

Microorganism	Control (normal urine)	Albumin (g/l)			Creatinine (g/l)		
		1.0	1.5	2.0	2.5	3.0	3.5
<i>Staphylococcus saprophyticus</i>	0.230	0.311	0.572	0.927	0.155	0.120	0.078
<i>Enterococcus faecalis</i>	0.111	0.486	0.915	1.281	0.446	0.531	0.611
<i>Streptococcus</i> sp.(B) group	0.115	0.744	1.211	1.947	0.576	0.600	0.650
<i>Escherichia coli</i>	0.107	0.617	1.429	2.111	0.045	0.033	0.010
<i>Klebsiella pneumoniae</i>	0.085	0.856	1.591	2.375	0.074	0.040	0.025
<i>Proteus mirabilis</i>	0.732	1.177	1.997	2.617	0.745	0.782	0.810
<i>Pseudomonas aeruginosa</i>	0.478	1.234	2.124	2.956	0.502	0.587	0.628
<i>Acinetobacter</i> sp.	0.130	0.755	1.045	1.476	0.075	0.030	0.015
<i>Enterobacter</i> sp.	0.090	0.688	1.211	2.305	0.343	0.392	0.485
<i>Citrobacter freundii</i>	0.250	1.732	2.139	3.275	0.294	0.460	0.535

spp. and *Enterobacter* spp.). Contrariwise Gram positive bacteria such as *Staphylococcus* spp., *Enterococcus* spp. and *Streptococcus* spp. showed an increase in the O.D of its growth as increasing the concentration of both  $\text{CaC}_2\text{O}_4$  and  $\text{CaCO}_3$ .

On the other hand the O.D of growth of Gram-positive bacteria was influenced by increasing concentration of citric acid, for example *Streptococcus* spp. recorded zero O.D at 2 g/l while *Staphylococcus saprophyticus* and *Enterococcus* recorded 0.077 and 0.073 O.D, respectively, at the same concentration.

As a matter of fact, glucose and albumin were the most favorable chemical constituents of urine for the growth of 10 tested genera. The growth is increased as the concentration increased. Similarly Moore et al. (2002) reported that the causative agents of UTI were propagated in the presence of hyperglycemia.

Interestingly, *Acinetobacter* spp. was sensitive to the presence of all chemical constituents of urine (except glucose and albumin); since their increase in concentration causes the growth to markedly decrease.

This indicates that *Acinetobacter* spp plays a minimal role in UTI.

*Enterococcus* spp., *Streptococcus* spp. and *Citrobacter* spp. were affected by increasing the concentration of NaCl and thereby recording a low growth rate.

### Bacterial resistance to antibiotics

It is widely known that bacteria are able to develop resistance to antibiotics. The development of antibiotic resistant is essentially an adaptive process since it reflects the ability of organisms to survive by adjusting themselves to adverse environmental conditions. The etiology of UTIs and the antibiotic susceptibility of urinary pathogens have been changing over the past years and recently resistance to antibiotics has become a major problem worldwide (Chomarat, 2000).

The prevalence of *Enterobacter* as a hospital-acquired pathogen has greatly increased since the introduction of extended-spectrum cephalosporins into clinical practice

**Table 8.** Resistance (%) of etiological agents of UTI to some antibiotics.

Microorganism	Am (%)	Amx (%)	TE (%)	S (%)	C (%)	CS (%)	NF (%)	UN (%)	Ofx (%)	AN (%)	Cip (%)	Nor (%)
<i>Staphylococcus saprophyticus</i> .	100	100	86.6	86.6	66.6	73.3	40	20	13.30	13.30	0.00	0.00
<i>Enterococcus</i> sp.	100	100	85.7	85.7	71.4	71.4	42.8	28.6	14.20	14.20	0.00	0.00
<i>Streptococcus</i> sp.	100	100	75	75	75	62.5	0	12.5	25	0.00	0.00	0.00
<i>Escherichia coli</i>	100	76.1	76.1	76.1	71.4	66.6	66.6	23.8	9.50	14.30	4.70	0.00
<i>Klebsiella pneumoniae</i>	100	100	83.3	83.3	83.3	83.3	66.6	33.3	16.60	16.60	0.00	0.00
<i>Proteus mirabilis</i>	100	75	75	75	75	50	50	0	25	0.00	0.00	0.00
<i>Pseudomonas aeruginosa</i>	100	100	100	100	100	100	100	33	33	33	0.00	0.00
<i>Acinetobacter</i> sp.	50	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00
<i>Enterobacter</i> sp.	85.7	71.4	71.4	71.4	57.1	85.7	42.8	14.2	28.60	14.20	0.00	0.00
<i>Citrobacter freundii</i>	80	60	40	20	40	20	0	0	0.00	0.00	0.00	0.00

Cip = Ciprofloxacin, S = Streptomycin, TE = Tetracycline, Amx = Amoxicillin, C = Chloramphenicol, CS = Colistinsulphate, Un = Uncisyn, Nor = Noracin, Am = Ampicillin, AN = Amkacin, NF = Nitrofurantoin, Ofx = Ofloxacin.

(Kaminska et al., 2002). Broad spectrum antimicrobial therapy promotes the acquisition of resistance to extended-spectrum cephalosporines, aminoglycosides and fluoroquinolones (Grandsen, 1997; Domin, 1998). "*Enterobacter*" in this study" showed resistance to aminoglycoside (71.4%) and ofloxacin (28.6%) as they contain quinoline constituents, while it was sensitive to other quinolones (ciprofloxacin and noracin) with a 100% rate (Table 8).

Akbar (2001) found ampicillin resistance strains of *E. coli* and *Pseudomonas* sensitive to gentamycin and ciprofloxacin. Also Lark et al. (2000) mentioned that *E. coli* could account for approximately 50% of hospital – acquired bacteriuria. It is often resistant to both sulfonamides and ampicillin. Conversely a study undertaken by Sotto et al. (2001) described that *E. coli* has a resistance rate of 20.3% for ampicillin and it is sensitive to gentamycin and ciprofloxacin.

However; in the present study *E. coli* showed an absolute resistance (100%) to ampicillin and an

absolute sensitivity to noracin (100%). Similarly *Proteus* showed the same ratio of resistance and sensitivity to both antibiotics, respectively. This is consistent with Chomarar (2000) results.

To the best of our knowledge we conclude that antibiotic resistance problem could arise from many reasons, including antibiotic use in animal feeds, inappropriate prescription of antibiotics, arbitrary administration of antibiotics, and poor infection control strategies.

Nonetheless *Acinetobacter* was very sensitive to all tested antibiotics, except ampicillin in which *Acinetobacter* shows 50% resistance rate. Therefore; this finding is consistent with previous conclusion that *Acinetobacter* has no important role in UTI.

#### Analysis of extra polymeric substance

A preliminary test of extra polymeric substance on sucrose agar medium revealed that some bacterial strains possess capsules or mucous. These

strains were *Staphylococcus saprophyticus*, *Streptococcus* sp. (B) group, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Tables 9 and 10). The presence of a capsule can be a major factor in determining the pathogenicity of these bacteria especially *K. pneumoniae*, *Proteus mirabilis* and *P. aeruginosa*. In some cases those bacteria have two variants; one that forms a capsule, representing the virulent pathogen, and a non capsulated one which is subjected to phagocytosis by blood cells involved in the immune response of the infected host organism. In this regard, Williams and Gibbons (1972) emphasized that the main function of SIgA antibody is to prevent bacterial adherence to the mucosal surface by coating bacterial cell wall.

On the other hand, phagocytes, blood cells involved in the immune response are properly unable or less able to engulf and digest those capsulated bacteria. Moreover, Van Demark and Batzing (1984) mentioned that because the organism can be sequestered within the capsular bag, adequate drug levels may not be present for

**Table 9.** Analysis of sugar in extrapolymeric substance of etiological agents of UTI.

Strain	D-glucose	D-galactose	D-fructose	D-mannose	Glucurano-lactone	L-rhamnose	Raffinose
<i>Staphylococcus saprophyticus</i>	+	+	-	-	-	-	-
<i>Streptococcus</i> sp. ( $\beta$ ) group	+	+	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	+	+	-	-	-	-	-
<i>Proteus mirabilis</i>	-	-	+	+	+	+	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-

**Table 10.** Analysis of amino acid in extrapolymeric substance of etiological agents of UTI.

Strain	Histidine	Methionine	Arginine	Glycine	Alanine	Valine	Isoleucine
<i>Staphylococcus saprophyticus</i>	-	-	+	-	+	-	+
<i>Streptococcus</i> sp. ( $\beta$ ) group	+	+	+	+	+	-	+
<i>Klebsiella pneumoniae</i>	-	-	+	-	+	-	+
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	-	-	+	-	-	+

sufficient time to kill the organism even after injection of antibiotics into the bag. As bacteria use their capsules as a mean of attachment and pathogenicity for UTI, the isolated bacterial capsules were subjected to structural analysis using this layer chromatography (TLC) technique. On the bases of the results shown in Tables 9 and 10 bacteria was classified as capsule carbohydrate – containing, or capsule amino acid associated with carbohydrate – containing bacteria. In this context; *Proteus mirabilis* was found to be capsule carbohydrate containing bacterium while *Pseudomonas aeruginosa* was found to be capsule amino acid containing. Meanwhile others strains were possesses capsule carbohydrate and amino acid containing. It is merit to mention that L-rhamnose, glucuranolactone, D-mannose and D-fructose were detected only in capsule of *Pseudomonas aeruginosa*. However; this result was in concomitant with Stanier et al (1970) study.

## Conclusion

*E. coli* and *Klebsiella* spp. were the most frequent organisms isolated from UTIs. However, glucose and albumin were the most favorable chemical constituents of urine for bacterial growth since their concentration increased the growth of bacteria increased. In addition increasing creatinine concentration increases the growth of *Enterococcus faecalis*, *Streptococcus* sp. ( $\beta$ ) group, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Citrobacter freundii*. Nevertheless, NaCl, and CaC<sub>2</sub>O<sub>4</sub> effect on bacterial growth were influenced by their concentration and the type of bacteria tested.

Noracin has an absolute inhibition effect on all tested bacteria. However, *Acinetobacter* shows 50% resistance

rate to Ampicillin while all other bacteria showed a resistance rate between 80.7 to 100 % to the same antibiotic.

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