

Rt  
00564  
c.1

INCIDENCE OF VIRULENCE  
FACTORS IN  
UROPATHOGENIC  
*ESCHERICHIA COLI* IN TWO  
LEBANESE HOSPITALS AND  
THE EVALUATION OF THE  
CPS ID3 CHROMOGENIC  
MEDIUM FOR URINE  
CULTURES

↓

by

**Majd J. Lahham**

A thesis submitted in partial  
fulfillment of the requirements for the  
degree of

Master of Science

Molecular Biology

Lebanese American University

2007

**Under the supervision of Dr. Sima Tokajian**



**Thesis approval Form (Annex III)**

Student Name: Majd Lahham I.D. #: 200401073

Thesis Title: Incidence of virulence factors in uropathogenic *Escherichia coli* in two Lebanese hospitals and the valuation of the cps ID3 chromogenic medium for urine cultures

Program : M.Sc in Molecular Biology  
Division/Dept : Natural Sciences

School : **School of Arts and Sciences**

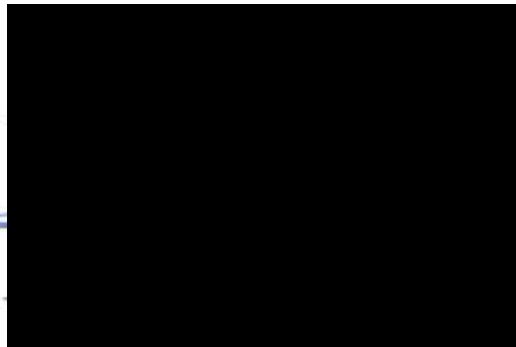
Approved by:

Thesis Advisor: Dr. Sima Tokajian

Member : Dr. Ziad Daoud

Member : Dr. Roy Khalaf

Date 17/9/07



I grant to the **Lebanese American University** the right to use this work, irrespective of any copyright, for the university's own purpose without cost the university or its students, agents and employees. I further agree that the university may reproduce and provide single copies of the work, in any format other than in or from microfilms, to be public for the cost of reproduction.

Majd J. Lahham

LEBANASE AMERICAN UNIVERSITY

ABSTRACT

PREVALENCE OF VIRULENCE FACTORS IN  
UROPATHOGENIC *ESCHERICHIA COLI* IN  
LEBANON AND THE EVALAUTION OF THE CPS  
ID3 CHROMOGENIC MEDIUM FOR URINE  
ANALYSIS

by Majd J. Lahham

Chairperson of the Supervisory Committee: Dr. Sima Tokajian  
Department of Natural science division

Urinary tract infections are among the most frequent encountered infections. The vast majority of urinary tract infections are caused by *Escherichia coli*. The invasion and colonization of the urinary tract by uropathogenic *E. coli* is mediated by its virulence factors. In this study, 160 uropathogenic *E. coli* strains were screened for pilus associated with pyelonephritis (*pap*), S fimbriae (*sfa*), afimbriae (*afa*), aerobactin (*aer*), hemolysin (*hly*), and cytotoxic necrotizing factor 1 (*cnf1*). The prevalence of these genes among the studied isolates were

33.6, 33.1, 8.8, 45.6, 32.5, and 18.1% for *pap*, *sfa*, *afa*, *aer*, *hly*, and *cnf*, respectively. Genes coding for adherence factors were present in 81% of the isolates confirming their importance role in tissue targeting and colonization of mucosal sites. *Cnf* gene was limited to hemolytic strains and was linked in most isolates to adhesins, while *sfa* was always associated with other virulence factors. Different combinations of genes were detected indicating possible synergism and/or the fact that the genes coding for these virulence factors are located on the bacterial chromosome. Detection of the virulence factors would be of a great value in understanding pathogenesis.

CPS ID3 chromogenic medium was compared to routine media for the isolation, and to API system for the identification of urinary tract pathogens. Positive predictive values were 100% for all species included in this study except *Enterococcus* spp. that was 66.7%. False negative results were observed in *E. coli* and *Enterococcus* spp. isolates (96% and 92.3% respectively) indicating that additional tests should be performed, particularly when white colonies are recovered. All organisms isolated from urine samples grew on this medium, but not all could be identified based on the morphological characteristics of their colonies. However, using this medium had the additional benefit of reducing the time needed for urine analysis when compared to the time needed with routine analysis. The most important advantage attributed to the use of CPS ID3 was the prompt identification of *E. coli* within 24 hours with no need to use additional biochemical tests. On the other hand, being expensive, having a short shelf life and not covering *Staphylococcus saprophyticus* were the only important drawbacks of this medium. CPS ID3 enables presumptive identification of a high proportion of urinary tract pathogens through the color of the colony, and even pathogens that were colorless were successfully isolated using this medium. The results of the present study suggest that use of chromogenic medium offers a time saving method for the reliable detection and presumptive identification of urinary tract pathogens. Moreover, it also revealed that the studying of bacterial virulence

determinants is an important aspect for the development of strategies to combat urinary tract infection.

## TABLE OF CONTENTS

Table of contents.....	i
List of figures .....	iii
List of tables.....	iv
Acknowledgement.....	v
Classification.....	vi
Glossary .....	vii
Chapter 1: Introduction.....	1
Chapter 2: Literature review .....	3
<b>2.1: Urinary Tract Infection</b> .....	3
<b>2.2: <i>Escherichia coli</i></b> .....	4
2.2.1: General overview	
2.2.2: <i>Escherichia coli</i> and urinary tract infection	
2.2.3: Molecular detection of <i>Escherichia coli</i>	
<b>2.3: Extraintestinal <i>Escherichia coli</i> virulence factors</b> .....	9
2.3.1: Extraintestinal <i>E. coli</i> virulence factors and pathogenic islands	
2.3.2: Adherence	
<b>2.3.2.1: P fimbriae: pilus associated with Pyelonephritis</b>	
<b>2.3.2.2 X Adhesion: Dr Family (Afimbriae) &amp; S fimbriae</b>	
2.3.3: Aerobactin	
2.3.4: Hemolysin	
2.3.5: Cytotoxic necrotizing factor	
<b>2.4: Detection of urinary tract pathogens:</b> .....	15
2.4.1: Conventional methods	
2.4.2: Chromogenic media	
2.4.3: Molecular techniques	
Chapter 3: Material and Methods:.....	19
<b>3.1: Sample collection:</b> .....	19
<b>3.2: Cultivation and enrichment of urine specimens on conventional and chromogenic media:</b> .....	19
<b>3.3: Reading of the chromogenic plates:</b> .....	19
<b>3.4: Identification using API system:</b> .....	20
<b>3.5: DNA extraction:</b> .....	20
<b>3.6: Detection of <i>E. coli</i> containing samples:</b> .....	20
<b>3.7: Detection of <i>hly</i> virulence gene:</b> .....	21
<b>3.8: Detection of <i>pap, sfa, cnf, aer, and afa</i> virulence genes:</b> ...	21
Chapter 4: Results.....	23
<b>4.1: Total number of each particular pathogen in the urine samples according to API system:</b> .....	23
<b>4.2: Results of CPS ID3 chromogenic medium:</b> .....	23
<b>4.3 Detection of <i>E. coli</i>-containing samples:</b> .....	30
<b>4.4 Detection of <i>hly</i> in <i>usp A</i> positive samples:</b> .....	31
<b>4.5: Detection of <i>pap, sfa, cnf, aer, and afa</i> genes using</b>	

<b><u>Multiplex PCR assay:</u></b> .....	32
<b><u>4.6: Number of virulence factors per sample:</u></b> .....	34
Chapter 5: Discussion.....	39
Chapter 6: conclusion.....	48
Bibliography.....	45



## LIST OF FIGURES

<i>Number</i>	<i>Page</i>
Fig 1: An early electron photomicrograph of a fimbriated <i>E. coli</i> cell.....	11
Fig 2: Overview of <i>pap</i> operon.....	12
Fig 3: Overview of <i>bly</i> operon.....	15
Fig 4: Number of samples that gave specific color.....	27
Fig 5: An example of urine sample that gave one species.....	28
Fig 6: An example of urine sample that gave two species.....	29
Fig 7: An example of urine sample that gave three species.....	30
Fig 8: PCR amplification of <i>usp A</i> gene.....	31
Fig 9: PCR amplification of <i>bly</i> gene.....	32
Fig 10: Multiplex PCR amplification of <i>pap</i> , <i>sfa</i> , <i>aer</i> , <i>cnf</i> , and <i>afa</i> genes.....	33
Fig 11: Number of samples VS number of virulence factors per sample.....	36
Fig 12: Number of samples that contain one virulence factor.....	36
Fig 13: Number of samples that contain two virulence factors.....	37
Fig 14: Number of samples that contain 4 virulence factors.....	37

## LIST OF TABLES

<i>Number</i>	<i>Page</i>
Table1: Pathogenic behaviors of the three main types of <i>Escherichia coli</i> .....	7
Table2: Etiology of uncomplicated versus complicated urinary tract infection.....	7
Table3: <i>Escherichia coli</i> CFT073: Genome contents.....	8
Table 4: Mannose resistant adhesins of uropathogenic <i>E. coli</i> .....	10
Table 5: Sequences of <i>pap</i> , <i>sfa</i> , <i>cnf</i> , <i>aer</i> , <i>afa</i> primers.....	22
Table 6: Number of pathogens recovered from positive samples and their percentage.....	24
Table 7: The sensitivity, specificity, and positive predictive value of pathogens recovered on CPS ID3 chromogenic medium in comparison with API system.....	26
Table 8: Number of samples positive for each particular gene.....	34

## ACKNOWLEDGMENTS

Thank GOD for the gifts of persistence, creativity and stubbornness.

I would like to express my deepest gratitude to Dr. Sima Tokajian, my supervisor, for being a source of inspiration and trust throughout the period extending from the proposal of the study till its closure.

My thanks are due to the members of the committee: Dr. Sima Tokajian, Dr. Ziad Daoud, and Dr. Roy Khalaf.

My sincere gratitude is as well dedicated to Dr. Fuad Hashwa, the dean of school of arts and sciences, and Dr. Costantine Daher, the chairperson of our department.

I am particularly grateful to Dr. James Johnson and his assistant Mr. Brian Johnston from the University of Minnesota for providing us with the *E. coli* reference strains, without whose generous contribution, this project would not have been possible.

I am grateful to Dr. Ziad Daoud and Dr. George Abdelnour for supplying us with the isolates needed to conduct this study.

I would like to thank Miss Fadia Karam and Miss Mirna Bejani, the technicians in the medical laboratory of St. George hospital, for the technical assistance.

I would like also to thanks Miss Helena bou farah for being there whenever we needed assistance.

Special thanks to my friend Mr. Bechara Meffarej for his assistance.

Last but not least, I would like to thank my family: Joseph, Antoinette, Nader, Nidal, Hanadi, and Rim for their patience and constant support.

## GLOSSARY

### Glossary:

UTI: urinary tract infection.

*Pap*: pilus associated with pyelonephritis

*Sfa*: S fimbriae

*Afa*: A fimbriae

*Cnf*: cytotoxic necrotizing factor

*Aer*: aerobactin

*Hly*: hemolysin

ExPEC: extrapathogenic *Escherichia coli*

UPEC: uropathogenic *Escherichia coli*

PAIs: pathogenicity islands

MRHA: mannose resistant hemagglutination

MSHA: mannose sensitive hemagglutination

PCR: polymerase chain reaction

## Chapter 1

### INTRODUCTION

Urinary tract infection (UTI) which is the presence of microorganisms in an otherwise sterile urinary system (Barnett and Stephens, 1997; Kunin, 1994; Zorc et al., 2005) is considered as a public health concern especially that wide range of gram-positive and gram-negative bacteria can be associated with this type of infection (Hanson et al., 1981). *Escherichia coli*, however, remains the most frequent isolated pathogen in UTI (Tobias et al., 2002; Stamm and Hootom, 1993; Gruneberg, 1984; Ronald, 2002), and were found to possess virulence factors that do not exist in commensal strains (Johnson, 1991; Donnenberg and Welch, 1996). Knowledge of these factors allow biologists to better understand the evolution and pathology of this organism (Usein et al., 2001), and consequently preventive therapies could be developed by targeting these virulence genes (Russo and Johnson, 2003).

Different chromogenic media can be used for the presumptive identification of UTIs' pathogens, since the enormous numbers of UTIs' cases all over the world along with the workload in medical labs acquire the necessity to design such media that can give rapid and cost effective results (Stevens, 1989; Emori and Gaynes, 1993).

#### **Objectives of the study:**

- 1- Evaluation of the chromogenic media CPS ID3 (BioMerieux) for the identification of pathogens associated with UTIs in comparison with API (BioMerieux).
- 2- Molecular detection of *E. coli* containing samples by the amplification of *uspA* specific gene.

- 3- Detection of virulence genes in *uspA* positive isolates including: *pap*, *sfa*, *afa*, *aer*, *bly*, and *cnf* using multiplex PCR.

LITERATURE REVIEW

2.1 Urinary Tract Infection:

Urinary tract infection is regarded as a group of infections of different clinical severity and prognosis (Hanson et al., 1980). The etiology of UTI has been established for several years (Ronald, 2002). The members of the family *Enterobacteriaceae* are considered as the major causes of the urinary tract infection (UTI) which is one of the most common infectious diseases in the world (Chomarat, 2000). Many statistical studies showed that UTI was the most frequent cause of nosocomial infections, after respiratory tract infections. UTI is the second reason responsible for hospitalization and is a common cause of infection in ambulatory patients in the US (Stamm and Hootom, 1993; Emori and Gaynes, 1993; Hootom and Stamm 1997). Two issues are raised when studying UTI as a public health concern: first a wide range of bacterial species can be associated with this type of infection (Hanson et al., 1981), second, as a result of antibiotic treatment; pathogens can become resistant to antimicrobial agents (Ronald, 2002). Both gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus spp.*) and gram-positive bacteria (*Staphylococcus spp* and *Streptococcus spp.*) can be associated with UTI. However, not all are pathogenic, with many isolates such as lactobacilli being contaminants from the surrounding area of the urethra and being non pathogenic (Barnett and Stephens, 1997).

Since a UTI definition does not specify neither the site of infection in the urinary tract nor the severity of the infection, two terms are used to diagnose such infections: pyelonephritis, infection of the upper urinary tract such as the renal pelvis or the renal parenchyma (Bergeron, 1995; Bass et al., 2003; Zorc et al, 2005), and cystitis, infection of the lower tract, such as the bladder or urethritis (Zorc et al., 2005). However, asymptomatic bacteruria is also a term



used in infections that are not associated with inflammatory symptoms (Hanson et al., 1981; Zorc et al., 2005). Moreover, uncomplicated UTI refers to infections in patients with anatomically-normal urinary tracts; while complicated UTI is used when patients have structurally or functionally abnormal urinary tracts (Bergeron, 1995; Bass et al., 2003).

Epidemiologic studies showed that all people are susceptible to UTI (Pezzlo, 1988). Host factors such as gender, age, diabetes, and catheterization were considered as important as bacterial pathogens in defining the severity of infection (Ronald, 2002). A large proportion of women, around 50%, experience UTI in their life, and 25% of this population experience another UTI within the following 6 months (Foxman, 1990), with sexual intercourse playing a major role in this incidence (Buckley et al., 1978). In infants younger than 90 days, the urinary tract is the major site exposed to infection, and according to Rushton (1997) the frequency of UTI at this age group is 1%. Abbot (1972) however, revealed that the incidence of UTI is higher in premature babies than full term infants. UTI additionally attacks elderly individuals without differentiation between males and females, which is due to functionally or structurally abnormal urinary tracts (Ackermann and Monroe, 1996). In general, over six million outpatients are infected by UTI every year (Kunin, 1996).

## 2.2 *Escherichia coli*

### 2.2.1 General overview of *E. coli*:

In 1885, Theodore Escherich isolated a new bacterial species when he was conducting studies on gastrointestinal bacterial contents. He named this species *bacterium coli commune* (Sussman, 1985). Between 1887 and 1902, scientists argued if this species is *Bacillus typhi*, which causes typhoid fever or not. Then many test including lactose fermentation showed that *bacterium coli commune* differs from *Bacillus typhi* (Ewing et al., 1981). In 1919, to honor

Theodore Escherich for his discovery, Castelani and Chalmers named this bacterium *Escherichia coli* (Bettelheim, 1991)

*Escherichia coli* –a member of the *Enterobacteriaceae* family- is gram-negative, a facultative anaerobic bacillus that constitutes a large part of human and warm-blooded animal flora. This species invades infants' intestines within few hours of life as a commensal organism (Drasar and Hill, 1974; Orskov and Orskov, 1985). *E. coli* is considered as an important member of the bacterial kingdom because of its ability to synthesize its own nutrients from single organic components and few minerals (Farmer et al., 1985; Schaechter and Neidhardt, 1987)

Although *E. coli* is a normal member of the human intestine, it can cause infections in the gastrointestinal tract and other organs in the body, including the nervous system and the urinary tracts. These infections can be attributed to the fact that this organism is an opportunistic pathogen and that it possesses virulence factors. Infection caused by *E. coli* which is considered as the leading cause of nosocomial infections (Emori and Gaynes, 1993) could be as a result of damage in the gastrointestinal barriers (Cavalieri et al., 1984; Orskov and Orskov, 1992; Nataro and Kaper, 1998; Tobias et al, 2002). Additionally, *E. coli* is a common cause of common community-onset bacteremia with the rates being higher compared to that established by *Staphylococcus aureus* or group A or group B streptococci (Jackson et al., 2005).

*E. coli* is classified as a commensal, intestinal and extraintestinal organism (Table 1) (Russo and Johnson, 2000), associated with enteric/diarrheal disease, urinary tract infection, and sepsis/meningitis (Nataro and Kaper, 1998). Extraintestinal *E. coli* infections are important causes of morbidity, mortality, and increased health-care costs (Russo and Johnson, 2003).

### 2.2.2 *Escherichia coli* and urinary tract infections:

Many studies showed that *E. coli* was the most common pathogen causing complicated and uncomplicated cases of urinary tract infections in immunocompromised and normal individuals (Table 2) (Gruneberg, 1984; Stamm and Hootom, 1993; Ronald, 2002; Tobias et al., 2002). Since the gastrointestinal tract is the habitat of this microorganism, the close proximity between urogenital sites and anus might indicate that the fecal flora was the source of urinary strains isolated in a UTI; supporting a so-called "fecal-urethral hypothesis" (Gruneberg, 1969). While Goetz et al. (1999) pointed out that extraintestinal *E. coli* was present in the normal flora without causing gastroenteritis.

*E. coli* isolated from patients with urinary tract infections have virulence factors that are not detected in commensal isolates. These factors allow the organism to invade and colonize the uroepithelial cells, evade the lymphatic system, and find its way into the blood stream (Johnson and Russo, 2002). Although 29 factors have been identified including adhesins, toxins, and proteases (Johnson and Stell, 2000), many pathogenic *E. coli* isolated from urinary tracts did not show any of the defined virulence factors. Indicating that there were many unidentified factors (Marrs et al., 2002). At the cellular level, virulence factors were classified into two categories: those found on the cell surface like adhesins, and those produced in the cell and exported to the site of action like hemolysin (Emody et al., 2003). Since such virulence factors were not unique to uropathogenic *E. coli*; meningitis-causing *E. coli* strains had these as well, this group of organisms was denoted as extraintestinal *Escherichia coli* (Johnson, 1991; Donnenberg and Welch, 1996; Johnson and Russo, 2002). Furthermore, Johnson et al. (2001) realized that extraintestinal *E. coli* belonged to the phylogenetic group B2 and to a lesser extent to the D group.

Because of its importance, Welch et al. (2002) sequenced the complete genome of an uropathogenic *E. coli* strain (CFT073) isolated from a woman

with acute Pyelonephritis and compared it with the nonpathogenic *E. coli* strain MG1655, and the enterohemorrhagic *E. coli* strain O157:H7 (EDL933) (Table 3). Being distinctly different from each other, the difference in disease potential between O157:H7 and CFT073 was attributed to the absence of toxin-encoding genes. The CFT073 genome carried genes that encoded potential fimbrial adhesins, autotransporters, iron-sequestration systems, and phase-switch recombinases.

Table 1: Pathogenic behavior of the three main types of *E. coli*: relative participation of each *E. coli* group in the particular clinical manifestation is indicated on a semiquantitative scale, from - (absent) to +++ (maximal) (Russo and Johnson, 2000)

<i>E. coli</i> pathotypic group	Asymptomatic intestinal colonization	Diarrhea	extraintestinal infection
Commensal	+++	-	+
Intestinal Pathogenic	-	+++	-
ExPec*	+	-	+++

\*ExPec: Extrapathogenic *Escherichia coli*

Table 2: Etiology of Uncomplicated Versus Complicated Urinary Tract Infection (UTI) (Ronald, 2002)

Uncomplicated UTI pathogens	Complicated UTI Pathogens
<i>Escherichia coli</i>	<i>Escherichia coli</i>
<i>Staphylococcus saprophyticus</i>	<i>Klebsiella</i> spp.
<i>Klebsiella</i> spp	<i>Enterobacter cloacae</i>
<i>Enterococcus faecalis</i>	<i>Serratia marcescens</i>
	<i>Proteus mirabilis</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Enterococcus faecalis</i>
	Group B streptococci

Table 3: *Escherichia coli* CFT073: genome contents: (Welch et al., 2002)

Genome length	5,231,428 bp
Plasmids	None
Protein-coding genes	5,533
rRNAs	22 genes in 7 operons
tRNAs	89 [1 pseudo, 1 novel (Arg),
1 extratandem (Arg), 3 phage-encoded]	
Miscellaneous RNAs annotated	11
G -C%	50.47%
Backbone genes	3,190
Island genes	1,827
Backbone regions	359 (3,925,047 bp; 75.02%)
CFT073-specific islands	247 (1,306,391 bp; 24.98%)
Cryptic prophage	5

### 2.2.3: Molecular detection of *Escherichia coli*:

*Escherichia coli* species harbor a unique protein known as the universal stress protein  $\Lambda$  (*uspA*), whose exact physiological role is still unknown. Since its intracellular level sharply increases under-stress conditions such as exposure to oxidants, heat, and antibodies, it could be essential for the survival of the organism when cellular growth arrests. Starvation due to the lack of nitrogen, carbon, sulfate, phosphate and amino acid, increased intracellular *uspA* level (Nyström and Neidhart, 1994; Gustavsson et al., 2002; Kvint et al., 2003). Mutation in *uspA* gene led to premature death during stasis (Nyström and Neidhart, 1993). On the other hand, over-expression of this protein kept *E. coli* in a growth-arrested stage (Nyström and Neidhart, 1996). The gene (434 bp) that encodes for *uspA* protein was found to be located on the *E. coli* chromosome (Nyström and Neidhart, 1992), and the protein was cytoplasmic with a molecular weight of 13.5 KDa (Freestone et al., 1997).

Chen and Griffiths (1998) took advantage of *uspA* specificity and developed a polymerase chain reaction to differentiate *E. coli* from other gram-negative bacteria using primers complementary to the flanking region of *uspA* gene.

## **2.3 Extraintestinal *Escherichia coli* virulence factors:**

### 2.3.1 Extraintestinal *E. coli* virulence factors and Pathogenic Islands:

Hacker et al. (1990) found out that the deletion of the gene coding for the fimbriae and hemolysin in two extraintestinal *E. coli* strains resulted in loss of the other, thus linkage between these two genes was deduced and termed “Pathogenicity Island”. Several studies followed showing that deletion of pathogenicity islands yielded non pathogenic *E. coli* strains (Blum et al., 1994), indicating that these islands were clusters of genes that conferred virulence to nonpathogenic strains (Groisman and Ochman, 1996). Their mobility and their high G+C content differentiated these islands from their surrounding DNA, therefore, their horizontal transfer provides nonpathogenic *E. coli* with pathogenic properties (Hacker and Kaper, 2000; Oelschlaeger et al., 2002).

### 2.3.2 Adherence:

Gram-negative bacilli have fimbriae that contain lectin-like domains. These domains have the ability to recognize receptors found on epitopes in cell surface glycoproteins or glycolipids (Hedlund et al., 2001). Adherence of extraintestinal *E. coli* to uroepithelial cells was considered the first step in the pathogenesis (Langermann et al., 1997; Beachey, 1981). Historically, Duguid et al. (1967) illustrated that the attachment of *E. coli* to erythrocytes was due to thin fiber-like appendages, known as fimbriae (Figure 1). Most strains of *E. coli*, isolated from urinary tracts, have such fimbriae needed for the attachment to the uroepithelial cells (Plos et al., 1995). Proteins involved in adherence were called adhesins and found on the bacterial surface. Those structures have the ability to bind glycoconjugate receptors on the epithelial cells and may or may not be associated with fimbriae (Lefflier and Svanborg Eden, 1980; Le Bouguenec, 1992). Thus, the globoseries of glycolipid receptors of certain human cells predispose them to infection. Thereby, cells that do not

have these receptors can avoid infection (Kallenius and Mollby, 1979). At the molecular level, attachment of *E. coli* occurred as, the Gal $\alpha$ 1 $\rightarrow$ 4 $\beta$ Gal, found on the surface of certain cells like group P1 and P2 erythrocytes, was recognized by adhesin proteins (Lefflier and Svanborg Eden, 1980; Kallenius et al., 1980). Interestingly, these receptors have the ability to bind mannose residues, competing with some fimbriae types like type 1 pili. This type of agglutination, however, was designated as mannose sensitive hemagglutination (MSHA). This was contrary to mannose resistant hemagglutination (MRHA), where no competition occurs between other fimbriae types and mannose (Chabanon et al., 1979; Wold et al, 1988). In turn, MRHA was divided into two groups according to receptor specificity: (i) those recognizing P blood serotype (named P fimbriae) and (ii) others (named X fimbriae or X adhesins) such as S, M, F, Dr (Table 4) (Johnson, 1991)

Table 4: Mannose resistant adhesins of uropathogenic *E. coli* (Johnson, 1991)

Adhesin type UTI'	Synonym(s)	Fimbriae present	Receptor	Role in
<u>P adhesion</u>				
P	Gal-Gal, <i>Pap</i>	+	Gal (1-4)Gal	+++
F	Prs, <i>Pap-2</i>	+	Forssman antigen	++
ONAP		+	Gal(al-4)Gal	+/-
<u>X adhesion</u>				
<b>Dr related</b>				
Dr	075-X	-	Dr blood group antigen	++
<i>AFA-1, AFA-III</i>		-	Dr blood group antigen	+
S		+	NeuNAc(a2-3)Gal	+/-
M		-	M blood group antigen	+/-
G		+	GlcNAc	+/-
<i>NFA-1, NFA-2</i>		-	?	+/-



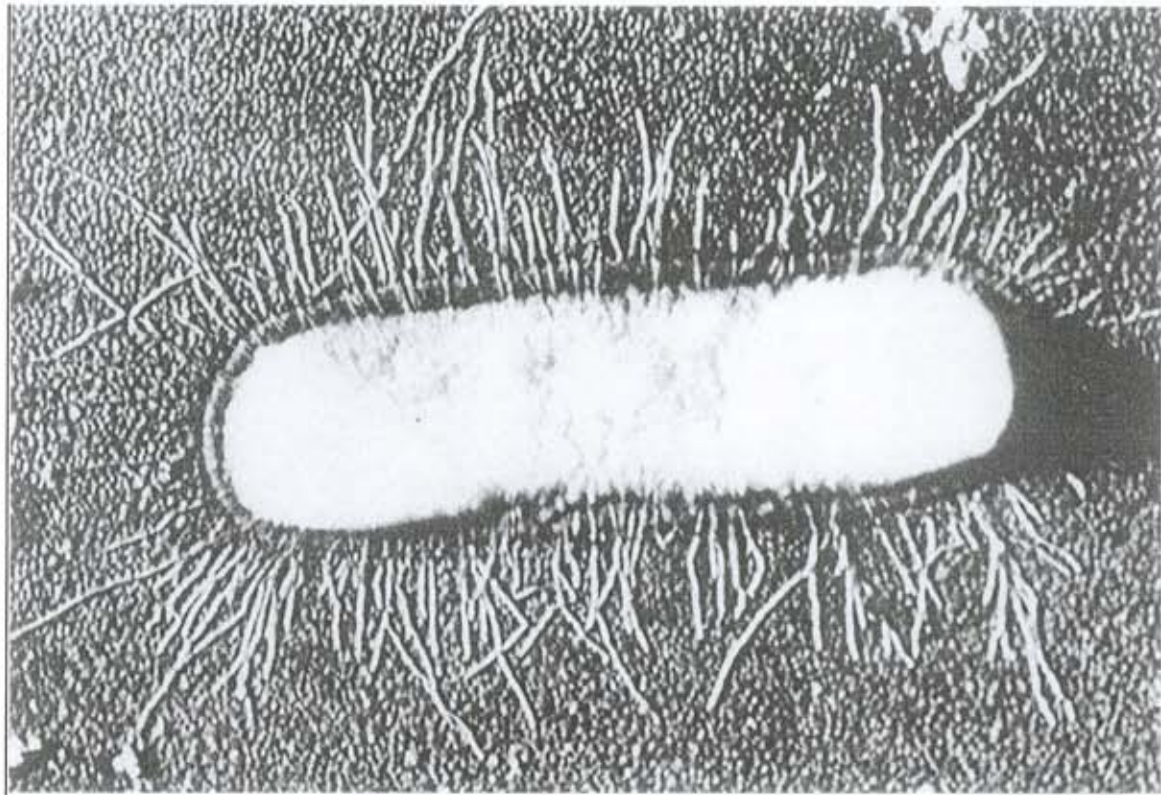


Figure1. An early electron photomicrograph of a fimbriated *E. coli* cell, showing protoplast, cell wall, about 200 fimbriae, and no flagella.(Duguid et al., 1955)

### 2.3.2.1: P fimbriae: pilus associated with pyelonephritis: (*pap*)

P fimbriae belong to a mannose-resistant hemagglutination (MRHA) category. The P abbreviation comes from the human blood group antigen P that contains Gal $\alpha$ 1 $\rightarrow$ 4 $\beta$ Gal moiety. This moiety was not only found on P blood group but also on certain mammalian cells like uroepithelial cells as carbohydrate components of glycosphingolipids (Marcus et al., 1981). Thus, cell lines expressing the Gal $\alpha$ 1 $\rightarrow$ 4 $\beta$ Gal moiety, like P blood group or uroepithelial cells, were predisposed to infection by P fimbriae-containing strains (De Man et al., 1987; Kallenius et al., 1982). Pilus associated with pyelonephritis is encoded by a cluster of genes (operon) termed *pap* (Figure 2), which consisted of *pap A*, *pap F*, *pap G*, *pap E* genes (Baga et al., 1984). *Pap* fimbriae is made of approximately  $10^3$  polymerized subunits with the major



subunit being *pap A*. *Pap E*, *pap F*, and *pap G* are minor subunits that existed in minute amount at the fimbrial tip (Lindberg et al., 1987). Although *pap A* is the major subunit of P fimbriae, it does not play any role in adherence. The ability to adhere was mainly correlated to *pap G*, which formed a complex with *pap F*. *Pap F* in turn was bound to *pap A* via *pap E* (Lund et al., 1987; Hoschutzky, 1989)

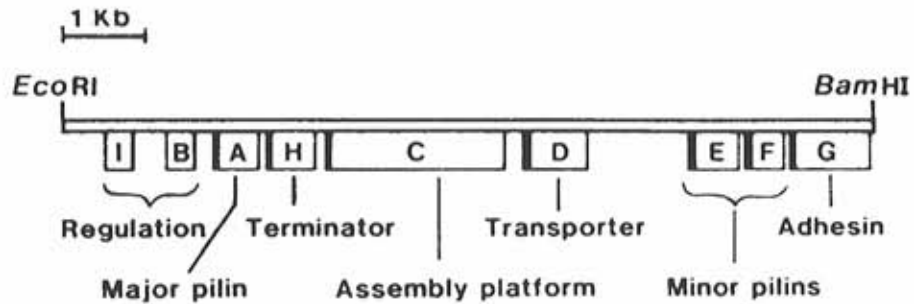


Figure 2: overview of *pap* operon. *PapA* is the major (structural) fimbrial subunit. *PapH* seems both to terminate fimbrial growth and to anchor the fully grown fimbriae to the cell surface. *PapC* is located in the outer membrane and forms the assembly platform for fimbrial growth. *PapD* is a periplasmic protein which forms complexes with the fimbrial subunits before assembly. *PapE*, *PapF*, and *PapG* are minor fimbrial components. *PapG* is the adhesin molecule conferring Gal(otl-4)Gal binding specificity, *PapF* complexes with *PapG*, and *PapE* attaches the *PapF*-*PapG* complex to fimbriae (Lindberg et al., 1987).

### 2.3.2.2: X Adhesion: Dr Family (Afimbriae) & S fimbriae

X adhesion belongs to the MRHA category, like P fimbriae. Two adhesins belong to this group: Dr Family (Afimbriae) and S fimbriae.

Dr family adhesins were first described by Väisänen-Rhen and Labigne-Roussel et al. (1984) who revealed that *E. coli* O75 had novel adhesins of unknown receptor specificity, and designated is as O75X. Following that, three separate groups cloned novel adhesin-encoding genes, later was found to have similar characteristics (Dr, afimbrial adhesins (*afa*), and F1845) (Nowicki et al., 1987; Labigne-Roussel and Falkow, 1988; Bilge et al., 1989). Nowicki et al. (1990) proved that those three adhesins and O75 X adhesin bind Dr blood group antigen. These types of adhesins structurally differ from other fimbriae in their appearance which appeared as fine mesh (Arthur et al., 1989). Dr adhesins were mostly isolated from infected pregnant women and children rather than adult men and non-pregnant women (Labigne-Roussel and Falkow, 1988). Dr Family protein was encoded by cluster of five genes. One of these genes was responsible for structural hemagglutinin (Labigne-Roussel et al., 1985).

S fimbriae were also MRHA and named S (*sfa*) because of its ability to bind to terminal sialyl-galactoside moiety (Parkkinen et al., 1983). Sialyl-galactoside receptors were found on uroepithelial cells of renal vascular endothelium, on the proximal and distal tubules, collecting ducts, and glomerulus (Korhonen et al., 1986). Ott et al. (1986) revealed that S-fimbriated *E. coli* strains were more commonly isolated from patients with bacteremia and meningitis than UTI patients, although genes that code for S fimbriae were first cloned from the uropathogenic *E. coli* strain O6:K15 (Korhonen et al., 1986).

### 2.3.3 Aerobactin:

Aerobactin (*aer*) is responsible for the extraction of  $Fe^{3+}$  from the iron-binding protein of the host which is then transferred through an outer membrane receptor (Carbonetti and Williams, 1984). One of the host mechanisms to fight infection is by shifting iron available in the plasma pool to intracellular storage in the form of iron-binding protein (Weinberg, 1978). *E. coli* strains having Aerobactin have a growth advantage over those without

aerobactin in the presence of low iron concentration (Braun et al., 1984; Montgomerie, 1984). Iron taken by aerobactin is used in *E. coli* for DNA synthesis, electron transport, oxygen transport and storage, and metabolism of peroxides (Bagg and Neilands, 1987, b). Aerobactin is composed of two lysine and one citrate molecules (Neilands et al., 1985). It is encoded by an operon with five genes. One of those genes is responsible for the outer membrane protein receptor while the others are involved in synthesizing the aerobactin (Gross et al., 1985). Aerobactin operon is controlled by the concentration of iron in *E. coli*, when the iron concentration is high, transcription of the repressor gene proceeds. Conversely, when iron concentrations are low, transcription of the repressor gene is blocked (Bagg and Neilands, 1987, a).

#### 2.3.4: Hemolysin:

Hemolysin protein (*hly*) lyses human erythrocyte to obtain calcium from the host cell (Short and Kurtz, 1971). Hemolysin inserts into the lipid membranes of erythrocytes and creates space channels, which are permeable mainly to calcium and other cations (Menestrina et al., 1987). Besides its ability to harm erythrocytes, hemolysin can induce tissue injury and inflammation in other host cells (Cavalieri et al., 1984). Furthermore, hemolysin induces renal tubular cells to liberate hydrogen peroxide and superoxide anion (Keane et al., 1987). Four-gene operon is responsible for producing hemolysin protein (Figure 3) (Goebel and Hedgpeth, 1982). This operon is found on the chromosome of *E. coli* strains isolated from humans, while it is located on a plasmid in *E. coli* strains isolated from animals (Welch et al., 1983). Hemolysin A is the main player in hemolytic activity and it does not need cleavage of a signal peptide or cellular lyses to be secreted. It needs activation by hemolysin C, and secretion is mediated by hemolysin B and D (Springer and Goebel, 1980).

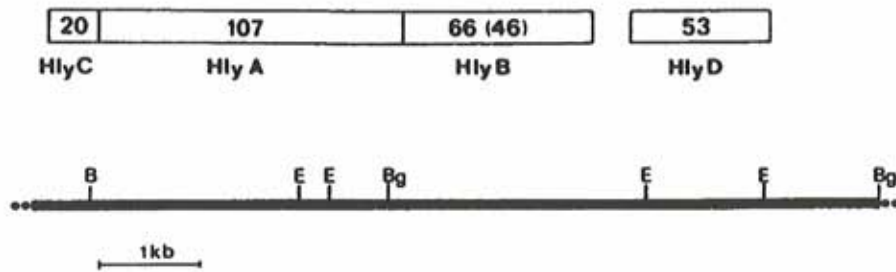


Fig 3: Overview of *hly* operon which consisted of *hly A*, *hly B*, *hly C*, and *hly D* (Mackman et al., 1986)

Cytotoxic necrotizing factor (*cnf*):

The gene encoding for this protein is found in isolates having the hemolysin gene (Caprioli et al., 1987). This 110 KD protein induce dermonecrosis in rabbits, and multinucleation in the ovary of Chinese hamster through changes in cytoskeletal actin and tubulin (Caprioli et al., 1983; Fiorentini et al., 1988). Furthermore, *cnf* promotes apoptosis in uroepithelial cells in the human bladder (Mills et al., 2000).

## 2.4: Detection of urinary tract pathogens:

### 2.4.1: Conventional methods:

Pezzlo (1988) introduced many rapid methods to detect UTI and classified them into six categories: microscopic, enzymatic, colorimetric, bioluminescence, and photometric methods. Culturing urine on agar media to enrich pathogenic bacteria is still the gold standard for UTI diagnosis (Zorc et al., 2005). Two kinds of media are routinely used: a non-selective medium, blood agar, and selective medium like MacConkey agar or Cysteine lactose electrolyte deficient medium (CLED) for gram-negative bacteria. MacConkey and CLED have lactose sugar to detect lactose fermenting

pathogens and to inhibit the swarming of *Proteus* spp. (Samra et al., 1998; Graham and Galloway, 2001). Urine culture is considered positive when it yields more than  $10^5$ cfu/ml (Williams and Schaeffer) while  $10^4$ cfu/ml is considered positive in young children (Zorc et al., 2005). Urine should be cultured before antibiotic treatment because antimicrobial agents reduce the bacterial count (Ramkrishnan and Scheid, 2005). However, occasionally, 100 cfu/ml is considered a positive value in the presence of significant infection symptoms (Kunin et al., 1993). Once the bacteria are cultured and isolated, chemical tests can be applied to determine the identity of the pathogens. One of the systems used in the routine work is the API, which is a plastic strip holding microtubes containing dehydrated substrates that react with bacterial enzymes. After 18-24 hours of incubation at 37 °C, the color changes on the strip are used to identify the strain (Shayegani et al., 1978). Evaluation studies showed that API system is a valuable method to routinely identify bacterial pathogens (Bulter et al., 1975; Shayegani et al., 1978). Moreover, simple tests can be additionally used to differentiate between different genera. For example, catalase test is positive for *Staphylococcus* spp. but not for *Streptococcus* spp. while the oxidase test is positive for *Pseudomonas* spp. but not for member of the *Enterobacteriaceae* family (Graham and Galloway, 2001)

#### 2.4.2: Chromogenic media:

Rapid identification improves the ability to specify the antimicrobial treatment and reduces the incidence of antibiotic resistance (Slak, 1984; Alon et al., 1987). To help diagnose UTI, many chromogenic media develop specific colors for each bacterial species based on the reaction between bacterial specific enzyme and substrates added to the media (Scarparo et al., 2002; Samra et al., 2002). Such as the chromogenic medium CPS ID3

(BioMerieux) used in this study, which gives burgundy color for *E. coli* due to the production of  $\beta$ -glucuronidase enzyme (Edberg and Kontnick, 1986).

#### 2.4.3: Molecular techniques:

In the last few decades, molecular techniques have played a major role in many fields, especially in the molecular diagnosis of microorganisms. Many techniques have been used in studying bacteria including restriction fragment length polymorphism (RFLP) and the polymerase chain reaction (PCR). Those techniques facilitated the conductance of large-scale epidemiological studies and the construction of gene banks and protein banks (Tang et al., 1997). Molecular techniques have additionally helped in studying the structure and species composition of microbial communities (Muyzer et al, 1993). The PCR reaction is based on the capability of DNA polymerase isolated from thermophilic bacteria to synthesize many copies of DNA using DNA template, oligonucleotide primers, and dNTPs under specific thermal conditions (Tang et al., 1997). PCR is a very sensitive powerful method that can be applied in many fields like forensic, environmental, and genetic sciences (Tsai and Olson, 1992). The advantage of using PCR in the field of microbiology lies in the ability to detect bacteria directly from the specimen without having to culture them first, with results that can be obtained within a few hours of sample collection (Tang et al., 1997; Hinata et al., 2004). Furthermore, the high PCR's sensitivity allow for the detection of any bacterial gene even when the pathogen is present at a very low concentration or is mixed with many species as is the case with intestinal flora (Lo, 1994). When the target of the study is more than one gene, a multiplex PCR can be used where several genes can be amplified simultaneously using different primers. This method decreases the time and the cost of reaction without reducing the efficiency of the results. However, to get best results, primers used in multiplex PCR should have the same annealing temperature, and the replicons need to have different sizes to

easily separate the bands by gel electrophoresis (Henegariu et al., 1997; Singh, 2006)

## MATERIAL AND METHODS

### **3.1: Sample collection and reference strains:**

Three hundred Mid-stream urine samples were thankfully collected By Dr. Ziad Daoud from St. George Hospital (n=232) and Dr. George Abdelnour from Notre Dame de Secoures hospital (n=68), from in and out-hospital patients. Samples with more than 100 white blood cells per mm<sup>3</sup> were examined for the presence of bacteria using direct microscopic examination. Reference strains PM9, 2H16, and 2H25 were kindly provided by Dr. James R. Johnson (University of Minnesota).

### **3.2: Cultivation and enrichment of urine samples on conventional and chromogenic media:**

Using 1 µl calibrated sterile loop (Himedia), urine was cultivated on CPS ID3 (Biomerieux), blood base agar (Biomerieux) with 5% Human blood, and MacConkey agar (Oxoid). Plates were incubated at 37°C overnight. After 24 hours of incubation, four criteria were examined: the color of the colonies on CPS ID3 plates, growth and hemolytic activity on blood agar plates, growth of gram-negative bacteria and lactose fermenting bacteria on MacConkey agar plates.

### **3.3: Reading of the chromogenic plates:**

Chromogenic plates were interpreted according to the manufacturer's instructions. Pink or burgundy colonies being produced in the presence of the β-glucoronidase enzyme produced by *E. coli*, light to dark Brown colonies due to deaminase of *Proteeae* tribe, turquoise colonies due to β-glucosidase of Enterococci and green to brownish green colonies in KESC group (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* ). However, metallic grey were specific for *Pseudomonas aeruginosa* colonies, while Violet colonies were specific for *Streptococcus agalactiae*.



### **3.4: Identification using API system:**

Well isolated bacterial colonies were suspended in 0.85% saline. The suspension was used to inoculate the API 20E strips according to manufacturer's instruction. After 24 hours of incubation at 37° C the strips were read by using the software provided by BioMerieux (APILAB PLUS Version 3.3.3). Staphylococci were identified using API Staph strips, while Streptococci using API Strep. The bacterial suspension for Streptococci prepared from organism cultivated on blood agar. The suspension was prepared according to the manufacturer's instructions. Strips were inoculated and incubated at 37° C. Results of API Strep were read after 4 hours and 24 hours according to the recommendation of the manufacturer. By using the software provided by BioMerieux (APILAB PLUS Version 3.3.3).

### **3.5: DNA extraction:**

A loopful of each strain was suspended in 200 µl DNase free water, boiled with mixing for 15 minutes and then centrifuged at 13000 xg for 3 min at 4C.

### **3.6: Detection of *E. coli* containing samples:**

*E. coli* was detected by the PCR-based amplification of the *uspA* gene (Chen and Griffiths, 1998). PCR was done in a total volume of 50 µl containing 5 µl of the DNA template, 0.1 mM forward (5'- CCG ATA CGC TGC CAA TCA gT -3') and reverse primers (5'- ACG CAG ACC GTA GGC CAG AT -3'), 0.2 mM of each deoxynucleotide triphosphate and 1X PCR buffer (50 mM KCl, 10 mM Tris HCl); 3mM MgCl<sub>2</sub>, and 1U Taq polymerase (Fermantas®). The PCR was performed with a Perkin-Elmer Gene-Amp 9700 thermal cycler under the following conditions: heating at 94°C for 5 min. 30 cycles of: 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min. Final extension 72° C for 5 min. DNA sample extracted from *Klebsiella pneumoniae*

isolate was used as a PCR negative control. The PCR amplicons (884 bp) were visualized using a UV light box after electrophoreses on 1% agarose gel containing 0.5 mg/ml ethidium bromide.

### **3.7: Detection of *hly* virulence gene:**

PCR was performed in a total volume of 50  $\mu$ l containing the following components: 5  $\mu$ l of DNA template; 30 mM of *hly* forward (5'-AACAAAGGATAAGCACTGTTCTGGCT-3') and reverse (5'-ACCATATAAGCGGTCATTCCTCGTCA-3') primers; 1mM of deoxinucleotide triphosphate; 1x buffer solution, (50 mM KCl, 10 mM Tris HCl); 3 mM MgCl<sub>2</sub>, 1.5 unit of Taq DNA polymerase (Fermantas). The PCR was performed with a Perkin-Elmer Gene-Amp 9700 thermal cycler under the following conditions: heating at 94°C for 2min; followed by 30 cycles of denaturation at 94° C for 1 min, annealing at 60°C for 1 min, and extension at 72° C for 1.30 min; and the a final extension for 5 min at 72°C. *E. coli* reference strain (2h16) was used as a positive control. Sample containing water instead of DNA was used as a negative control. The PCR amplicons (1177 bp) were visualized using a UV light box after electrophoreses on 1% agarose gel containing 0.5 mg/ml ethidium bromide.

### **3.8: Detection of *pap*, *sfa*, *cnf*, *aer*, *afa* virulence genes:**

Multiplex PCR was performed in a final volume of 50  $\mu$ l containing the following: 5  $\mu$ l of DNA template, 10 mM each of *pap* and *sfa* forward and reverse primers, 12 mM of *cnf*, *aer*, and *afa* forward and reverse primers (Table 5), 1 mM of deoxinucleotide triphosphate mixture, 1X buffer solution, 3mM MgCl<sub>2</sub>; 1.5 unit of Taq DNA polymerase (Fermantas). The PCR was performed with a Perkin-Elmer Gene-Amp 9700 thermal cycler under the specific the following conditions: heating at 94°C for 2 min; 30 cycles of denaturation at 94° C for 1 min, annealing at 60°C for 1 min, and extension at 72° C for 1 min and a final extension for 5 min at 72°C. *E. coli*

reference strain (PM9, 2h16, and 2h25) were used as positive controls. Sample containing water instead of DNA was used as a negative control. The PCR amplicons (328, 410, 498, 602, 750 bp respectively) were visualized using a UV light box after electrophoreses on 2.5% agarose gel containing 0.5 mg/ml ethidium bromide.

Table 5: sequences of *pap*, *sfa cnf*, *aer*, *afa*, primers, adapted from Yamamoto et al. (1995).

<i>Pap</i> forward primer	5'-gACGGCTGTACTGCAGGGTGTGGCG-3'
<i>Pap</i> reverse primer	5'-ATATCCTTTCTGCAGGGATGCAATA-3'
<i>Sfa</i> forward primer	5'-CTCCGGA <sub>g</sub> AACTGGGTGCATCTTAC-3'
<i>Sfa</i> reverse primer	5'-CGGAGGAGTAATTACAAACCTGGCA-3'
<i>Cnf</i> forward primer	5'-AAGATGGAGTTTCCTATGCAGGAG-3'
<i>Cnf</i> reverse primer	5'-CATTTCAGAGTCCTGCCCTCATTATT-3'
<i>Aer</i> forward primer	5'-TACCGGATTGTCATATGCAGACCGT-3'
<i>Aer</i> reverse primer	5'-AATATCTTCCTCCAGTCCGGAGAAG-3'
<i>Afa</i> forward primer	5'-GCTGGGCAGCAA <sub>g</sub> ACTGATAACTCTC-3'
<i>Afa</i> reverse primer	5'-CATCAAGCTGTTTGTTCGTCCGCCG-3'

## RESULTS

### **4.1: Total number of each pathogen in the urine samples according to API system:**

A total of 300 samples were collected from in and outpatients were donated to support this study by St. George hospital and Notre Dame de Secoure hospital, over a period of fifteen months from October 2006 to December 2007. Growth was detected in 241 samples, while 59 samples yielded no growth. Among all urinary tract pathogens, *Escherichia coli* was the most common, representing 68.85% of the studied population ( $n = 179$ ), whereas *Citrobacter* and *Serratia* had the lowest percentage being 0.38% each (Table 6)

### **4.2: Results of CPS ID3 Chromogenic media:**

The cost of the CPS ID3 was 2\$ for each plate and the shelf life was just 2 months. Colonies on chromogenic media will have a different color depending on reaction between enzymes produced by the cultivated organism and substrates available in the medium. The positive predictive value is calculated based on the specificity of the medium (Table 7). The positive predictive value of Chromogenic media was 100% for all species except for *Enterococci* (66.7%) as compared to the API system. (Figures 4, 5, 6, 7,). Sensitivity (the probability that the new test will be positive when the patient truly is infected), specificity (the probability that the new test will be negative when the patient is not infected, and the positive predictive value (true and false negative results/ true and false positive results) were calculated for each species.

Table 6: Number of pathogens recovered and Identified from positive samples and their percentage using the API system.

<u>Species</u>	<u>Number</u>	<u>Percentage</u>
<i>Escherichia coli</i>	179	68.85%
<i>Klebsiella pneumonia</i>	21	8.08%
<i>Klebsiella oxytoca</i>	2	0.77%
<i>Pseudomonas aeruginosa</i>	4	1.52%
<i>Proteus mirabilis</i>	10	3.85%
<i>Proteus vulgaris</i>	2	0.77%
<i>Enterobacter aerogenes</i>	2	0.77%
<i>Citrobacter spp.</i>	1	0.38%
<i>Serratia spp.</i>	1	0.38%
<i>Staphylococcus epidermidis</i>	5	1.92%
<i>Staphylococcus aureus</i>	3	1.15%
<i>Staphylococcus hemolyticus</i>	2	0.77%
<i>Staphylococcus auricularis</i>	2	0.77%
<i>Staphylococcus sciuri</i>	2	0.77%
<i>Staphylococcus saprophyticus</i>	1	0.38%

<u>Species</u>	<u>Number</u>	<u>Percentage</u>
<i>Staphylococcus capitis</i>	1	0.38%
<i>Staphylococcus xylosum</i>	1	0.38%
<i>Streptococcus faecalis</i>	12	4.61%
<i>Streptococcus agalactiae</i>	6	2.31%
<i>Streptococcus faecium</i>	1	0.38%
<i>Streptococcus porcinus</i>	1	0.38%
<i>Streptococcus cloacae</i>	1	0.38%

Table 7: : the sensitivity, specificity and positive predictive value of pathogens recovered on CPS ID3 chromogenic media in comparison with API system which is the reference method in this study. All KESC group, *Proteus* spp., and *streptococcus agalactia* isolates gave the expected colony color. Of 179 *E. coli* recovered in this study, 7 strains gave white colonies.

	Total number of isolates according to API system	Burgundy	Green	Brown	Turquoise	Metallic	Violet	White	Sensitivity	Specificity	Positive predictive value (PPV)
<i>Escherichia coli</i>	179	172	-	-	-	-	-	7	96%	100%	100%
KESC group	27	-	27	-	-	-	-	-	100%	100%	100%
<i>Proteus</i> spp	4	-	-	-	-	3	-	1	75%	100%	100%
<i>Streptococcus</i> spp	12	-	-	12	-	-	-	-	100%	100%	100%
<i>Streptococcus agalactia</i>	13	-	-	-	12	-	-	1	92.3%	97.6%	66.7%
<i>Streptococcus pneumoniae</i>	6	-	-	-	-	-	6	-	100%	100%	100%
<i>Streptococcus</i> spp	2	-	-	-	2	-	-	-	ND	ND	ND
<i>Streptococcus</i> spp	17	-	-	-	4	-	-	13	ND	ND	ND

ND: Not Determined

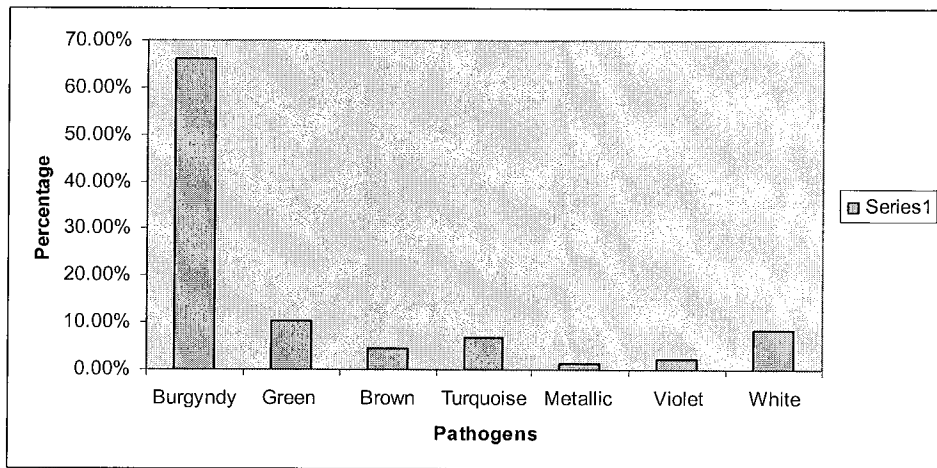


Figure 4: Number of samples that gave specific color. There were six specific colors in this medium for most prevalent isolates. The isolates that were included in this system were grown as white colonies.



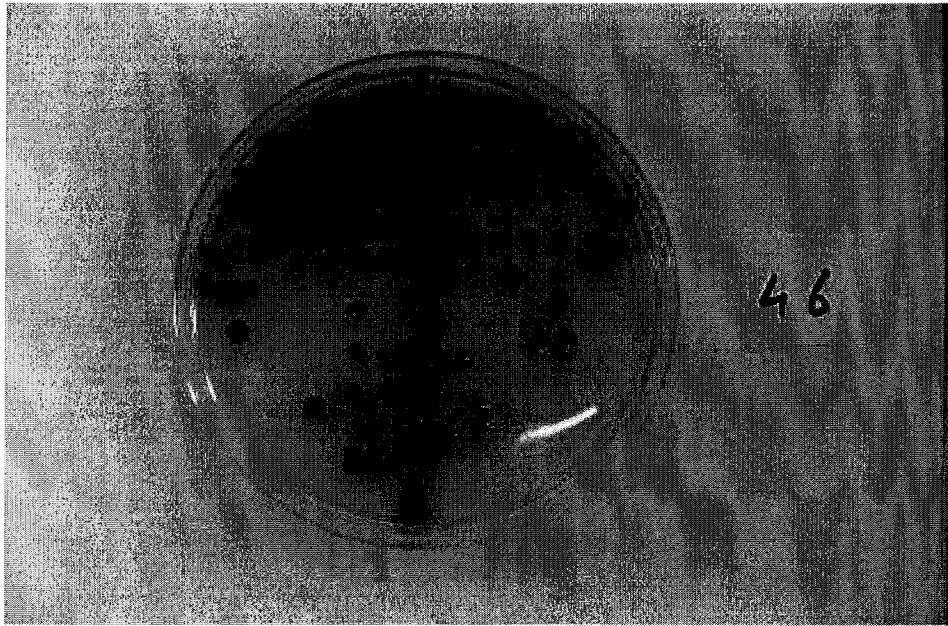


Figure 5: CPS ID 3 plate: The burgundy color in the above CPS plate indicates that the urine sample harbors *E. coli* which is the only pathogen recovered in this sample.

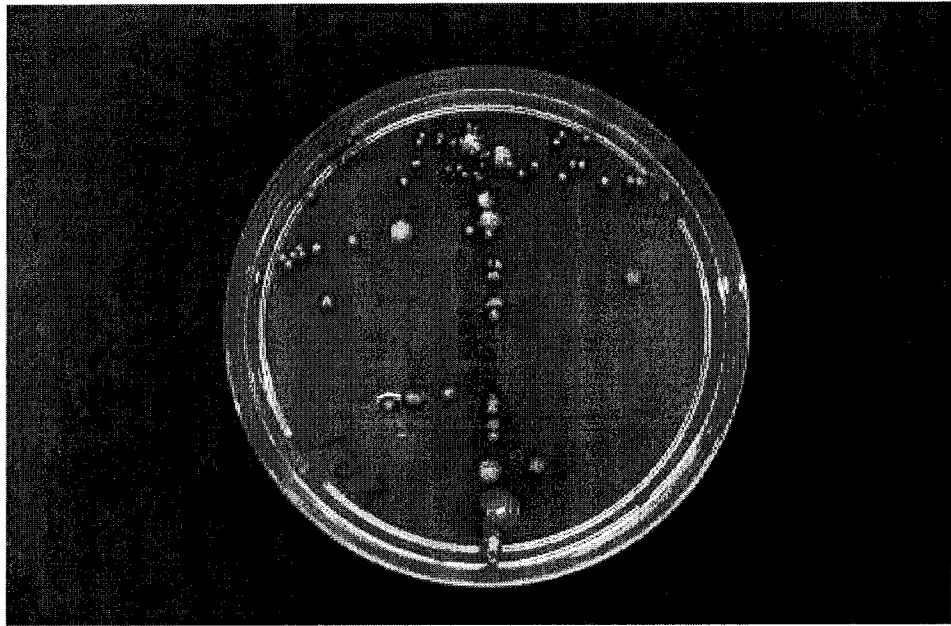


Figure 6: CPS ID 3 plate: The two colors (turquoise and white) in the above CPS plate indicated the presence of 2 pathogens: *Streptococcus* D (turquoise) and another pathogen (white) which in turn requires isolation and further testing.

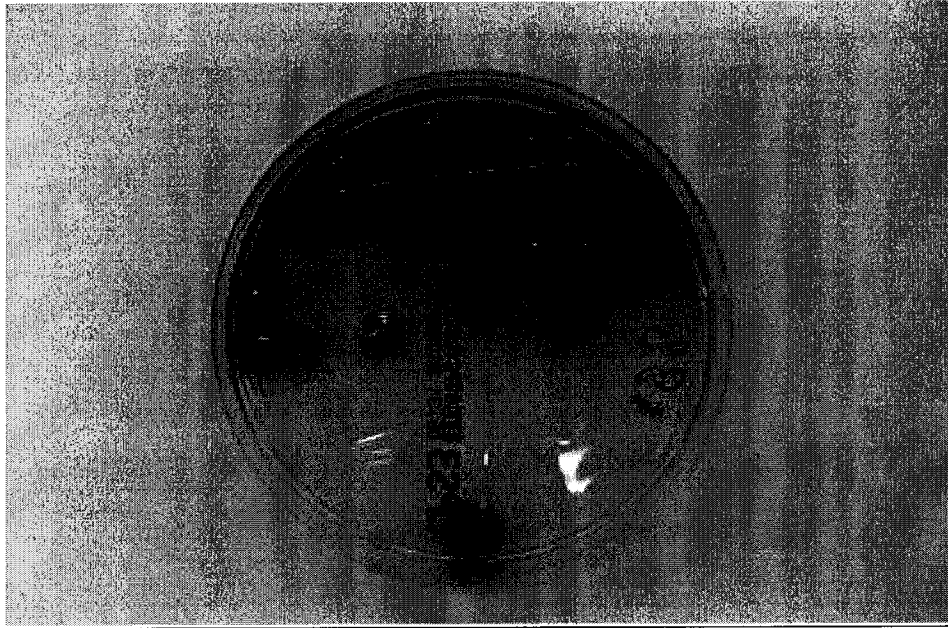


Figure 7: CPS ID 3: The three colors (burgundy and green and brown) in the above CPS plate indicated the presence of 3 pathogens: *E. coli* (burgundy), KESC group (green), and *Proteus* spp (brown).

#### **4.3: Detection of *E. coli*-containing samples:**

All 160 *E. coli*-containing samples (identified by API system) were further tested by PCR for the presence of the *uspA* gene. A gene harbored by all *E. coli* pathogens, hence confirming their identity (Figure 8). These were the samples that were later subjected to the PCR-detection of virulence genes according to Yamamoto et al. (1995).

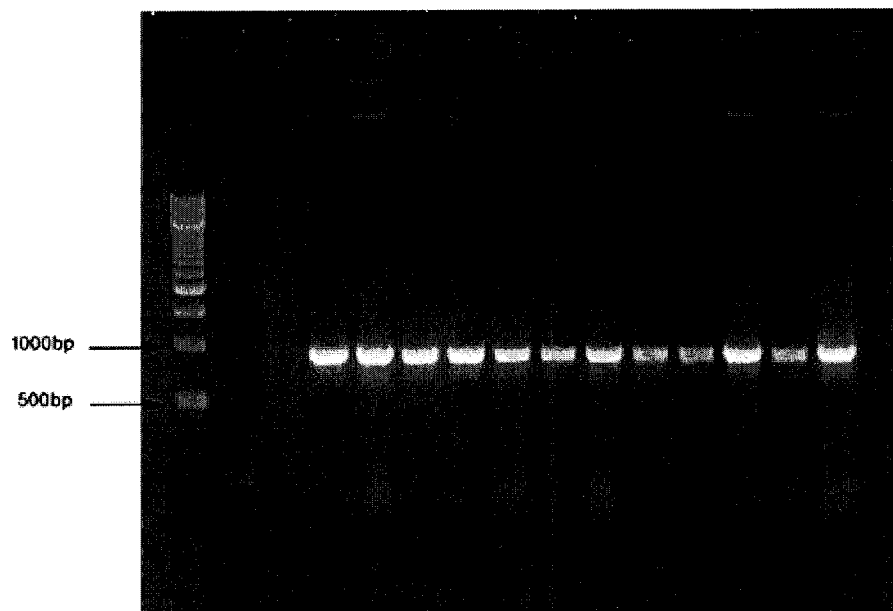


Figure 8: PCR amplification of *uspA* gene. Lane 1: 500 bp ladder; Lane 2: negative control containing water instead of DNA; Lane 3: negative PCR control containing DNA from *Klebsiella pneumoniae*; Lanes 4-15: DNA extracted from uropathogenic *E. coli* strains recovered from urine samples collected from St. George and Notre Dame de Secures hospitals. 884 bp-amplicons confirmed the presence of *Escherichia coli* bacterium in those samples.

#### **4.4: Detection of *hly* gene in *uspA* positive samples:**

PCR screening for the 160 *E. coli* samples (those positive for *uspA* gene) to check the *hly* gene, indicated that 52 of them harbored this gene (Figure 9).

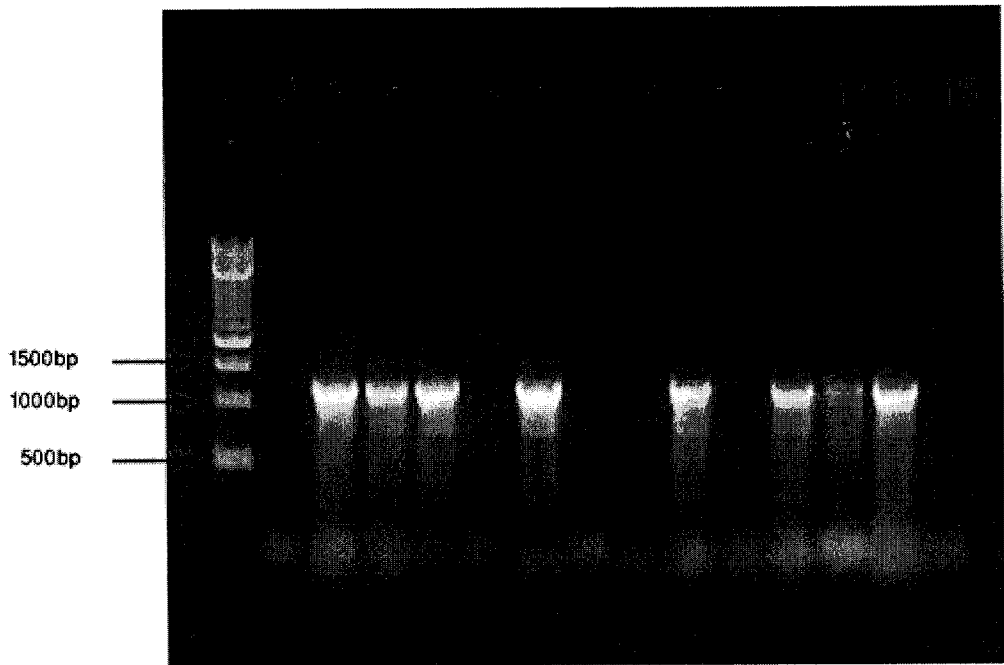


Figure 9: PCR amplification of hemolysin gene. Lane 1: 500 bp ladder. Lane 2 is negative control containing water instead of DNA. Lane 3 positive *hly* references strain 2H16 (provided by Dr. James J. Johnson, University of Minnesota). Lanes 4-15 are DNA extracted from *Escherichia coli* strains isolated from the urine of patients of St. George and Notre Dame of Secures hospitals. The 1177 bp products indicated the presence of *hly* gene in those samples.

#### **4.5: Detection of *pap*, *sfa*, *cnf*, *aer*, *afa* genes using multiplex PCR:**

*uspA* positive samples were subjected to PCR-detection of five virulence genes. The results of the PCR amplification using gene-specific primers revealed that the highest frequency (45.6%) pertains to the *Aer* gene and the least (8.7%) for *afa* gene (Table 8). Representative PCR results are presented in Figure 10.

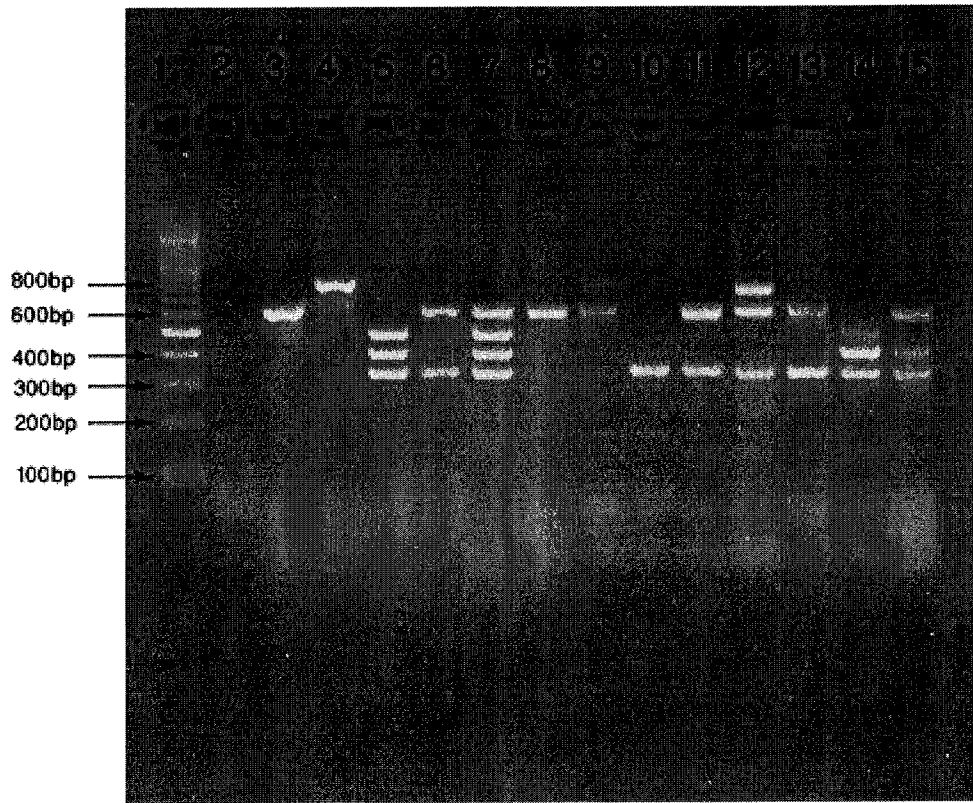


Figure 10: Multiplex PCR of *pap*, *sfa*, *cnf*, *aer*, and *afa* genes. Lane 1 is 100 bp ladder. Lane 2 is a negative control containing water instead of DNA. Lane 3 is *E. coli* reference strain PM9 that was *aer* gene positive. Lane 4 is *E. coli* reference strain 2H16 that contains *afa* gene. Lane 5 is *E. coli* reference strain 2H25 that contain *pap*, *sfa*, and *cnf* genes (the three *E. coli* reference strains PM9, 2H16, and 2H25 were provided by Dr. Johnson, University of Minnesota). Lanes 6-15 were DNA extracted from uropathogenic *E. coli* strains isolated from the urine of patients of St. George and Notre Dame of Secures hospitals. The 328, 410, 498, 602, and 750 bp amplicons indicated the presence of *pap*, *sfa*, *cnf*, *aer*, and *afa* genes respectively.

Table 8: Number of samples positive for each particular gene. The most prevalent virulence factor is aerobactin (45.625%) and the least is Afimbriae (*afa*) (8.75%)

<u>Gene</u>	<i>pap</i>	<i>sfa</i>	<i>Cnf</i>	<i>Aer</i>	<i>Hly</i>	<i>afa</i>
<u>Number</u>	54	53	29	73	52	14
<b>Total number</b> <b>160</b>						
<u>percentage</u>	33.57%	33.125%	18.125%	45.625%	32.5%	8.75%

#### **4.6: Number of virulence factors per sample:**

Of the 160 *uspA*-positive samples, the presence of virulence factors ranged from none to five of them per sample. Figure 11 illustrates the number of genes positive for each sample.

Of the 38 strains that gave one virulence factor, 27 strains harbored *aer* gene. As for the rest of the genes, the number of samples harboring them was generally less than five (Figure 12)

Of the 33 strains that gave two virulence factors, and among 15 possible combinations, only five patterns were significant, of which *pap* + *aer* was the most frequently detected (Figure13).

In contrast, seven patterns were observed in the 16 strains that gave three virulence factors, while four combinations were observed with those having 4 virulence factors (Figure 14).

The 11 strains that gave 5 virulence factors had the same combination, which is *pap*, *sfa*, *cnf*, *aer*, *bly* (*afa* gene was not observed in that strains).

Of the 14 strains that gave *afa* gene, just three strains gave *afa* associated with *pap* gene. Ten strains gave *afa* gene that associated with *aer*. *Sfa* was associated with all factors except *afa*. *Sfa* was detected along with *pap* 34 times.

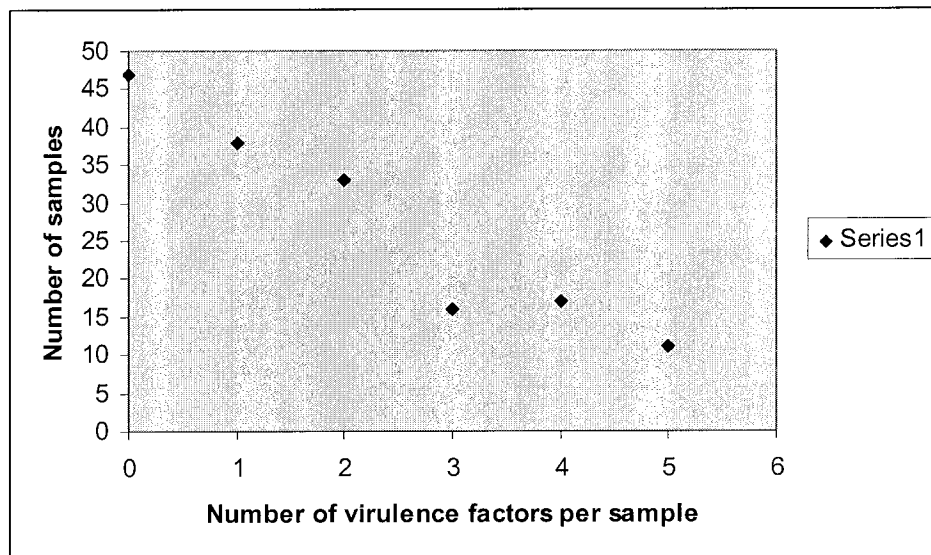




Figure 11: Number of samples VS number of virulence factors per sample. 47 samples did not show any type of virulence factor whereas just 11 strains had 5 virulence factors.

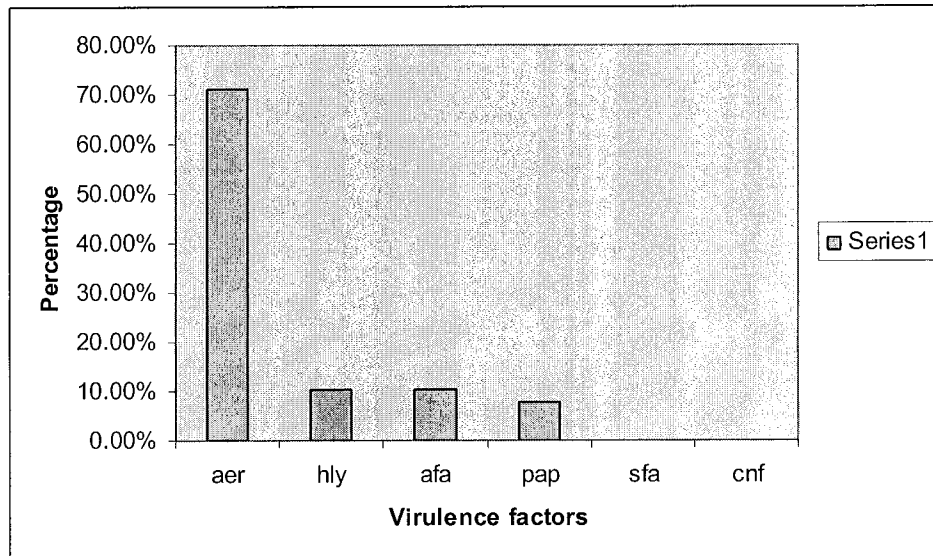


Figure 12: Number of samples that contain one virulence factor. Most of those samples had aerobactin where none of those samples had only S fimbriae or Cytotoxic necrotizing factor genes.

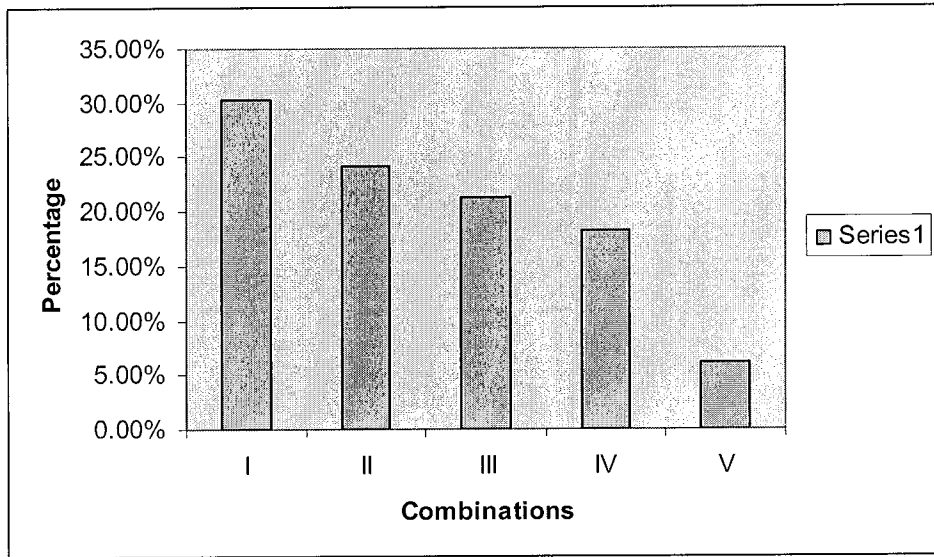


Figure 13: Number of samples that contain two virulence factors. Of the 160 uropathogenic *E. coli* strains included in this study, 33 strains harbored 2 virulence factors. (I: *pap+aer*, II: *sfa+aer*, III: *sfa+hly*, IV: *aer+afa*, V: *pap+sfa*)

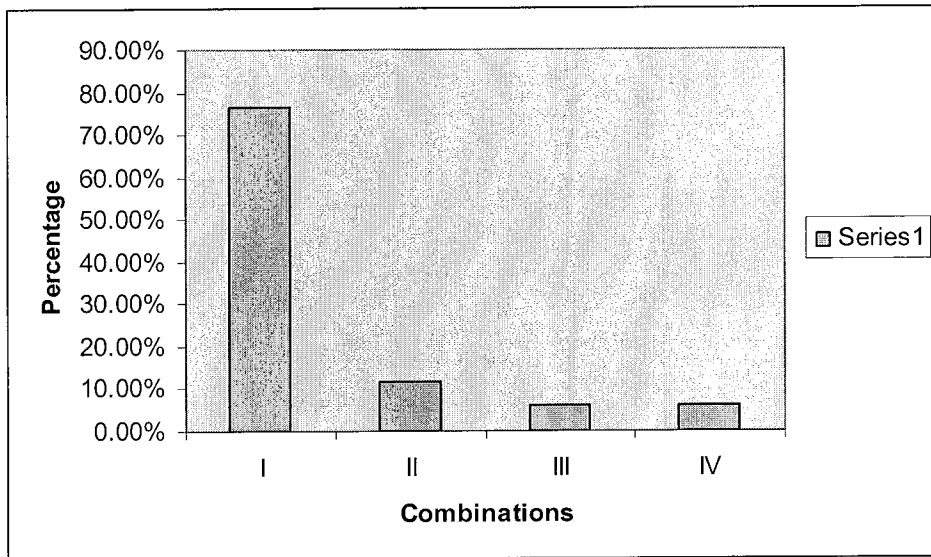


Figure 14: Number of samples that contain 4 virulence factors. The combination *pap+sfa+cnf+hly* was the most prevalent among other

combination. (I: *pap*<sup>+</sup> *sfa*<sup>+</sup> *cnf*<sup>+</sup> *hly*. II: *pap* + *sfa*<sup>+</sup> *aer*<sup>+</sup> *hly*, III: *pap*<sup>+</sup> *cnf*<sup>+</sup> *aer*<sup>+</sup> *hly*, IV: *sfa*<sup>+</sup> *cnf*<sup>+</sup> *aer*<sup>+</sup> *hly*)

## Chapter 5

### Discussion

In this study, CPS ID3 was evaluated as a direct isolation medium for urine samples. The enormous advantage of this medium was the ability to identify microorganisms growing within a mixed population according to colony color, as well as the ability to perform anti-microbial susceptibility tests without the need for further isolation. Bacteria that gave small colonies such as *Enterococcus* spp. were more frequently recovered on CPS ID3 medium than from conventional media because the specific colony color characterizing these pathogens facilitated the detection especially when growing along with other gram-negative bacteria. Furthermore, when two organisms have the same colonial morphology like *E. coli* and *Klebsiella pneumoniae* they could be distinguished depending on their colony color on chromogenic media. Another advantage of CPS ID3 was that it did not require any additional equipment to read the culture results and the test was easy to perform.

Our study agreed with many other (Samra et al., 1998; Scarparo et al., 2002) showing that chromogenic media could support the growth of all UTI pathogens. However, Aspevall et al. (2002) reported that chromogenic media did not consistently support the growth of gram-positive bacteria and blood agar was recommended to detect these pathogens. In this study, around 15% of the recovered isolates were gram-positive.

CPS ID3 had similar to some other Chromogenic media, inhibited the swarming of many organisms such as *Proteus* spp. (Samra et al., 1998; Chaux et al., 2002; Hengstler et al., 1997; Milles and Wren, 2005); a property not seen with blood agar (Hengstler et al., 1997); Thus allowing the detection of other isolates within a mixed population. On the other hand, CPS ID3 could not be used to differentiate between *Proteus mirabilis* and other *Proteus*

spp., and accordingly Indole test is recommended for the specific identification of *P. mirabilis* which was indole negative where other *proteus* spp. are indole positive. Moreover, *Klebsiella pneumonia*, *Enterobacter* spp., *Serratia* spp. and *Citrobacter* spp. could not be differentiated from each other, since all these bacteria gave similar colonies, and accordingly additional biochemical test should be performed. This was in harmony with the findings of Scarparo et al. (2002).

Hengstler et al. (1997), on the other hand, using BBL CHROMagar Orientation found that the fastidious strains of *Streptococcus agalactia* may not grow or grow as tiny colonies on CLED agar (cysteine lactose electrolyte deficient) or MacConkey agar. *Streptococcus agalactia* strains can be easily differentiated on CPS ID3 by their violet color.

Studies that evaluated CPS ID2 showed that about 95 to 98% of *E. coli* strains produced  $\beta$ -glucuronidase enzyme which was used in CPS ID media to identify *E. coli* (Mazoyer et al., 1995; Nunez et al., 1995; Orenge et al., 1993). The majority of *E. coli* strains (96%) isolated in this study produced this enzyme. Although *Salmonella* and *Shigella* were also  $\beta$ -glucuronidase positive (Le Minor et al., 1978), these organisms were rarely isolated from urine (Kilian and Bulow, 1979). Hansen and Yourassowsky (1984) indicated that *Shigella* was never isolated from urine samples, while Scarparo et al. (2002) recommended that indole test should be done on all positive  $\beta$ -glucuronidase colonies, since *Salmonella* and *Shigella* spp. were indole negative. However, and according to the results of this study, where *Salmonella* nor *Shigella* spp. were detected, we do not recommend doing the indole test especially that it is time consuming. The biggest advantage of using Chromogenic media was the ability to identify *E. coli* strains within 24 hours without the need to do any additional biochemical tests. However, indole test should be conducted in the case of gram -negative bacteria appearing white colonies to avoid false negative results with *E. coli*.

Hengstler et al. (1997) revealed that *Citrobacter* spp. on ChromAgar Orientation and CPS ID2 plates gave false positive results as it appeared on the form of pink to red colonies similar to *E. coli*. Moreover, in this study also indicated that *Proteus* spp. and *Enterobacter* spp. appeared again as red to pink colonies which additionally interfered with proper *E. coli* identification. On the other hand, using Oxoid UTI medium Fallon et al. (2003) showed that out of 18 *Citrobacter* strains, 11 appeared as purple colonies similar to *E. coli*, while only one *Citrobacter* was misidentified using CPS ID2 medium. Accordingly, it was recommended that all strains that gave pink to red colonies should be subjected to the indole test to rule out *E. coli* isolates. In this study, none of these organisms gave a burgundy colonies which was specific for *E. coli*, and thus indole test was not needed using this medium. However, the absence of false positive results in our study could be possibly attributed to the low number of *Enterobacter* (n=2), *Citrobacter* (n=1), and *Proteus* (n=12) recovered. In addition, CPS ID3 and contrary to CPS ID2 could be used for the identification of *Citrobacter* spp. which reduced the error of misidentifying *Citrobacter* as *E. coli*. Moreover, in CPS ID2, *Streptococcus agalactia* could appear as blue colonies like *Enterococcus* spp., while in CPS ID3 *Streptococcus agalactia* gave violet colonies, which was easily distinguishable from *Enterococcus* colonies appearing as turquoise colonies. Ciragil et al. (2006) showed that CPS ID3 medium helped in the detection of all *S. agalactia*, enterococci, staphylococci that grew on blood agar. However, these results revealed that *Staphylococcus* spp. appeared as colorless colonies, and additional chemical tests were needed to confirm the isolation of staphylococci. Both *Enterococcus faecalis* and *faecium* had colonial morphology, and therefore could not be distinguished at the species level. This was in harmony with studies based on the use of chromogenic media (Merlino et al., 1995; Willinger and Manfi, 1995; Hengstler et al., 1997; Scarparo et al., 2002).

The positive predictive value of CPS ID3 with *Enterococcus* spp. was low (PPV=66.7%), which was possibly due to the fact that 4 *Staphylococcus* spp.

and 2 *Streptococcus* spp gave also turquoise colonies. Perry et al. (2003) using the *S. aureus* ID chromogenic medium, found that some *Staphylococcus* species might occasionally produce colored colonies as a results of the hydrolysis of a chromogenic  $\beta$ -glucosidase substrate. Ahmet et al., (1995) showed that *Streptococcus milleri* could be identified by the testing for  $\beta$ -glucosidase enzyme.

On CPS ID 3 medium, *E. coli* usually appeared as pink to burgundy colonies. Two *E. coli* variants were detected in this study in one of the urine specimens. Those two variants were distinguished as the first appeared as a pink colony and the second variant as burgundy. This was a situation where more biochemical tests were recommended to determine whether these two variants corresponded to two different infecting strains so as to determine the suitable antibiotic treatment. Similar results were reported by Carricajo et al. (1999) and by Scarparo et al. (2002), where also two *E. coli* variants were recovered from on urine in sample and were distinguishable based on their colony colors on Chromogenic media.

One of the disadvantages of CPS ID3 was that *Staphylococcus saprophyticus* is not included in the system although it is considered as one of the common pathogens that colonized the urinary tracts (Barnett and Stephens, 1997). Furthermore, oxidase test was recommended to differentiate between *Pseudomonas* spp. (oxidase positive) and *Enterobacteriaceae* family (oxidase negative). The sensitivity of CPS ID3 to *Pseudomonas* spp. was 75%. The low sensitivity of the medium might be due to the low number of strains recovered from the urine samples (n=4). The catalase test was recommended to differentiate between *Staphylococcus* spp. (catalase positive) and *Streptococcus* spp. (catalase negative), especially that in this study 4 *Staphylococcus* spp. appeared as turquoise colonies, which was specific for *Enterococcus* spp. Results of oxidase and catalase test could be obtained in few minutes.

CPS ID3 reduced 50% of the time needed for the identification (24 hours instead of 48 hours) of isolates without the need for any additional tests. In our study, 72 % of isolated strains were directly identified using this medium, and it reduced the time needed when working with mixed cultures.

Concerning cost effective studies, Scarparo et al. (2002) showed that the price of CPS ID is 0.44\$/plate. We have estimated our cost at 2\$/plate. This variation in price was attributed to delivery charges. It is not wise to determine cost effectiveness based only on the price of a chromogenic media and a conventional media, as we should also be taking into consideration the additional biochemical tests that should be done.

Finally, CPS ID3 enables presumptive identification of a high proportion of urinary tract pathogens through the color of the colony, and even pathogens that were colorless were successfully isolated using this medium. Time and biochemical tests can be sharply reduced enabling the clinician to recommend the appropriate therapy immediately after growth. The false positive results within the *Enterobacteriaceae* were very low (high specificity), allowing technicians in the clinical labs to be confident enough to report results without doing any further tests. However, the conventional biochemical tests should be applied whenever white colonies are recovered. White colored colonies are an indicator either to false negative results or are due to organisms not included in the system such as *Staphylococcus* spp. Finally, as the CPS ID3 and according to our results can be used for the identification of the members of the *Enterobacteriaceae* family, the use of other media such as MacConkey agar was not recommended anymore.

In this study, virulence factors in uropathogenic *E. coli* were detected using a multiplex PCR assay. Blanco et al. (1997) revealed that there was a correlation between results obtained using PCR assay and those obtained by phenotypic methods. Additionally, PCR-based detection was found to be quicker easier and inexpensive, facilitating detection of virulence related



genes in *E. coli*. Time required to obtain results using PCR assay was much less than time needed with colony hybridization test (Le Bouguenec et al., 1992), with Yamamoto et al.(1995a) revealing that multiplex PCR was a useful method for the rapid detection of *E. coli* virulence factors.

Of the 17 strains that were positive for four tested virulence genes, 14 strains were positive for the combination of *pap*<sup>+</sup>, *sfa*<sup>+</sup>, *cnf*<sup>+</sup>, and *hly*<sup>+</sup> which was in accordance with Yamamoto et al (1995 b), who reported that this combination was the most dominant among isolates associated with cystitis in Japan. There was a genetic linkage between those genes as shown by Hacker et al. (1990) who showed that deletion of gene coding for fimbriae or hemolysin resulted in the loss of the other.

On the other hand, and regarding the hemolysin (*hly*) gene and the cytotoxic necrotizing factor (*cnf*), the results of this study agreed with many others and showed that presence of *cnf* was limited to hemolytic strains (Caprioli et al., 1987; Caprioli et al., 1989; Ruiz et al., 2002). However, not all *E. coli* strains that produced *hly* were *cnf* producing. It is noteworthy that in this study none of the tested strains had only these two genes, but rather these were associated with other virulence factors. In contrast, *hly* gene was detected as the only virulence factor in four of the tested strains. However, although *cnf* was limited to hemolytic strains, it required other adhesion gene to be virulent. Blanco et al. (1997) showed that there was a direct chromosomal link between *cnf*, *hly* and *pap* operons, which partly was in harmony with our results where we detected in three strains the presence of the *hly* and *cnf* genes in the absence of the *pap*. Hacker et al. (2002) reported that different pathogenicity islands had different combinations of genes.

Johnson et al. (1991) indicated that uropathogenic *E. coli* strains usually had adhesin genes, while in this study and in 19% of the tested isolates, we were not able to detect any adhesin. Most of these samples (87%) not having adhesin factors were aerobactin positive. There could be two explanations

for this co- occurrence of virulence genes: strains that contain *aer* gene do not require adhesin proteins to colonize urinary tracts, or *aer* were linked to classes of adhesins that were not undertaken in this study such as type 1 fimbriae, which was mannose sensitive hemaagglutination. Since type1 fimbria was found to be consistently associated with extraintestinal *E. coli* strains (O'Hanley et al., 1985; Usein et al., 2001), it was excluded from this study. *Sfa* adhesin was detected in 33% of the strains and this was an obvious indication that uropathogenic *E. coli* strains examined in this study had this type of adhesin. Ott et al. (1986), studied the *sfa* adhesins and reported that S-fimbriated strains were mainly associated with meningitis rather than with urinary tract infection.

Of the 11 strains that were positive for 5 virulence factors (amongst 6 tested), *afa* gene was not detected and this was in accordance with the finding of Usein et al. (2001). Usein et al. also screened for the same six virulence factors and found that the *afa* and *sfa* genes were not detected together in any of the tested strains, which was again in harmony with our results. Moreover, results of this study also agreed with those of Usein et al. (2001) with respect to the association between the *afa* and *aer* genes.

In this study, 32.5 and 45% of the strains were found to be positive for *hly* and *aer* respectively, while Santo et al. (2006) reported that 96 and 76% of the strains were positive for *hly* and *aer*, respectively. The difference between both studies might be attributed to the methods used in the evaluation, where Santo et al. (2006) relied basically on phenotypic methods, or alternatively it could be simply due to differences correlated to geographical factors (Lebanon versus Brazil).

Johnson et al. (1991) reported that Mannose resistant hemagglutination (MRHA) were common among hemolytic strains. Our results agreed with this observation were only around 8% of the tested strains did not have of the known adhesins. Johnson et al. (1991) suggested that there was a

synergism between MRHA adhesins and other virulence factors. Further studies are recommended to elucidate the presence of any functional correlation between MRHA adhesins and *hly*.

*Aer* gene occurred with all types of adhesins, whereas Jacobson et al. (1998) reported that *aer* was commonly found with *pap*. Johnson et al. (1988) revealed that *aer* locus could be either chromosomal or plasmid gene, with the chromosomal being associated with *pap* gene. Since the extraction method undertaken in this study recovered both chromosomal and plasmid DNA we could not have any conclusion with this respect especially the *aer* gene was detected with all other studied virulence factors.

S fimbriae adhesin (*sfa*) was not observed alone among the samples included in this study. No one of articles published concerning this type of adhesin reported about this observation. Furthermore, *sfa* was not limited to any type of the studied genes like the case of *hly* and *cnf* suggesting that *sfa* played a role in adherence but it could not play a major role in pathogenicity without the presence of the other studied genes.

*Hly* gene was not amplified in the multiplex PCR assay where the primers for *hlyA* were designed to amplify a fragment with a size of 1177bp. It is usually difficult to amplify such a big fragment using a multiplex PCR reaction. Therefore, *hly* gene was detected separately in a simplex PCR assay.

### **Future work:**

Blood agar medium is routinely used to determine bacterial counts in urine samples. It would be of great interest if the CPS ID3 could be used instead of blood agar for the above purpose.

*E. coli* isolation was confirmed through the amplification of the *uspA* gene. A multiplex PCR designed to detect virulence genes should also be standardized to include primers that can simultaneously detect the *uspA* gene.

It is recommended to screen the same samples for the O: K: H serotypes to find out if there is any correlation between *E. coli* serotypes and the prevalence of the studied genes. Furthermore, determination of the antibiogram for the isolates having adhesin specific genes is another aspect that needs to be studied.

## Chapter 6

### Conclusion

- The use of CPS ID3 helped in differentiating between different organisms in mixed populations and supported the growth of all types of organisms frequently involved in UTI.
- Chromogenic media inhibited swarming of *Proteus* spp.
- CPS ID3 could be used routinely in clinical laboratories since it saved 50% of the time compared to routine analysis.
- The positive predictive values were 100% for all organisms included in this study except *Enterococcus* which was 66.7%.
- CPS ID 3 could differentiate between *E. coli* and other members of the *Enterobacteriaceae* family based on the differences in the morphology of the colonies.
- Additional biochemical tests should be performed with white colonies recovered on CPS ID3 medium.
- Non of the isolates included in this study had the morphological characteristics as that of *E. coli*. Hence, false positive results were not encountered (specificity of *E. coli* 100%).
- *Staphylococcus saprophyticus*, which is considered as one of the pathogens that cause urinary tract infection, did not give any specific color on CPS ID3 medium.

- CPS ID3 had the disadvantage of being expensive and having a short shelf life.
- The prevalence of the studied virulence factors among 160 strains of *E. coli* were 33.6, 33.1, 8.8, 45.6, 32.5, and 18.1% for *pap*, *sfa*, *afa*, *aer*, *hly*, and *cnf*, respectively.
- Most strains possessed adherence factors confirming the importance of these proteins in mediating the colonization of the urinary tracts.
- This study revealed, like many others, that *cnf* was limited to hemolytic strains and most hemolytic strains were linked to genes coding for adhesins genes.
- None of the samples had only *sfa* gene, which existed in combination with other genes. Neither *sfa* nor the *afa* genes were detected in any of the tested strains.
- In many isolates, the *aer* gene was not associated with genes encoding for adhesins. This could indicate that such isolates either do not require adherence factors to colonize the urinary tract, or that the *aer* gene was linked to other factors not undertaken in this study.
- *Sfa* was detected in 33% of the isolates although it was a gene mainly detected in *E. coli* associated with meningitis.
- Of the 17 strains that were positive for four tested virulence genes, the combination *pap*<sup>+</sup>, *sfa*<sup>+</sup>, *cnf*<sup>+</sup>, and *hly*<sup>+</sup> was observed in 14 of these isolates.
- Within the 11 isolates that were positive for five of the studied virulence factors, none had the *afa* gene.

## BIBLIOGRAPHY

- Abbott, G. D. (1972). Neonatal bacteriuria: A prospective study in 1,460 infants. *British Medical Journal*, 1, 267-269.
- Ackermann, R. J., & Monroe, P.W. (1996). Bacteremic urinary tract infection in older people. *Journal of the American Geriatrics Society*, 44, 927-33
- Alon, U., Davidai, G., Berant, M., & Merzbach, D.(1987). Five-year survey of changing patterns of susceptibility of bacterial uropathogens to trimethoprim-sulfamethoxazole and other antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, 31, 126-128.
- Arthur, M., Johnson, C. E., Rubin, R. H., Arbeit, R. D., Campanelli, C., Kim C., et al. (1989). Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. *Infection and Immunity*, 57(2), 303-313.
- Aspevall, O., Osterman , B., Dittmer R., Sten, L., Lindback E., & Frosum.U. (2002). Performance of four chromogenic urine culture media after one or two days of incubation compared with reference media. *Journal of clinical microbiology*, 40(4), 1500-1503.
- Baga, M., Ormark, S., Hardy, J., O'Hanley, P., Lark, D., Olsson, O., et al. (1984). Nucleotide sequence of the *papA* gene encoding the *pap* pilus subunit of human uropathogenic *Escherichia coli*. *Journal of Bacteriology*, 157(1), 330- 333.
- Bagg, A., & Neilands, J.B. (1987). Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. *Biochemistry*, 26, 5471-5477.
- Bagg, A., & Neilands, J.B. (1987). Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiological Reviews*, 51, 509-518.
- Barnett, B.J. & Stephens, D.S. (1997). Urinary tract infection an overview. *The American Journal of the Medical Sciences*, 341(4), 245-249
- Bass, P. F., Jarvis, J.A., & Mitchell, C.K. (2003). Urinary tract infections. *Primary Care*, 30, 41-61.
- Beachey, E. H. (1981). Bacterial adhesion. *Journal of Infectious Diseases*, 143, 325-345.

- Bergeron, M. G. 1995. Treatment of pyelonephritis in adults. *The Medical clinics of North America*, 79, 619-49.
- Bettelheim, K. A. (1991). The genus *Escherichia*. In *The prokaryotes: A handbook on the biology of bacteria, ecophysiology, isolation, identification, applications* (pp. 2696-2736). New York, N.Y.: Springer-Verlag
- Bilge, S.S., Clausen, C.R., La, W., & Moseley, S.L. (1989). Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. *Journal of Bacteriology*, 171(8), 4281-4289.
- Blanco, M., Blanco, J.E., Rodriguez, E.L., Abalia, I., Alonso, M.P., & Blanco, J. (1997). Detection of virulence genes in uropathogenic *Escherichia coli* by polymerase chain reaction (PCR): Comparison with results obtained using phenotypic methods. *Journal of Microbiological Methods*, 31, 37-43.
- Blum, G., Ott, M., Lischewski, A., Ritter, A., Imrich, H, Tschäpe, H., et al. (1994). Excision of large DNA regions termed pathogenicity islands from tRNA-specific loci in the chromosome of an *Escherichia coli* wild-type pathogen. *Infection and Immunity*, 62(2), 606-614
- Braun, V., Brazel-Faisst, C., & Schneider, R. (1984). Growth stimulation of *Escherichia coli* in serum by iron (III) aerobactin. Recycling of aerobactin. *FEMS Microbiology Letters*, 21, 99-103.
- Buckley, R. M., McGuckin, M., & MacGregor, R.R. (1978). Urine bacterial counts after sexual intercourse. *The New England Journal of Medicine*, 298, 321-324.
- Bulter D.A., Lobrecat, C.M., Gavan, T.L. (1975), Reproducibility of the analytab (API 20E) System. *Journal of Clinical Microbiology*, 2(4), 322-326
- Caprioli, A., Falbo, V., Roda, L.G., Ruggeri, F. M., & Zona.C. (1983). Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. *Infection and Immunity*, 39(3), 1300-1306.
- Caprioli, A., Falbo, V., Ruggeri, F.M., Baldassarri, L., Bisicchia, R., Ippolito, G., et al. (1987). Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. *Journal of Clinical Microbiology*, 25(1), 146-149.
- Caprioli, A., Falbo, V., Ruggeri, F.M., Minelli, F., Orskov, I., & Donelli, G. (1989). Relationship between cytotoxic necrotizing factor production and serotype in hemolytic *Escherichia coli*. *Journal of Clinical Microbiology*, 27(4), 758-761.



- Carbonetti, N. H., & Williams, P.H. (1984). A cluster of five genes specifying the aerobactin iron uptake system of plasmid ColV-K30. *Infection and Immunity*, 46(1), 7-12.
- Carricajo, A., Boiste, S., Thore, J., Aubert, G., Gille, Y., & Freydiere, A.M. (1999). Comparative evaluation of five chromogenic media for detection, enumeration and identification of urinary tract pathogens. *European Journal of Clinical Microbiology & Infectious Diseases*, 18, 796-803
- Cavaliere, S. J., Bohach, G.A. & Snyder, I.S. (1984). *Escherichia coli*  $\alpha$ -hemolysin: Characteristics and probable role in pathogenicity. *Microbiological Reviews*, 48, 326-343.
- Chabanon, G., Hartley, C. L. & Richmond, M.H. (1979). Adhesion to a human cell line by *Escherichia coli* strains isolated during urinary tract infections. *Journal of Clinical Microbiology*, 10(4), 563-566.
- Chaux, C., Crepy, M., Xueref, S., Roure, C., Gille, Y., & Freydiere, A.M. (2002). Comparison of three chromogenic agar plates for isolation and identification of urinary tract pathogens. *Clinical Microbiology and Infection*, 8, 641-645
- Chen, J., & Griffiths, M.W. (1998). PCR differentiation of *Escherichia coli* from other gram negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress protein. *Letters in Applied Microbiology*, 27, 369-371.
- Chomarat, M. (2000). Resistance of bacteria in urinary tract infections. *International Journal of Antimicrobial Agents*, 16, 483-487.
- Ciragil, P., Gul, M., Aral, M., & Ekerbicer, (2006). Evaluation of a new chromogenic medium for isolation and identification of common urinary tract pathogens. *European Journal of Clinical Microbiology & Infectious Diseases*, 25, 108-111
- De Man, P., Cedergren, B., Enerback, S., Larsson, A.C., Leffler, H., Lundell, A.L., et al. (1987). Receptor-specific agglutination tests for detection of bacteria that bind globoseries glycolipids. *Journal of Clinical Microbiology*, 25(2), 401- 406.
- Donnenberg, M.S. & Welch, R.A. (1996). Virulence determinants of uropathogenic *Escherichia coli*. In H.L.T. Mobley & J.W. Warren (Eds.), *Urinary tract infections: Molecular pathogenesis and clinical management* (pp. 145-174). Washington, DC: ASM Press.

- Drasar, B. S., & Hill, M.J. (1974). *Human intestinal flora* (pp. 36-43). London: Academic Press.
- Duguid, J. P., & Anderson, E. S. (1967). Terminology of bacterial fimbriae, or pili, and their types. *Nature*, 215, 89-90.
- Duguid, J. P., Smith, I.W., Dempster, G., & Edmunds, P.N. (1955). Nonflagellar filamentous appendages ("fimbriae") an haemagglutinating activity in *Bacterium coli*. *The Journal of Pathology and Bacteriology*, 70, 335-349.
- Edberg, S. C., & Kontnick, C.M. (1986). Comparison of 3-Glucuronidase-Based Substrate Systems for Identification of *Escherichia coli*. *Journal of Clinical Microbiology*, 24(3), 368-371
- Emody, L., Kerenyi, M., & Nagy, G. (2003). Virulence factors of Uropathogenic *Escherichia coli*. *International Journal of Microbial Agents*, 22, S29-S33
- Emori, T.G. & Gaynes, R.P. (1993). An overview of nosocomial infections, including the role of the microbiology laboratory. *Clinical Microbiology Review*, 6(4), 428-442
- Ewnig, H.R., Davis, B.R., & Martin, W.J. (1981). *Biochemical characterization of Escherichia coli, publication no. 81-8109* (pp. 1-15). Atlanta, GA.: U.S. department of health and human services, central for disease control and prevention.
- Fallon, D., Ackland, G., Andrews, N., Frpdsjam, D., Howe, S., Howells, K., et al. (2003). A comparison of the performance of commercially available chromogenic agars for the isolation and presumptive identification of organisms from urine. *Journal of Clinical Pathology*, 56, 608-612.
- Farmer, J. J., Davis, B.R., Hickman-Brenner, F.W., McWhorter, A., Huntley-Carter, G.P., Asbury, M.A., et al. (1985). Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *Journal of Clinical Microbiology*, 21(1): 46-76.
- Fiorentini, C., Arancia, G., Caprioli, A., Falbo, V., Ruggeri, F.M., & Donelli, G. (1988). Cytoskeletal changes induced in HEp-2 cells by the cytotoxic necrotizing factor of *Escherichia coli*. *Toxicon*, 26, 1047-1056.
- Foxman, B. (1990). Recurring urinary tract infections: Incidence and risk factors. *Journal of Public Health*, 880, 331-333.
- Freestone, P., Nystrom, T., Trinei, M., & Norris, V. (1997). The universal stress protein, UspA, of *Escherichia coli* is phosphorylated in response to stasis. *Journal of Molecular Biology*, 274, 318-324

- Goebel, W. & Hedgpeth, J. (1982). Cloning and functional characterization of the plasmid-encoded hemolysin determinant of *Escherichia coli*. *Journal of Bacteriology*, 151(3), 1290-1298.
- Goetz, G. S., Mahmood, A., & Hultgren, S.J. (1999). Binding of pili from uropathogenic *Escherichia coli* to membranes secreted by human colonocytes and enterocytes. *Infection and Immunity*, 67(11), 6161–6163.
- Graham, J. C. & Galloway, A. (2001). ACP best practice no 167: The laboratory diagnosis of urinary tract infection. *Journal of Clinical Pathology*, 54, 911-919
- Groisman, E.A. & Ochman, H. (1996). Pathogenicity islands: Bacterial evolution in quantum leaps. *Cell*, 87, 791-794
- Gross, R., Engelbrecht, F., & Braun V. (1985). Identification of the genes and their polypeptide products responsible for aerobactin synthesis by pColV plasmids. *Molecular and General Genetics*, 201, 204-212.
- Gruneberg, R. N. (1984). Antibiotic sensitivities of urinary pathogens, 1971-1982. *The Journal of Antimicrobial Chemotherapy*, 14, 17-23.
- Gruneberg, R. N. (1969). Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet*, 1, 766–768.
- Gustavsson, N., Diez, A., & Nyström, T. (2002). The universal stress protein paralogues of *Escherichia coli* are co-ordinately regulated and co-operate in the defence against DNA damage. *Molecular Microbiology*, 43, 107-117
- Hacker, J., Bender, L., Ott, M., Wingender, J., Lund, B., Marre, R., et al. (1990). Deletions of chromosomal regions coding for fimbriae and hemolysins occur *in vitro* and *in vivo* in various extraintestinal *Escherichia coli* isolates. *Microbial Pathogenesis*, 8, 213-225
- Hacker, J., Knapp, S., & Goebel, W. (1983). Spontaneous deletions and flanking regions of the chromosomally inherited hemolysin determinant of an *Escherichia coli* O6 strain. *Journal of Bacteriology*, 154(3), 1145-1152.
- Hacker, J. & Kaper, J.B. (2000). Pathogenicity islands and the evolution of microbes. *Annual Review of Microbiology*, 54, 641-79
- Hansen, W. & Yourassowsky, E. (1984). Detection of  $\beta$ -Glucuronidase in lactose fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. *Journal of Clinical Microbiology*, 20(6), 1177-1179.

- Hanson, L. A., Fasth, A., Jodal, U., Kaijser, B., & Eden, C.S. (1981). Biology and pathology of urinary tract infections. *Journal of Clinical Pathology*, *34*, 695-700.
- Hedlund, M., Duan, R., Nilsson, A., Svensson, M., Karpman, D., & Svanborg, C.(2001). Fimbriae, transmembrane signaling, and cell activation. *The Journal of Infectious Diseases*, *183*, S47-S50
- Henegariu O. , Heerema, N.A., Dlouhy, S.R., Vance, G.H., & Vogt, P.H. (1997). Multiplex PCR: Critical parameters and step-by-step protocol. *BioTechniques*, *23*, 504-511
- Hengstler, K. A., Hammann, R., & Fahr, A.M. (1997). Evaluation of BBL CHROMagar orientation medium for detection and presumptive identification of urinary tract pathogens. *Journal of Clinical Microbiology*, *35*(11), 2773-2777
- Hinata, N., Shirakawa, T., Okada, H., Shigemura, K., Kamidono, S., Gotoh, A. (2004). Quantitive detection of *Escherichia coli* from urine of patients with bacteruria by real time PCR. *Molecular Diagnosis*, *8*, 179-184
- Hooton, T. & Stamm, W. (1997). Diagnosis and treatment of uncomplicated urinary tract infection. *Infectious Disease Clinics of North America*, *11*, 551–581.
- Hoschutzky, H., Lottspeich, F., & Jann, K. (1989). Isolation and characterization of the  $\alpha$ -galactosyl-1,4-,  $\beta$ -galactosyl-specific adhesin (P adhesin) from fimbriated *Escherichia coli*. *Infection and Immunity*, *57*(1), 76-81.
- Jacobson, S. H., Tullus, K., Wretling, B., & Brauner, A. (1988). Aerobactin-mediated uptake of iron by strains of *Escherichia coli* causing acute pyelonephritis and bacteremia. *The Journal of Infection*, *16*, 147-152.
- Johnson, J. R., Moseley, S. L., Roberts, P. L., & Stamm, W. E. (1988). Aerobactin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: Association with patient characteristics. *Infection and Immunity*, *56*(2), 405-412.
- Johnson, J. R. (1991). Virulence factors in *Escherichia coli* urinary tract infection. *Clinical Microbiology Review*, *4*(1): 80-128
- Johnson, J. R. & Russo, T. (2002). Extraintestinal pathogenic *Escherichia coli* (ExPEC): The 'other bad *E. coli*. *The Journal of Laboratory and Clinical Medicine*, *139*, 155–162.

- Johnson, J. R. & Stell, A. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *The Journal of Infectious Diseases*, 181, 261–272.
- Johnson, J. R., Delavari, P., Kuskowski, M., Stell, A. L. (2001). Phylogenetic distribution of extraintestinal-virulence associated traits in *Escherichia coli*. *The Journal of Infectious Diseases*, 183, 78-88
- Kallenius, G. & Mollby, R. (1979). Adhesion of *E. coli* to human periurethral epithelial cells correlated to mannose resistant agglutination of human erythrocytes. *FEMS Microbiology Letters*, 5, 295-299.
- Kallenius, G., Mollby, R., Svensson, S. B., Winberg, J., Lundblad, A., Svensson, S., et al. (1980). The pk antigen as receptor for the haemagglutination of pyelonephritogenic *Escherichia coli*. *FEMS Microbiology Letters*, 7, 297-302.
- Kallenius, G., Svenson, S. B., Mollby, R., Korhonen, T., Winberg, J., Cedergren, B., et al. (1982). Carbohydrate receptor structures recognized by uropathogenic *E. coli*. *Scandinavian Journal of Infectious Diseases*, 33, 52-60.
- Keane, W. F., Welch, R., Gekker, G., & Peterson, P.K. (1987). Mechanism of *Escherichia coli*  $\alpha$ -hemolysin-induced injury to isolated renal tubular cells. *The American journal of Pathology*, 126, 350-357.
- Kilian, M., & Bulow, P. (1976). Rapid diagnosis of Enterobacteriaceae : Use of a  $\beta$ - glucuronidase detecting agar medium for the identification of *Escherichia coli* in primary cultures of urine samples. *Acta Pathologica et Microbiologica Scandinavica*, B87, 271-276.
- Korhonen, T. K., Vaisanen, V., Saxen, H., Hultberg, H., & Svenson, S.B. (1982). P-antigen-recognizing fimbriae from humanuropathogenic *Escherichia coli* strains. *Infection and Immunity*, 37(1), 286-291.
- Korhonen, T. K., Parkkinen, J., Hacker, J., Finne, J., Pere, A., Rhen, M., et al. (1986). Binding of *Escherichia coli* S fimbriae to human kidney epithelium. *Infection and Immunity*, 54(2), 322-327.
- Kunin, C. M., White, L.V., & Hua, T.H. (1993). A reassessment of the importance of low- count bactiruria in young women with acute urinary symptoms. *Annals of Internal Medicine*, 119, 454-460
- Kunin, C. M. (1996). Urinary tract infection and Pyelonephritis. In J.C. Bennett & F. Plum (Eds.), *Cecil textbook of medicine* (20<sup>th</sup> ed., pp. 602-605). Philadelphia: WB Saunders Company.

- Kunin C. M. (1994). Urinary tract infections in females. *Clinical Infectious Diseases*, 18, 1-12
- Kvint, K., Nachin, L., Diez, A., & Nyström, T. (2003). The bacterial universal stress protein: Function and regulation. *Current Opinion in Microbiology*, 6, 140-145.
- Labigne-Roussel, A., Lark, D., Schoolnik, G., & Falkow, S. (1984). Cloning and expression of an afimbrial adhesin (*Afa-I*) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. *Infection and Immunity*, 46(1), 251-259.
- Labigne-Roussel, A., & Falkow S. (1988) Distribution and degree of heterogeneity of the afimbrial adhesin-encoding operon (*afa*) among uropathogenic *Escherichia coli* isolates. *Infection and Immunity*, 56(3), 640-648
- Labigne-Roussel, A., Schmidt, M.A., Walz, W., & Falkow, S. (1985). Genetic organization of the afimbrial adhesin operon and nucleotide sequence from a uropathogenic *Escherichia coli* gene encoding an afimbrial adhesin. *Journal of Bacteriology*, 162(3), 1285-1292.
- Langermann, S., Palszunski, S., Barnhart, M., Auguste, G., Pinkner, J.S. & Burlein, J. (1997). Prevention of mucosal *Escherichia coli* infection by Fim H-adhesion-based systematic vaccination. *Science*, 276, 607-611.
- Le Bouguenec, C., Archambaud M., & Labigne A. (1992). Rapid and specific detection of the *pap*, and *sfa* adhesion encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *Journal of Clinical Microbiology*, 30(5), 1189-1193
- Lefflier, H., & Svanborg Eden, C. (1980). Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. *FEMS Microbiology Letters*, 8, 127-134.
- Le Minor, L., Buisserie, J., Novel, C., & Novel, M. (1978). Relation entre le serotype et l'activite  $\beta$ -glucuronidase chez les Salmonella. *Annals of Microbiology*, 129B, 155-165
- Lindberg, F., Lund, B., Johansson, L., & Normark, S. (1987). Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature (London)*, 328, 84-87.
- Lo, Y.M.D. (1994). Detection of minority nucleic acid populations by PCR. *Journal of Pathology*, 174(1), 1-6.

- Lund, B., Lindberg, F., Marklund, B.I., & Normark, S.(1987).The *papG* protein is the  $\alpha$ -D-galactopyranosyl-(1-4)-p-D-galactopyranose- binding adhesin of uropathogenic *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 84, 5898-5902.
- Mackman, N., Nicaud, J. M., Gray, L., & Holland, I.B. (1986). Secretion of haemolysin by *Escherichia coli*. *Current Topics in Microbiology and Immunology*, 125, 159-181.
- Marcus, D. M., Kundu, S.K., & Suzuki, A. (1981). The P blood group system: Recent progress in immunochemistry and genetics. *Seminars in Hematology*, 18, 63-71.
- Marrs, C. F., Zhang, L., Tallman, P., Manning, S.D., Somsel, P., Raz, P., et al. (2002). Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*. *Journal of Medical Microbiology*, 51,138-142
- Mazoyer, M. A., Orenga, S., Doleans, F., & Freney, J. (1995). Evaluation of CPS ID2 medium for detection of urinary tract bacterial isolates in specimens from a rehabilitation center. *Journal of Clinical Microbiology*, 33(4), 1025–1027.
- Menestrina, G., Mackman, N., Holland, I.B., & Bhakdi, S. (1987). *Escherichia coli* hemolysin forms voltage-dependent ion channels in lipid membranes. *Biochimica et Biophysica Acta*, 905,109-117.
- Merlino, J., Siarakas, S., Robertson, G.J., Funnell, G. R., Gottlieb, T., & Bradbury, R. (1995). Evaluation of CHROMagar Orientation for the differentiation and presumptive identification of gram negative bacilli and Enterococcus species, poster 17. *Abstracts of the Annual Meeting of the Australian Society for Microbiology*.
- Mills, M., Meysick, K.C., & O'Brien, A.D. (2000). Cytotoxic necrotizing factor type 1 of uropathogenic *Escherichia coli* kills cultured human uroepithelial 5637 cells by an apoptotic mechanism. *Infection and Immunity*, 68(10), 5869-5880
- Milles, K. I. & Wren, M. W. D. (2005). Evaluation of three commercial agar preparations for the presumptive identification of significant urinary isolates. *British Journal of Biomedical Science*, 62, 179-182.
- Montgomerie, J. Z., Bindereif, A., Nielands, J.B., Kalmanson, G. M., & Guze, L. B. (1984). Association of hydroxamate siderophore (aerobactin) with *Escherichia coli* isolated from patients with bacteremia. *Infection and Immunity*, 46(3), 835-838.

- Muyzer, G., De Waal, E., & Uitierlinden, A.G. (1993). Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA. *Applied and Environmental Microbiology*, 59(3), 695-700
- Nataro, P.N. & Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, 11(1), 142-201
- Neilands, J. B., Bindereif, A., & Montgomerie, J.Z. (1985). Genetic basis of iron assimilation in pathogenic *Escherichia coli*. *Current Topics in Microbiology and Immunology*, 118, 179-195.
- Nowicki, B, Barrish, J.P., Korhonen, T., Hull, R.A., & Hull, S.I. (1987). Molecular cloning of the *Escherichia coli* O75X adhesin. *Infection and Immunity*, 55(12), 3168–3173.
- Nowicki, B., Labigne, A., Moseley, S., Hull, R., Hull, S., & Moulds, J. (1990). The Dr hemagglutinin, afimbrial adhesins *Afa-I* and *Afa-III*, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. *Infection and Immunity*, 58(1), 279–281.
- Nuñez, M. L., Diaz, J., Lorente, I., Pe´rez, I., & Ruiz, J. (1995). Evaluation of CPS ID2 medium for diagnosis of urinary infections. *European Journal of Clinical Microbiology & Infectious Diseases*, 14, 1111–1113.
- Nystro¨m, T., & Neidhardt, F.C. (1993). Isolation and properties of a mutant of *Escherichia coli* with an insertional inactivation of the *uspA* gene, which encodes a universal stress protein. *Journal of Bacteriology*, 175(13), 3949-3956.
- Nyström, T., & Neidhart, F.C. (1994). Expression and role of the universal stress protein, UspA, of *Escherichia coli* during growth arrest. *Molecular Microbiology*, 11, 537-544
- Nystro¨m, T., & Neidhardt F.C. (1996). Effects of overproducing the universal stress protein, UspA, in *Escherichia coli* K-12. *Journal of Bacteriology*, 178(3), 927-930.
- Oelschlaeger, T.A., Dobrint, U., Hacker, J. (2002). Pathogenicity islands of uropathogenic *E. coli* and the evolution of virulence. *International Journal of Antimicrobial Agents*, 19, 517-21
- O'Hanley, P., Low, D., Romero, I., Lark, D., Vosti, K., Falkow, S., et al. (1985). Gal-Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. *The New England Journal of Medicine*, 313, 414-420.



- Orenga, S., Barbaux, L., Berenguer, L., Doleans, F., Monget, D., Moser, C., et al. (1993). Urinary tract infections: improved CPS ID, a new ready-to-use medium for enumeration and identification of *Escherichia coli*, *Proteae*, and *Enterococcus*. *Journal of Rapid Methods and Automation in Microbiology*, 11, 32-33.
- Orskov, F., & Orskov, I. (1992). *Escherichia coli* serotyping and disease in man and animals. *Canadian Journal of Microbiology*, 38, 699-704
- Orskov, I. & Orskov, F. (1985). *Escherichia coli* in extraintestinal infections. *Journal of Hygiene*, 95, 551-575.
- Ott, M., Hacker, J., Schmoll, T., Jarchau, T., Korhonen, T. K. & Goebel, W. (1986). Analysis of the genetic determinants coding for the S-fimbrial adhesin (*sfA*) in different *Escherichia coli* strains causing meningitis or urinary tract infections. *Infection and Immunity*, 54(3), 646-653.
- Parkkinen, J., Finne, J., Achtman, M., Vaisanen, V., & Korhonen, T.K. (1983). *Escherichia coli* strains binding neuraminyl  $\alpha$ 2-3 galactosides. *Biochemicals and Biophysical Research Communications*, 111, 456-461
- Perry, J.D., Rennison, C., Butterworth, L.A., Hopley, A.L.J., & Gould, F.K. (2003). Evaluation of S. aureus ID, a new chromogenic agar medium for detection of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 41(12), 5695-5698
- Pezzlo, M. (1988). Detection of urinary tract infections by rapid methods. *Clinical Microbiology Reviews*, 1(3), 268-280
- Plos, K., Connell, H., Jodal, U., Marlund, B.I., Marild, S., Wettergren, B. (1995). Intestinal carriage of P fimbriated *Escherichia coli* and the susceptibility to urinary tract infection. *Journal of Infectious Diseases*, 171, 625-31
- Ramkrishnan, K. & Scheid, D.C. (2005). Diagnosis and management of acute pyelonephritis in adults. *American Family Physician*, 71(5), 933-42
- Ronald, A. (2002). The etiology of urinary tract infection: Traditional and emerging pathogens. *The American Journal of Medicine*, 113(1A), 15S
- Ruis, J., Simon, K., Horcajada, J.P., Velasco, M., Barranco, M., Roig, G., et al. (2002). Differences in virulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men. *Journal of Clinical Microbiology*, 40(12), 445-4449

- Rushton, H. G. (1997). Urinary tract infections in children. Epidemiology, evaluation, and management. *Pediatrics Clinics of North America*, 44, 1133–1169.
- Russo, T.A. & Johnson J.R. (2000). A proposal for an inclusive designation for extraintestinal pathogenic *Escherichia coli*: ExPEC. *Journal of Infectious Diseases*, 181, 1753-1754
- Russo, T.A. & Johnson, J.R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and Infection*, 5, 449–456
- Samra, Z., Heifetz, M., Talmor, J., Bain, E., & Bahar J. (1998). Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. *Journal of Clinical Microbiology*, 36(4), 990-994
- Santo, E., Macedo, C., & Marin, J.M. (2006). Virulence factors of uropathogenic *Escherichia coli* from a University Hospital in Ribeirão Preto, São Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao. Paulo*, 48(4), 185-188.
- Scarpato, C., Piccoli, P., Ricordi, P., & Scagnelli, M. (2002). Comparative evaluation of two commercial media for detection and presumptive identification of urinary tract pathogens. *European Journal of Clinical Microbiology & Infectious Diseases*, 21, 283-289
- Schaechter, M. & Neidhardt, F.C. (1987). Introduction. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, & H. E. Umbarger (Eds.), *Escherichia coli and Salmonella typhimurium: Cellular and molecular biology* (Vol. 1, pp. 1-2). Washington, D.C.: American Society for Microbiology.
- Shayegani M., Maupin, P.S. & McGlynn, D.M. (1978). Evaluation of the API 20E system for identification of nonfermentative gram negative bacteria. *Journal of Clinical Microbiology*, 7(6), 539-545
- Short, E. C. & Kurtz, H.J. (1971). Properties of the hemolytic activities of *Escherichia coli*. *Infection and Immunity*, 3(5), 678-687.
- Singh, A., Goering, R.V., Simjee, S., Foley, S.L., & Zervos, M.J. (2006). Application of molecular techniques to the study of hospital infection. *Clinical Microbiology Reviews*, 19, 512-530
- Slak, R. (1984). Review of bacterial resistance: A challenge to the treatment of urinary infection. *The Journal of Antimicrobial Chemotherapy*, 13(Suppl. B): 1-7.

- Springer, W. & Goebel, W. (1980). Synthesis and secretion of hemolysin by *Escherichia coli*. *Journal of Bacteriology*, 144(1), 53-59.
- Stamm, W. & Hooton T. (1993). Management of urinary tract infection in adults. *The New England Journal of Medicine*, 329, 1328–1334.
- Stevens, M. (1989). Screening urine for bactiruria. *Medical Laboratory Sciences*, 46, 194-206.
- Sussman, M. (1985). Theodore Escherich (1857-1911): A biographical note. In M. Sussman (Ed.), *The virulence of Escherichia coli: reviews and methods* (pp. 1-4). London, United Kingdom: Academic Press.
- Tang, Y.W, Gary, W., Procop, F., David, H., & Persing, M. (1997). Molecular diagnostics of infectious diseases. *Clinical Chemistry*, 43, 2021-2038
- Tobias, A. O., Dobrindt, U., & Hacker, J. (2002). Virulence factors of uropathogens. *Current Opinion in Urology*, 12, 33-38.
- Tsai, Y.L. & Olson, B.H. (1992). Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied and Environmental Microbiology*, 58(7), 2292-2295.
- Usein, C.R., Damian, M., & Tatu-Chitou, D. (2001). Prevalence of virulence genes in *Escherichia coli* strains isolated from romanian adult urinary tract infection cases. *Journal of Cellular and Medical Medicine*, 5, 303-310.
- Väisänen-Rhen, V. (1984). Fimbria-like hemagglutinin of *Escherichia coli* O75 strains. *Infection and Immunity*, 46(2), 401–407
- Weinberg, E. D. (1978). Iron and infection. *Microbiological Reviews*, 42, 45-66.
- Welch, R. A., Hull, R., & Falkow, S. (1983). Molecular cloning and physical characterization of a chromosomal hemolysin from *Escherichia coli*. *Infection and Immunity*, 42(1), 178-186.
- Welch, R.A., Burland, V., Plunkett, G., Redford, P., Roesch, P., Rasko, D., et al. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *PNAS*, 99, 17020–17024.
- Welsh, J., McClelland, M., 1990. Fingerprinting genomes using using arbitrary primers. *Nucleic Acids Research*, 18(24), 7213- 7218.
- Williams, D.H. & Schaeffer A.J. (2004). Current concepts in urinary tract infections. *Minerva Urologica e Nefrologica*, 56(1), 15-31.

- Willinger, B. & Manafi, M. (1995). Evaluation of a new chromogenic agar medium for the identification of urinary tract pathogens. *Letters in Applied Microbiology*, 20, 300–302.
- Wold, A. E., Thorsen, M., Hull, S., & Svanborg Eden, C. (1988). Attachment of *Escherichia coli* via mannose- or Gal $\alpha$ 1-4Gal, B-containing receptors to human colonic epithelial cells. *Infection and Immunity*, 56(10), 2531-2537.
- Yamamoto, S., Terai, A., Yuri, K., Kurazono, H., Takeda, Y., Yoshida, O. (1995). Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immunology and Medical Microbiology*, 12, 85-90
- Yamamoto, S., Tsukamoto, A., Terai, A., Kurazono, Y., Takeda, Y., Yoshida, O. (1995). Distribution of virulence factors in *Escherichia coli* isolated from urine of cystitis patients. *Microbiology and Immunology*, 39, 401-404.
- Zorc, J. J., Kiddoo, D.A., & Shaw, K.N. (2005). Diagnosis and management of pediatric urinary tract infections. *Clinical Microbiology Reviews*, 18(2), 417-422.