Study of in Vitro Susceptibility of Fosfomycin among *Enterobacteriaceae* Clinical Isolates Causing Community Acquired Urinary Tract Infection

Thesis

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بسم الله الرحمن الرحيم

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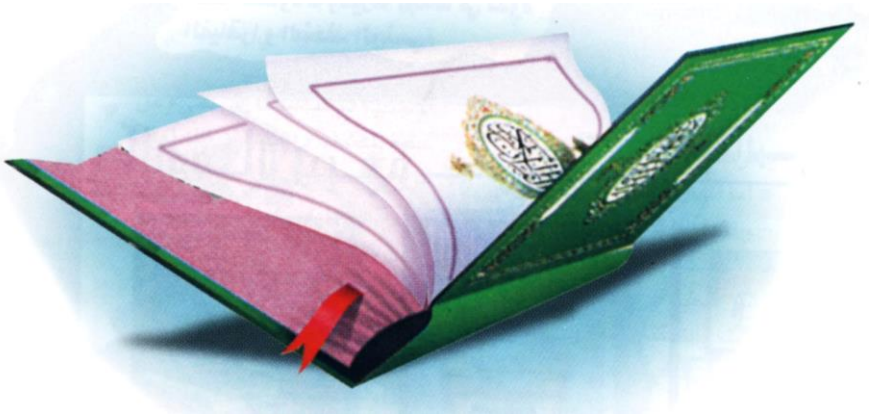

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List of Abbreviations

Abb.	Full term
A. baumanii	*Acinetobacter baumanii*
AK	*Amikacin*
AMC	*Amoxicillin-Clavulanate*
AMP	*Antimicrobial peptide*
AmpC	*Ampicillin class C beta-lactamase*
cAMP	*Cyclic adenosine monophosphate*
CAZ	*Ceftazidime*
CD	*Cluster of differentiation*
CIP	*Ciprofloxacin*
CLSI	*Clinical & Laboratory Standards Institute*
CN	*Gentamycin*
COT	*Trimethoprim-Sulfamethoxazole*
CRE	*Carbapenem-resistant Enterobacteriaceae*
CTX	*Cefotaxime*
CTX-M	*Active on Cefotaxime, first isolated in Munich*
DDST	*Double disc synergy screening test*
DNase	*Deoxyribonuclease*
dsDNA	*Double-stranded Deoxyribonucleic acid*
E test	*Epsilometer test*
E. coli	*Escherichia coli*
EDTA	*Ethylene diamine tetra-acetic acid*
ESBL	*Extended-Spectrum β-Lactamase*
F	*Nitrofurantoin*
FEP	*Cefepime*
FO	*Fosfomycin*
ICAM-1	*Intercellular adhesion molecule-1*
Ig	*Immunogloulin*
IL	*Interleukin*
IMP	*Imipenemase*
IPM	*Imipenem*
K. pneumoniae	*Klebsiella pneumoniae*
KPC	*Klebsiella pneumoniae carbapenemase*
MBL	*Class B Metallo-β-Lactamase*
MDR	*Multi-drug resistant*

List of Abbreviations cont...

Abb.	Full term
MGE	*Mobile genetic element*
MIC	*Minimum inhibitory concentration*
MRSA	*Methicillin resistant S. aureus*
NOR	*Norfloxacin*
OXA	*Oxacillin hydrolyzing enzymes*
P. aerogenosa	*Pseudomonas aerogenosa*
PBP	*Protein binding protein*
PCR	*Polymerase chain reaction*
Rho GTPase	*Ras homologous (Rho) guanine tri-phosphatase*
RNase	*Ribonuclease*
S. aureus	*Staphylococcus aureus*
SHV	*Sulfhydryl variable*
TEM	*Temoniera*
TLR	*Toll like receptor*
UPEC	*Uropathogenic E. coli*
UTI	*Urinary tract infection*

INTRODUCTION

Urinary tract infections (UTIs) are the most common type of human bacterial infections ***(Maraki et al., 2009)***. Over 150 million cases of UTIs occur annually in the world ***(Sultan et al., 2015)***. It is caused by a range of pathogens, but most commonly by *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae) subspecies aerogenes, and Proteus mirabilis* ***(Orhue et al., 2012 Flores - Mireles et al., 2015)***.

The prevalence of antibiotic resistance among uropathogenic *Enterobacteriaceae* is remarkably high ***(Yeganeh-Sefidan et al., 2016)***.These cases represent a therapeutic challenge to physicians as the availability of treatment options is limited ***(Linsenmeyer et al., 2016)***. This rapid increase in antibiotic resistance necessitates searching for alternatives. Since the availability of new antimicrobial agents is limited, so reevaluation of older antibiotic agents may be of help ***(Mashaly, 2016)***.

Among older antibiotics, Fosfomycin is an attractive choice since it is a broad spectrum antibiotic that is indicated in treatment of UTI ***(Michalooulos et al., 2011- Matthews et al., 2016)***. It is a bactericidal antibiotic that interferes with cell wall synthesis in both Gram-negative and Gram-positive bacteria ***(Michalooulos et al., 2011)***.

Unfortunately, limited data is available about the effectiveness of Fosfomycin against *Enterobacteriaceae* as agents of UTI in Egypt. Moreover, many studies reported resistance to Fosfomycin in areas where it is widely used ***(Oteo et al., 2009)***. Several mechanisms are implicated in this resistance such as reduced permeability, modification of *murA* gene target but the most important is plasmid mediated Fosfomycin modifying enzymes that act by inactivating the antibiotic ***(Karageorgopoulos et al., 2011)***.

AIM OF THE WORK

The aim of this study is to determine the susceptibility profile of *Enterobacteriaceae* isolated from UTIs to Fosfomycin and to detect plasmid mediated Fosfomycin resistance genes (*fosA*, *fosB* and *fosA3*) by using polymerase chain reaction (PCR).

Chapter 1

URINARY TRACT INFECTION

Urinary tract infections (UTIs) are the most common type of human bacterial infections ***(Maraki et al., 2009)***. Over 150 million cases of UTIs occur annually in the world ***(Sultan et al., 2015)***.This represents a high financial burden on health care system. UTI can be community-acquired or health-care related occurring in both males and females ***(Mann et al., 2017)***.

UTI is classified into complicated and uncomplicated UTI. Uncomplicated UTI occurs in healthy individual having no problems in the urinary tract. It usually affects children, females and old people. It can be further divided into upper UTI, most commonly pyelonephritis, and lower UTI, most commonly cystitis ***(Hooton, 2012)***.

Manifestations of lower UTI include dysuria, frequency and urgency, whereas manifestations of upper UTI include fever and loin pain which are usually in association with manifestations of lower UTI ***(Stapleton, 2014)***. Bloody urine is a rare finding ***(Salvatore et al., 2011)***. Uncomplicated UTI usually resolves by host's immunity even without antibiotic treatment and rarely causes serious damage ***(Hooton, 2012)***. Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection,

diabetes, obesity and genetic susceptibility ***(Hannan et al., 2012 - Foxman, 2014)***.

Complicated UTI occurs in patients with urinary tract abnormalities such as obstruction, retention, immunosuppression and renal failure and in cases of previous antibiotic exposure. These factors increase the risk of serious complications and treatment failure. Prolonged treatment and increased risk of chronicity and/or recurrence are usual associations with complicated UTI ***(Mann et al., 2017)***.

UTI is caused by a range of pathogens, most commonly bacteria, but also fungi and some viruses have some role. Considering bacteria, Gram-negative bacteria of the *Enterobacteriaceae* family, such as *E. coli*, *Klebsiella*, *Proteus* species, *etc*., are the most common agents. Some Gram-positive organisms namely; *Staphylococcus aureus (S. aureus), Staphylococcus saprophyticus* and *Streptococcus agalactiae*, also have a role especially among young females ***(Samie, 2017)***. Among them, Uropathogenic *E. coli* (UPEC) accounts for up to 75% of all cases and 95% of community-acquired cases ***(Mann et al., 2017)***.

Pathogenesis:

UTI usually starts when a pathogen residing in the gut contaminates and colonizes the urethra and migrates up to the bladder followed by invading its epithelium. In the bladder, the outcome of host–pathogen interactions determines whether the

uropathogen will colonize or will be eliminated ***(Flores-Mireles et al., 2015)***. UTI can progress from the bladder, through the ureters to the kidney, to cause pyelonephritis which may lead to kidney damage resulting in kidney failure. Moreover uropathogens may get access to the blood stream, resulting in septicemia ***(Baby et al., 2016)***.

Virulence Factors of UPEC:

Virulence Factors are the specific features of organisms that allow them to overcome host defenses and cause disease ***(Samie, 2017)***. A number of virulence factors encoded by UPEC allows the bacteria to colonize the urinary tract and overcomes the highly effective host defense. In UPEC, specialized virulence genes are found on mobile genetic elements called pathogenicity islands. These virulence factors can be divided into two groups: (1) surface virulence factors and (2) secreted virulence factors ***(Bien et al., 2012)***.