

Detection of resistance patterns among nosocomial urinary tract infection

Thesis

**Submitted for fulfillment of degree of master in pharmaceutical science
(Microbiology and Immunology)**

Presented by

Mahmoud Abd El- Ati Fouad

B.Sc. Faculty of pharmacy (MUST)

Under the supervision of

Prof. Dr: Abd El-Gawad M.Hashem

**Chairman of Microbiology and immunology department, Cairo University
And Professor of microbiology and immunology faculty of pharmacy
(MUST)**

Dr: Wael Mostafa Tawakkol

**Chairman of and assistant Professor of microbiology and immunology
department faculty of pharmacy (MUST)**

Faculty of pharmacy

Cairo University

2009

Acknowledgment

*I am deeply thankful to **Allah** who helps and supports me in all my steps and who has already granted me the strength and patience to achieve this work.*

*On the accomplishment of the present study I would like to take this opportunity to extend my deepest sense of gratitude and words of appreciation towards those, who helped me during the pursuit of this study. I deem it a proud privilege and feel immense pleasure to acknowledge all those who are directly or indirectly involved. Words are inadequate in the available lexicon to avouch the excellent guidance given by my major advisor **Prof. Dr: Abd El-Gawad M. Hashem**, head of microbiology and immunology department; faculty of pharmacy, Cairo university. His dedication to research, meticulous planning, consecutive counsel and unreserved help served as a beacon light throughout the present study, research work and completion of this work.*

*I express my heart-felt gratitude to, **Dr. Weal Tawakkol**, Assistant Professor of and head of microbiology and immunology department faculty of pharmacy at Misr University for science and technology (MUST), for his consistent and invaluable, inspirations, prolific and introspective guidance with constructive suggestions, deliberative discussions and active persuasion throughout this study.*

*I respectfully acknowledge and express my profound thanks to staff members of Department of Microbiology and Immunology faculty of pharmacy (MUST) for their unreserved help and continuous motivation. I am really falling short of words to express my gratitude to **Tamer Mohamed samir** for his keen interest, sustained encouragement and support during the entire work.*

*I respectfully acknowledge and express my profound thanks to staff members of faculty of biotechnology at MUST for their timely help during the experiments. I extend my affable thanks to Dr. **Mohamed Mostafa**, for his keen interest, sustained encouragement and providing use of essential equipments for successful completion of this research work, also special thanks to Mr. **Mohamed A. Ezz-Alregal** bioinformatics specialist for actively participating in the bioinformatics analysis of this thesis.*

My vocabulary utterly fails in expressing my accolade to my revered parents who brought me to this stage. I deeply express my sincere thanks to my family members whose continuous inspiration, encouragement and affection, boosted up my morale during the period of study. I apologize for the faux pass of the persons who have extended the help in a way or other and deserved such thanks.

LIST OF CONTENT

CONTENT	Page
1-INTRODUCTION	1
2-AIM OF WORK	2
3- LITERATURE REVIEW	3
1) Nosocomial infection	3
1.1) Definitions of nosocomial infection	3
1.2) Impact of nosocomial infections	3
1.3) Frequency of infection	4
1.4) Nosocomial infection sites	4
2) Nosocomial urinary tract infections (NUTIs)	6
2.1) Epidemiology	7
2.2) Epidemics of Nosocomial UTIs	8
2.3) Magnitude of the Problem	9
2.3.1. Incidence and Costs	9
2.3.2. Mortality	10
2.3.3. Morbidity.....	11
2.4) Pathogenesis	12
2.4.1. Pathways of Infection	13
2.4.2. Host Factors	15
2.4.3. Role of catheter	16
2.5) Etiologic agents	18
<i>E. coli</i>	19
<i>Klebsiella spp</i>	20
<i>Pseudomonas aeruginosa</i>	20
<i>Proteus mirabilis</i>	21
<i>Acinetobacter spp</i>	22
<i>Staphylococcus spp</i>	22

3) Treatment of Urinary tract infection	23
3.1) β -lactam antibiotics	23
3.1.1. Mechanism of action of β -lactam	25
3.1.2. Mechanism of resistance to β -lactam	27
3.2) Quinolones	31
3.2.1. Quinolones class members	32
3.2.2. Mechanisms of Quinolone Action	32
3.2.3. Mechanisms of Quinolone Resistance	35
3.3) Nitrofurantoin	35
3.3.1. Mechanism of action of nitrofurantoin	36
4) Chemical antimicrobial agents (biocides) and the prevention of infectious disease	37
▪ Pathogenic organisms persist in the environment	40
5) Plasmid	42
▪ Resistance Plasmids and its Transfer	42
6) Prevention of nosocomial urinary tract infection	44
6.1. Limiting Catheter Usage	44
6.2. Catheters Containing Antimicrobial Agents	45
6.3) Probiotics	45
4-MATERIAL AND METHODS	46
i. Patients	46
ii. Urine samples	46
iii. Materials	46
A) Chemicals	46
B) Media	47
C) Reagents and Solutions	53
iv. Methods	59
(1) Collection and processing of urine samples	59
(2) Collection of data from the patient	60
(3) Isolation and identification of bacteria	62
(4) Determination of the Susceptibility to antimicrobial agents	67
(5) Detection of β -lactamase	69
(6) Phenotypic identification of the β -lactamases type	69
(7) Detection of efflux activity	70
(8) Plasmid DNA	71
(9) Random Amplified Polymorphic DNA – PCR	73

5- RESULTS	74
6-DISCUSSION	121
7- Recommendation for Multidrug- resistant organisms (MDRO) Prevention and Control	139
8-SUMMARY	146
9- CONCLUSION	148
10-REFERENCE	150
11- ARABIC SUMMARY	

LIST OF TABLES

Title	Page
Table (1): distribution and frequency of site of nosocomial infections	4
Table (2): β –lactam antibiotics generation	26
Table (3): Classification scheme for bacterial β -lactamase.....	30
Table (4): quinolone classes& members	32
Table (5): <i>Chemical structures and uses of biocides in antiseptics and disinfectants</i>	38
Table (6): <i>summary of mechanism of action of various disinfectants and antiseptics</i>	40
Table (7): Relation between different microorganisms (M.O) with variable age and gender ...	75
Table (8): The distribution of varied age ranges with the gender.....	76
Table (9): Distribution of catheter associated urinary tract infection among hospitalized patients	77
Table (10): Antimicrobial susceptibility level of <i>E .coli</i> isolated from out-patient.....	79
Table (11): one – way analysis of variance (ANOVA) of resistance level data of <i>E .coli</i> isolated from out-patient	80
Table (12): Antimicrobial susceptibility level of <i>Klebsiella</i> isolated from out-patient.....	81
Table (13): one – way analysis of variance (ANOVA) of resistance level data of <i>Klebsiella pneumonia</i> isolated from out-patient.....	81
Table (14): Antimicrobial susceptibility level of <i>Pseudomonas aeruginosa</i> isolated from out-patient	82
Table (15): one – way analysis of variance (ANOVA) of resistance level data of <i>Pseudomonas aeruginosa</i> isolated from out-patient.....	82
Table (16): Antimicrobial susceptibility level of <i>Proteus mirabilis</i> isolated from out-patient...	83
Table (17): one – way analysis of variance (ANOVA) of resistance level data of <i>Proteus mirabilis</i> isolated from out-patient.....	83
Table (18): Antimicrobial susceptibility level of <i>Staphylococcus</i> spp. isolated from out-patient.....	84
Table (19): one – way analysis of variance (ANOVA) of resistance level data of <i>Staphylococcus spp.</i> isolated from out-patient.....	84
Table (20): Antimicrobial susceptibility level of <i>Streptococcus</i> spp. isolated from out-patient..	85
Table (21): one – way analysis of variance (ANOVA) of resistance level data of <i>Streptococcus spp.</i> isolated from out-patient.....	85
Table (22): Total community isolates susceptibility level.....	86
Table (23): one – way analysis of variance (ANOVA) of resistance level data of Total community isolates.....	86
Table (24): Frequency and resistance pattern of most frequently occurring community-UTI pathogens against 5 selected antimicrobial agents tested	88
Table (25): Antimicrobial susceptibility level of <i>E .coli</i> isolated from in-patient.....	89
Table (26): one – way analysis of variance (ANOVA) of resistance level data of <i>E .coli</i> isolated from in-patient.....	90
Table (27): Antimicrobial susceptibility level of <i>Klebsiella</i> isolated from in-patient.....	91

Table (28): one – way analysis of variance (ANOVA) of resistance level data of <i>Klebsiella pneumoniae</i> isolated from in-patient.....	91
Table (29): Antimicrobial susceptibility level of <i>Pseudomonas</i> isolated from in-patient.....	92
Table (30): one – way analysis of variance (ANOVA) of resistance level data of <i>Pseudomonas aeruginosa</i> isolated from in-patient.....	92
Table (31): Antimicrobial susceptibility level of <i>Proteus mirabilis</i> isolated from in-patient	93
Table (32): one – way analysis of variance (ANOVA) of resistance level data of <i>Proteus mirabilis</i> isolated from in-patient.....	93
Table (33): Antimicrobial susceptibility level of <i>Staphylococcus spp.</i> isolated from in-patient.	94
Table (34): one – way analysis of variance (ANOVA) of resistance level data of <i>Staphylococcus spp</i> isolated from in-patient.....	94
Table (35): Antimicrobial susceptibility level of <i>Acinetobacter spp.</i> isolated from in-patient...	95
Table (36): one – way analysis of variance (ANOVA) of resistance level data of <i>Acinetobacter baumannii</i> isolated from in-patient.....	95
Table (37): Total antimicrobial susceptibility level of isolates from in-patient	96
Table (38): one – way analysis of variance (ANOVA) of resistance level data of Total isolates from in-patient.....	97
Table (39): Frequency and resistance pattern of most frequently occurring nosocomial UTI pathogens against 5 selected antimicrobial agents tested	99
Table (40): Relationship between antimicrobial resistance to different chemotherapeutics agents	100
Table (41): Antibiotic susceptibility profile for the eleven MDR isolates.....	102
Table (42): Minimum inhibitory concentration of antimicrobial chemotherapeutic agent.....	103
Table (43): Minimum inhibitory concentration of antimicrobial chemical biocide	103
Table (44): Result of double disc synergy test for ESBL detection.....	105
Table (45): Double disc synergy test for detection of AmpC.....	107
Table (46): Minimum inhibitory concentrations values of tested antimicrobial agent in presence and absence of efflux pump inhibitor	109
Table (47): Molecular size (kb) of Plasmid DNA molecules extracted from Tested MDR-isolates.....	112
Table (48): Molecular size (kb) of Plasmid DNA molecules extracted from wild type and SDS treated of the tested bacterial strains	114
Table (49): Minimum inhibitory concentrations values of both wild type and plasmid cured isolates.....	115
Table (50): Patient profile data of the MDR-isolates.....	116

LIST OF FIGURES

Title	Page
Figure (1): distribution and frequency of sites of nosocomial infections	5
Figure (2): member of the β -lactam family.....	24
Figure (3): Nitrofurantoin structure	36
Figure (4): identification of organisms isolated from urine	60
Figure (5): Flow diagram depicting the LOGIC system for biochemical identification of Enterobacteriaceae isolates from urine.....	61
Figure (6): Gender distribution among hospital and community acquired UTI.....	74
Figure (7): Age and gender distribution of nosocomial urinary tract infection.....	76
Figure (8): Distribution of catheter associated urinary tract infection among hospitalized patients.....	77
Figure (9): Incidence of microorganisms isolated from urine samples of out-patients suffering from UTI	78
Figure (10): Incidence of microorganisms isolated from urine samples of in-patients suffering from UTI	78
Figure (11): <i>E. coli</i> susceptibility level among community isolates.....	79
Figure (12): <i>Klebsiella</i> susceptibility level among community isolates.....	80
Figure (13): <i>Pseudomonas aeruginosa</i> susceptibility level among community isolates.....	81
Figure (14): <i>Proteus mirabilis</i> susceptibility level among community isolates.....	82
Figure (15): <i>Staphylococci spp.</i> susceptibility level among community isolates.....	84
Figure (16): <i>Streptococci spp.</i> susceptibility level among community isolates.....	85
Figure (17): Total susceptibility level among community isolates.....	86
Figure (18): Resistance level of microorganisms isolated from out-patient suffering (UTI) to different antimicrobial chemotherapy	87
Figure (19): <i>E. coli</i> susceptibility level among nosocomial isolates.....	89
Figure (20): <i>Klebsiella pneumonia</i> susceptibility level among nosocomial isolates.....	90
Figure (21): <i>Pseudomonas aeruginosa</i> susceptibility level among nosocomial isolates.....	92
Figure (22): <i>Proteus mirabilis</i> susceptibility level among nosocomial isolates.....	93
Figure (23): <i>Staphylococci spp.</i> susceptibility level among nosocomial isolates.....	94
Figure (24): <i>Acinetobacter</i> susceptibility level among nosocomial isolates.....	95
Figure (25): Total susceptibility level among nosocomial isolates.....	96
Figure (26): Resistance level of microorganisms isolated from in-patient suffering (NUTI) to different antimicrobial chemotherapy	98
Figure (27): Relationship and interaction of antimicrobial resistance to different chemotherapeutics agents	100
Figure (28): Double disc synergy test indicates Positive <i>ESBL</i> in <i>Klebsiella pneumonia</i>	106
Figure (29): Double disc synergy test indicates Positive <i>ESBL</i> in <i>E. coli</i>	106
Figure (30): Double disc synergy test indicates Positive <i>ESBL</i> in <i>pseudomonas aeruginosa</i>	106
Figure (31): Double disc synergy test indicates Positive <i>ESBL</i> in <i>Acinetobacter baumannii</i>	106
Figure (32): Double synergy test show positive AmpC in MDR <i>Klebsiella pneumonia</i>	108
Figure (33): Double synergy test show positive AmpC in MDR <i>E coli</i>	108
Figure (34): Double synergy test show positive AmpC in MDR <i>Pseudomonas aeruginosa</i>	108
Figure (35): Double synergy test show positive AmpC in <i>Acinetobacter baumannii</i>	108
Figure (36): plasmid extraction of multi drug resistant <i>E.coli</i> and <i>Klebsiella pneumonia</i>	110

Figure (37): plasmid extraction of multi drug resistant <i>pseudomonas aeruginosa</i> and <i>Acinetobacter baumannii</i>	111
Figure (38): Plasmid extraction results of both wild type and SDS cured isolates	113
Figure (39): Random amplified polymorphic-PCR typing of the MDR-isolates	117
Figure (40): Analysis of the Random amplified polymorphic-PCR (RAPD-PCR) results	118
Figure (41): Dendrogram illustrate the relation between the MDR-isolates	119
Figure (42): Dendrogram illustrate the correlation between the analyzed data and PCR result of the MDR-isolates	120
Figure (43): Comparison between species distribution of uropathogens in the current study and other urinary tract infection studies	123

LIST OF ABBREVIATION

• AmpC	β -lactamase (Class C)
• ASC	Active Surveillance Culture
• AZT	Aztreonam
• BAC	Benzalkonium chloride
• Cb	Carbacillin
• CDC	Centers for Disease Control
• Ceph	Cephaloridine
• CLSI	Clinical and Laboratory Standards Institute
• CMY	Class (C) β -lactamases active on cephamycin
• CNF1	Cytotoxic necrotizing factor 1
• CoNS	Coagulase-negative staphylococci
• CPC	Cetylpyridinium chloride
• CSU	Catheter sample urine
• CTAB	Cetyl trimethyl ammonium bromide
• CTX-M	Active on Cefotaxime
• CX	Cefotaxime
• DDS	Double disc synergisme
• DMF	Di-Methyl Forfamide
• DMSO	Di-Methyl sulphoxide
• DNP	Dinitrophenol
• EDTA	Ethylene diamine tetra acetic acid
• EPI	Efflux pump inhibitors
• EPS	Extra-cellular polymeric substances
• ESBLs	Extended spectrum β -lactamase
• GIM	German imipenemase
• GNB	Gram Negative Bacteria
• HCP	Hand hygiene, Standard and Contact Precautions
• ICU	intensive care unit
• IMP	active on Imipenem
• IP	Imipenem
• LB	Luria-Bretani
• LTCF	Long-term care facilities
• MBLs	Metallo- β -lactmase
• MDR-GNB	Multidrug resistance gram negative bacteria

• MDRO	Multidrug resistance organisms
• MIC	Minimum inhibitory concentration
• MR- VP medium	Methyl red – voges proskauer
• MRSA	Methicillin-resistant Staphylococcus aureus
• MSA	Mannitol salt agar
• MSSA	Methicillin sensitive Staphylococcus aureus
• NAG	N-acetylglucosamine
• NAM	N-acetyl muramic acid
• NAUTIs	Nosocomial acquired urinary tract infections
• NCCLS	National committee for clinical laboratory standards
• NICU	Neonatal intensive care unit
• NNIS	National Nosocomial Infection Surveillance
• NUTI	Nosocomial urinary tract infection
• Omp	outer membrane protein
• Oxa	Oxacillin
• OXA	Oxacillinase group of β -lactamases (Class D)
• PBPs	penicillin-binding-proteins
• PCR	polymerase chain reaction
• PER	pseudomonas extended resistant
• PFGE	Pulsed Field Gel Electrophoresis
• PG	peptidoglycan
• Pn	penicillin
• QRDR	qunilone resistance-determing region
• RAPD	Random amplified polymorphic DNA
• RND	Resistance nodulation division family
• SDS	Sodium dodecyle sulafate
• SHV	Sulphahydryl region variable
• SPA	Suprapubic aspirate
• SPM	Sao Paulo metallo beta lactmase
• SXT	Cotrimoxazole
• TAE	Tris acetic acid edta
• TE	Tris-edta
• TEM	Named after the patient (Temoneira)
• TSI	Triple sugar iron
• TSN	The Surveillance Network
• UPEC	Uropathogenic E.coli

• UTI	Urinary tract infection
• VF	Virulence factor
• VIM	Verona integron-encoded metallo-b-lactamase
• VRE	vancomycin resistance enterococci

ABSTRACT

Urinary tract infection (UTI) is the most common type of nosocomial infections, whereby urinary tract represents the main site for 40% of nosocomial infections . UTI can be associated with substantial morbidity and significant expenditure.

In relation to the antimicrobial susceptibility of the out- patients isolates, its clear that nitrofurantoin appeared to be the most effective antimicrobial agent against *E. coli* as 93.75% of *E. coli* isolates were susceptible to nitrofurantoin followed by ceftazidime as 76.5% *E. coli* were susceptible; while for other isolates including gram negative bacteria, *Staphylococci spp* and *streptococci spp* isolates ciprofloxacin and norfloxacin were the most effective antimicrobial agent comparative with the tested antimicrobial agent, as 100% of *klebsiella* isolates; and 78% of the total isolates were susceptible to the tested quinolone. While within the hoaspital isolates *E. coli* was responsible for 50% of urinary tract infection, followed by *klebsiella spp.* 30%, *P. aeruginosa* 10.5%, *S. aureus* 3.5%, *Proteus mirabilis* 4.5%, and *Acinetobacter baumannii* 1.5%, Also, infection more distributed in female than male 57%, 43% respectively.

Escherichia coli was also the commonest cause of nosocomial urinary tract infections (UTIs). It was responsible for 50% of infections, followed by *Klebsiella spp* 30%, *Pseudomonas aeruginosa* 10.5%, *Proteus mirabilis* 4.5%, *Acinetobacter spp.* 1.5% and *Staphylococci spp.* 3.5%.

Our data also showed a substantial reduction in susceptibility to antibiotics in hospital associated infection rather than that associated with community whereby, eleven isolates among the hospital isolates and none among the community isolates showed 100% resistance to the five tested antimicrobials and also to other antimicrobial classes with exception of amikacin which founded to be effective against the MDR-isolates.

Plasmid profile analysis of these isolates revealed that all of them harbor at least one plasmid. Plasmid curing revealed the role of plasmid in mediating both β -lactam and quinolone resistance resistances of those isolates. Molecular typing using random amplified polymorphic PCR prove the presence of nosocomial infection as it showed the responsibility of one isolate for infection among different patients.

INTRODUCTION

1- Introduction:

First of all, urinary tract infection (UTI) is the first type of nosocomial infections whereby urinary tract represents the main site for 40% of nosocomial infections; in addition, it is the second most common infectious presentation in community practice. Worldwide, about 150 million are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars annually. Urinary tract infections (UTIs) can be associated with substantial morbidity and significant expenditure. Nosocomial UTI may lead to bacteremia with a subsequent mortality up to 30%. The single greatest risk factor for nosocomial UTI is urinary catheterization, approximately 10% of all patients will be catheterized during their hospital stay for a mean of four days. The risk of developing bacteriuria is about 5% for each day a patient is catheterized. Up to 20% of catheterized patients will develop bacteriuria and up to 6% develop symptoms of UTI; therefore, it is the reason behind having such an incidence rate.

An indwelling urinary catheter can predispose a patient to a UTI in several different ways. Trauma occurring during instrumentation and host factors such as advanced age, general debilitation or the postpartum state, may also predispose a patient to an infection. Additionally, urinary tract infections can be caused by both endogenous and exogenous transmission. Normal flora from the gastrointestinal tract can spread to the urinary tract, or pathogens can be transmitted by caregivers carrying out tasks related to the catheter or drainage bag. Consequently, pathogens are transmitted through urologic equipment that has not been adequately disinfected.

Microbiologically, nosocomial urinary tract infections are usually caused by gram-negative pathogens, the most common being *Escherichia coli*, *Proteus mirabilis*, *Klebsiella spp.*, and *P. aeruginosa*, other causal pathogens include *enterococci* and *Enterobacter spp.* The morbidity associated with UTIs makes treatment of these infections a serious problem because when choosing an appropriate agent to combat these infections, there are several factors for clinicians to consider the drug of choice that should have good *in vitro* and *in vivo* activity against many of the organisms known to cause UTIs, and should be able to achieve high and prolonged concentrations in the urine and surrounding urinary tract tissues without loss of activity. Therefore, UTIs are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms.

The extensive use of antimicrobial agents has invariably resulted in the development of antibiotic resistance, which is, in recent years, has become a major problem worldwide. A current phenomenon of great concern in the medical community is the rise in multi-drug resistant organisms, which are defined as bacteria with simultaneous resistance to three or more different classes of antibiotics, patients infected with such organisms experience significantly higher rates of treatment failures', prolonging antibiotics usage, and morbidity associated with infections.