



**Value of E-test in the Determination of
Synergistic Antimicrobial Combinations
Active against Multi-Drug Resistant
*Enterobacteriaceae***

Thesis

*Submitted for Partial Fulfilment
of MD Degree in Clinical Pathology*

By

Marwa Ramadan Mohamed Abd-Elhalem

M.B.B.Ch

*Master of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Under Supervision of

Professor / Névine Nabil Kassem

*Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Professor/ Ghada Abdel-Wahed Ismail

*Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Professor / Hala Mahmoud Hafez

*Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Assistant Professor /Fatma Alzahraa Mohamed Gomaa

*Assistant Professor of Microbiology and Immunology
Faculty of Pharmacy - Alazhar University*

Doctor /Noha Alaa EL-Din Mohammed Fahim

*Lecturer of Clinical Pathology
Faculty of Medicine - Ain Shams University*

Faculty of Medicine - Ain Shams University

2019

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgments

*First and foremost, I feel always indebted to **Allah** the Most Beneficent and Merciful.*

*I wish to express my deepest thanks, gratitude and appreciation to **Professor / Mévène Nabil Kassem**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her meticulous supervision, kind guidance, valuable instructions and generous help.*

*Special thanks are due to **Professor/ Ghada Abdel-Wahed Ismail**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her sincere efforts, fruitful encouragement.*

*I am deeply thankful to **Assistant Professor / Hala Mahmoud Hafez**, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her great help, outstanding support, active participation and guidance.*

*Really I can hardly find the words to express my gratitude to **Assistant Professor / Fatma Alzahraa Mohamed Goma**, Assistant Professor of Microbiology and Immunology, Faculty of Pharmacy, Alazhar University, for her supervision, continuous help.*

*Thanks to **Doctor / Moha Alaa El-Din Mohammed Fahim**, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her encouragement throughout this work and tremendous effort.*

Marwa Ramadan Mohamed Abd-Elhalem

List of Contents

Title	Page No.
List of Tables	5
List of Figures	6
List of Abbreviations.....	8
Introduction.....	- 1 -
Aim of the Work	14
Review of Literature	
▪ Antimicrobial Resistance.....	15
▪ Multidrug Resistant (MDR) <i>Enterobacteriaceae</i>	38
▪ Treatment Options for MDR <i>Enterobacteriaceae</i>	48
▪ Laboratory Methods Used to Assess the Activity of Antimicrobial Combinations	59
▪ Prevention and Control of Antimicrobial Resistant Bacteria	72
Materials and Methods	79
Results	112
Discussion.....	132
Summary	143
Conclusion and Recommendations	149
References	153
Master Sheet	186
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Classification schemes for β -lactamases	22
Table (2):	Susceptibility break points of vitek 2 system (AST GN73) cards	84
Table (3):	MIC values of antibiotics according to <i>CLSI (2015)</i> for <i>Enterobacteriaceae</i>	94
Table (4):	Results of the MIC by the Vitek2C system.....	115
Table (5):	Results of the MIC as determined by the broth microdilution test.....	117
Table (6):	MIC results as determined by the E-test method.....	118
Table (7):	Comparison between the Vitek2C and the BMD test regarding the MIC results for amikacin, meropenem and ceftazidime.....	121
Table (8):	Comparison between the Vitek2C and the E-test method regarding the MIC results for amikacin, meropenem, ceftazidime and ampicillin/sulbactam.....	124
Table (9):	Comparison between the BMD and the E-test method regarding the MIC results for amikacin, meropenem, ceftazidime and ampicillin/sulbactam.....	126
Table (10):	Results of the antibiotic combinations as determined by the checkerboard BMD method.....	128
Table (11):	Results of the antibiotic combinations as determined by the E-test method.....	130
Table (12):	Comparison between the checkerboard BMD method and the E-test method regarding the results of the studied antibiotic combinations.....	131

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Evolution of antimicrobial resistance	15
Figure (2):	Phenotypic mechanisms of acquired antimicrobial resistance	18
Figure (3):	Multidrug, extensively drug and pandrug-resistant bacteria	39
Figure (4):	Selective Pressure as a Primary Driving Force for Antimicrobial Resistance (AMR).....	44
Figure (5):	Antimicrobial Synergy Study - Checkerboard Assay	61
Figure (6):	Time-kill analyses of test isolates.....	64
Figure (7):	Double disk synergy test.....	66
Figure (8):	<i>E</i> - test cross method	67
Figure (9):	E-test strip experiments on antimicrobial combinations labeled A and B	68
Figure (10):	<i>E</i> -test fixed ratio method	69
Figure (11):	<i>E</i> -test agar method	70
Figure (12):	<i>E</i> -test minimum inhibitory concentration: minimum inhibitory concentration method	71
Figure (13):	Checkerboard Broth micodilution method for antimicrobial synergy.	93
Figure (14):	Synergistic activity between ceftazidime and amikacin.	97
Figure (15):	Additive effect between meropenem and amikacin.	98
Figure (16):	Indifferent effect between ceftazidime and amikacin.....	99
Figure (17):	Detection of antimicrobial MIC by E-test.....	103
Figure (18):	The setup of an agar plate for E-test combination testing	105

List of Figures cont...

Fig. No.	Title	Page No.
Figure (19):	Combination E-test strips between amikacin and meropenem showing synergy.....	107
Figure (20):	Combination E-test strips between amikacin and ampicillin/ sulbactam showing additive.....	108
Figure (21):	Combination E-test strips between amikacin and ceftazidime showing indifferent effect.	109
Figure (22):	Percent of MDR and XDR among <i>Enterobacteriaceae</i>	113
Figure (23):	Type of samples from which MDR <i>Enterobacteriaceae</i> were isolated.	113
Figure (24):	The most commonly identified MDR species.	114
Figure (25):	MIC results of the studied isolates as determined by the VITEK 2 Compact system.	116
Figure (26):	Results of the MIC as determined by the broth microdilution test.	117
Figure (27):	MIC results as determined by the E-test method.	119
Figure (28):	Results of the antibiotic combination as determined by the checkerboard BMD method.	128
Figure (29):	Results of the antibiotic combinations using the E-test method.	130

List of Abbreviations

Abb.	Full term
<i>ACT</i>	<i>AmpC type</i>
<i>AK</i>	<i>Amikacin</i>
<i>AME</i>	<i>Aminoglycoside modifying enzyme</i>
<i>AmpC</i>	<i>Ambler Class C β-lactamases</i>
<i>AMR</i>	<i>Antimicrobial resistance</i>
<i>AST</i>	<i>Antimicrobial susceptibility test</i>
<i>BC</i>	<i>Bacillus cereus</i>
<i>BLICs</i>	<i>Beta lactamase inhibitors</i>
<i>BMD</i>	<i>Broth micro-dilution</i>
<i>CA</i>	<i>Categorical agreement</i>
<i>CARB</i>	<i>Carbenicillinase</i>
<i>CAZ</i>	<i>Ceftazidime</i>
<i>CB</i>	<i>Checker Board</i>
<i>CDC</i>	<i>Centers for Disease Control and Prevention, United States</i>
<i>CFU</i>	<i>Colony forming unit</i>
<i>CGB-1</i>	<i>Chryseobacterium gleum</i>
<i>CLSI</i>	<i>Clinical Laboratory Standards Institute</i>
<i>CMY</i>	<i>Cephameycins</i>
<i>CphA</i>	<i>Aeromonas hydrophila</i>
<i>CRE</i>	<i>Carbapenem-resistant Enterobacteriaceae</i>
<i>CRKP</i>	<i>Carbapenem-resistant Klebsiella pneumoniae</i>
<i>CSF</i>	<i>Cerebrospinal fluid</i>
<i>CTX-M</i>	<i>Active on cefotaxime</i>
<i>DHA</i>	<i>Dhahran hospital</i>
<i>DNA</i>	<i>Deoxyribonucleic acid</i>
<i>E. coli</i>	<i>Escherichia coli</i>

List of Abbreviations cont...

Abb.	Full term
<i>E.cloacae</i>	<i>Enterobacter cloacae</i>
<i>EARS-Net</i>	<i>European Antimicrobial Resistance Surveillance Network</i>
<i>EDTA</i>	<i>Ethylenediamine tetraacetic acid</i>
<i>ESBL</i>	<i>Extended-spectrum β-lactamase</i>
<i>ESCMID</i>	<i>European Society of Clinical Microbiology and Infectious Diseases</i>
<i>E-test</i>	<i>Epsilometer</i>
<i>EU/EEA</i>	<i>European Union / European Economic Area</i>
<i>EUCAST</i>	<i>European Committee on Antimicrobial Susceptibility Testing</i>
<i>FDA</i>	<i>Food and Drug Administration</i>
<i>FICI</i>	<i>Fractional inhibitory concentration index</i>
<i>FOX</i>	<i>Cefoxitin</i>
<i>GES</i>	<i>Guiana-extended spectrum</i>
<i>GIM</i>	<i>German imipenemase</i>
<i>GNB</i>	<i>Gram negative bacteria</i>
<i>HAI</i>	<i>Healthcare associated infection</i>
<i>HCW</i>	<i>Healthcare worker</i>
<i>HGT</i>	<i>Horizontal gene transfer</i>
<i>hr</i>	<i>Hour</i>
<i>I</i>	<i>Intermediate</i>
<i>ICU</i>	<i>Intensive care unit</i>
<i>IMI</i>	<i>Imipenem-hydrolysing β-lactamases</i>
<i>INDs</i>	<i>Chryseobacterium indologenes</i>
<i>Ipm</i>	<i>Active on imipenem</i>
<i>KPC</i>	<i>Klebsiella pneumoniae carbapenemase</i>
<i>L1</i>	<i>Stenotrophomonas maltophilia,</i>

List of Abbreviations *cont...*

Abb.	Full term
<i>LAT</i>	<i>Latamoxef</i>
<i>Log</i>	<i>Logarithm</i>
<i>LTACs</i>	<i>Long-term acute care centers</i>
<i>LTCF</i>	<i>Long-term care facility</i>
<i>MBL</i>	<i>Metallo-β-lactamase</i>
<i>MDR</i>	<i>Multidrug-resistant</i>
<i>ME</i>	<i>Major error</i>
<i>MEM</i>	<i>Meropenem</i>
<i>MHA</i>	<i>Muller hinton agar</i>
<i>MHB</i>	<i>Muller hinton broth</i>
<i>MICs</i>	<i>Minimum inhibitory concentrations</i>
<i>MiE</i>	<i>Minor error</i>
<i>MIR</i>	<i>Miriam Hospital</i>
<i>MOX</i>	<i>Moxalactam</i>
<i>NC</i>	<i>Negative control</i>
<i>NDM-1</i>	<i>New Delhi metallo-β-lactamase-1</i>
<i>NmcA</i>	<i>Not metalloenzyme carbapenemase</i>
<i>OXA</i>	<i>Oxacillin hydrolyzing capabilities</i>
<i>OXA</i>	<i>Oxacillinase</i>
<i>PC</i>	<i>Positive control</i>
<i>PC1</i>	<i>Penicillinase 1</i>
<i>PCR</i>	<i>Polymerase chain reaction</i>
<i>PDR</i>	<i>Pandrug-resistant</i>
<i>PER</i>	<i>Pseudomonas extended resistant</i>
<i>R</i>	<i>Resistant</i>
<i>S</i>	<i>Sensitive</i>
<i>SAM</i>	<i>Ampicillin / sulbactam</i>
<i>SD</i>	<i>Standard deviation</i>

List of Abbreviations cont...

Abb.	Full term
<i>SFH-1</i>	<i>Serratia fonticola.</i>
<i>SHV</i>	<i>Sulphydryl variable</i>
<i>SIM</i>	<i>Seoul imipenemase</i>
<i>SME</i>	<i>Serratia marcescens</i>
<i>SPM</i>	<i>Sao Paulo metallo-β-lactamase</i>
<i>TEM</i>	<i>Temoneira</i>
<i>TKA</i>	<i>Time kill assay</i>
<i>TOHO</i>	<i>Toho university</i>
<i>TPs</i>	<i>Transpeptidases</i>
<i>USA</i>	<i>United states of America</i>
<i>UTI</i>	<i>Urinary tract infection</i>
<i>VEB</i>	<i>Vietnamese extended-spectrum beta-lactamases</i>
<i>VIM</i>	<i>Verona integron-encoded metallo-β-lactamase</i>
<i>WHO</i>	<i>World Health Organization</i>
<i>XDR</i>	<i>Extreme drug-resistant</i>

INTRODUCTION

Because of the widespread use of antimicrobial drugs over several decades, antimicrobial resistance has become a serious global threat to the public health. Multidrug-resistant (MDR) bacteria have emerged as major pathogens causing serious infections in hospitalized patients, especially in critically ill patients (*Kim et al., 2016*).

Infections caused by multidrug-resistant *Enterobacteriaceae* are associated with increased morbidity and mortality compared to infections caused by their susceptible counterparts. This is may be due to delay in providing active therapy, and also some alternative drugs are not as effective as first-line antibiotics (*Rottier et al., 2012*).

As the prevalence of infections caused by MDR bacteria continues to increase, demand for combination antimicrobial therapies is growing rapidly since the development of new antimicrobial drugs cannot overcome the occurrence of antimicrobial resistance (*Kim et al., 2016*).

The advantages of antimicrobial combination over monotherapy include a broader antibacterial spectrum, synergistic effects, and reduced risk for emerging resistance during therapy. Combination between synergistic antimicrobials enhance their antibacterial effects against multidrug-resistant strains (*Tängdén, 2014*).

A number of methods have been used to study the in-vitro synergy between antibiotics with the checker board titration and time-kill curve methods being the most widely described. Although the checkerboard titration method is a relatively easy test to perform, it only measures the inhibitory activity. On the other hand, the time-kill method of synergy testing assesses bactericidal activity but is time-consuming and labor-intensive (*White et al., 1996*).

The E-test is characterized by its simplicity compared to the above time kill or checkerboard assays, making it easy for routine use in a diagnostic laboratory, providing the clinician with rapid valuable information when critical decisions are needed (*Arezzo et al., 2016*).

AIM OF THE WORK

The aim of the present thesis is to determine the prevalence of multi-drug resistance (MDR) among *Enterobacteriaceae* and to compare between the E-test and the checkerboard titration method as rapid in-vitro diagnostic tests that can help to determine the synergy between selected antimicrobial combinations thought to be active against multi-drug resistant *Enterobacteriaceae*.

Chapter 1

ANTIMICROBIAL RESISTANCE

The development of antimicrobial resistance among gram-negative pathogens has been progressive and relentless. Pathogens of particular concern include extended-spectrum β -lactamase (ESBL) – producing *Enterobacteriaceae*, and carbapenem-resistant *Enterobacteriaceae* (CRE). Classic agents used to treat these pathogens have become outdated. Moreover, of the few new drugs available, many have already become targets for bacterial resistance (*Kanj and Kanafani, 2011*).

Mechanisms of Acquired Antimicrobial Resistance:

The evolution of resistant strains is a natural phenomenon that occurs through selection pressure on the micro organism population from the antibiotic (Figure 1) (*Chellat et al., 2016*).

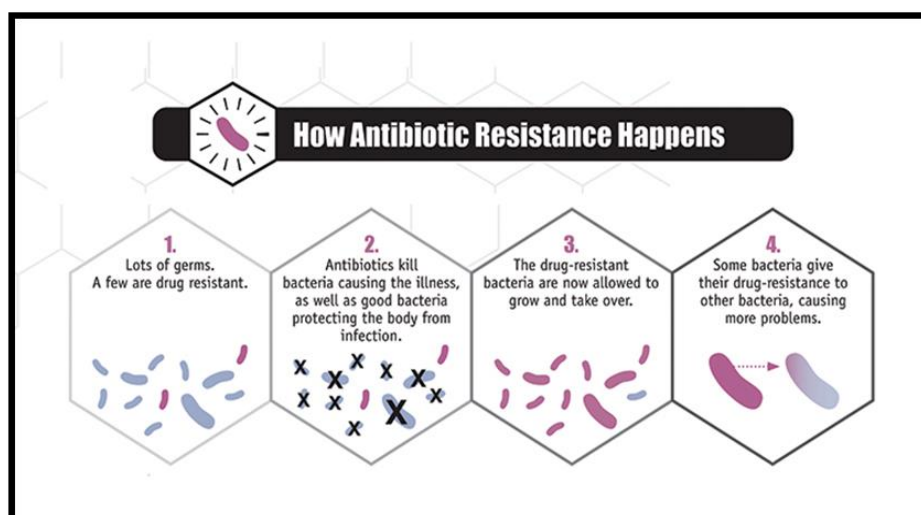


Figure (1): Evolution of antimicrobial resistance (*Chellat et al., 2016*).