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

Thesis

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List of Abbreviations

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

List of Abbreviations cont...

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

List of Abbreviations cont...

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

List of Abbreviations cont...

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

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 *Enterobacteriacae* 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 *Enterobacteriacae*.

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).***

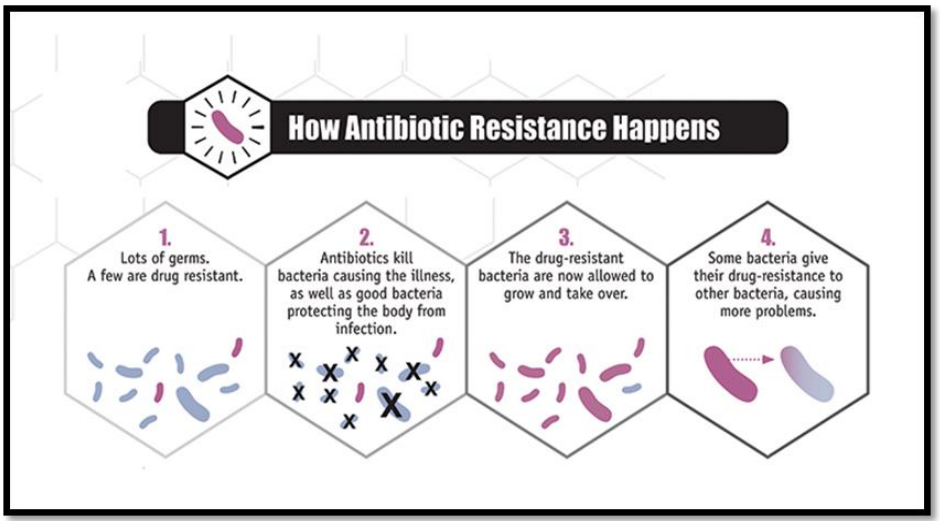

Figure (1): Evolution of antimicrobial resistance (***Chellat et al., 2016).***