Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Biological Costs Paid by Uropathogenic *Escherichia coli* as a Result of Developing Antimicrobial Resistance

A Thesis

Submitted in Partial Fulfillment of the Requirements of the

Master degree

In

Pharmaceutical Sciences

(Microbiology and **Immunology)**

By

Miran Yousri El Sayed El-Far

Bachelor of Pharmaceutical Sciences,

Faculty of Pharmacy, Cairo University, 2006

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Miran Yousri El-Far

Abstract

Biological Costs Paid by Uropathogenic Escherichia coli as a Result of Developing Amikacin Resistance

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It was found that a biological cost due to antimicrobial resistance may be exerted and affect virulence determinants and/or bacterial fitness. This phenomenon was detected in the present study for developed amikacin resistant *Escherichia coli* (*E. coli*) mutants using some measurable virulence factors as well fitness cost. Fifty-eight mutants were recovered from five selected uropathogenic *E. coli* isolates under stress of high amikacin concentrations. Determining the minimum inhibitory concentrations (MICs) of the recovered mutants and their corresponding parent isolates by microdilution technique showed variable resistance levels to amikacin for the tested strains. There is no relationship between biological cost levels of mutants and their MIC values. About 12-27% of amikacin recovered mutants showed 2- >16 fold increase in MIC values over those of the corresponding parent isolates. Surprisingly, 6.8% of the tested mutants showed no fold increase in their MICs relative to the corresponding parent isolates. About 55% of the collected mutants showed lowered adherence (39.6-99.9% cost), while 64% of the mutants showed lowered abilities of *in vitro* biofilm formation (11.4-100% cost) and 50% of the mutants showed lowered abilities to secrete cell free hemolysins (12.8-100% cost), 2% of the mutants showed lowered invasiveness (18.6-99% cost), and 73% of the selected mutants showed lower cytotoxicity (22-57% cost), all when compared to the corresponding parent isolates. Five out of five mutants tested for their fitness cost showed lowered relative growth rates (0.19-0.92) compared to the corresponding parent isolates when grown separately in monocultures. Four out of five mutants tested for their fitness cost showed lowered competition index values (0.0002-0.03) compared to the corresponding parent isolates, after 4 hours of competition in mixed cultures. Seven out of fifteen mutants tested for their fitness cost showed lowered relative number of generations (0.65-0.92) compared to the corresponding parent isolates, after 10 days of competition in mixed cultures.

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Abbreviation	Definition
ABU	Asymptomatic bacteruria
BMD	Broth microdilution method
CDT	Cytolethal distended toxin
cfu	Colony forming unit
C.I	Competition index
CI	Cytotoxicity index
CLSI	Clinical and laboratory standards institute
CNF-1	Cytotoxic necrotizing factor-1
CV	Crystal violet
D.S	Double strength
E	*E. coli* parent isolate
EM	*E. coli* mutant recovered from parent isolate
EMB	Eosin-methylene blue
EUCAST	European committee for antimicrobial susceptibility testing
ESCMID	European society of clinical microbiology and infectious diseases
G	Number of generations
HICPAC	Hospital infection control practices advisory committee
HlyA	Alpha hemolysin
MEM EARL'S	Earls minimum essential medium
MIC	Minimum inhibitory concentration
MOI	Multiplicity of infection
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD	Optical density
PAIs	Pathogenicity islands
PBS	Phosphate buffered saline
Rpm	Revolution per minute
SAT	Secreted autotransporter toxin
TC	Tissue culture
TCP	Toll domain containing protein
TIR	Toll/interleukin receptor
TMP-SMX	Trimethoprim-sulfamthoxazole
TSB	Tryptic soy broth
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
VACSERA	The holding company for biological products and vaccines
VFs	Virulence factors
WHO	World health organization

INTRODUCTION

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The excessive use of antimicrobial agents for therapy causes our relationship with microorganisms to be worse more and more. As most pathogenic microbes acquire resistance and many costs should be paid as a result of this bad relationship. The patient will pay one of these costs by consequent suffering poor prognosis, high mortality, and length of hospital stay. The patient, in addition will pay economic burdens due to extra need for procedures, newer medications and imaging (Niederman, 2001). Also, the health care system will suffer an economic crisis due to utilization of other infection control measures like vaccines and other measures. This will lead to more costs for the development of new drugs since drug choices for common infections will increasingly become limited and expensive (Cosgrove, 2006).

It is obvious that this bad relationship is in favor of microorganisms. But hope started to show up recently, when it is found that microbes pay cost to antimicrobial resistance too (Andersson and Levin, 1999, Bjorkman and Andersson, 2000). Since then a new concept emerges which is the biological cost of antimicrobial resistance.

In general, acquisition of bacterial resistance occurs via mutation in chromosomal loci or horizontal transfer of mobile genetics like plasmids. Mechanisms of action of most antimicrobials target majority of metabolic elements e.g. the ribosome, DNA gyrase, cell wall, etc. which are essential for bacterial growth. So any mutation in these genes for antibiotic resistance may incur deleterious effects on vital physiological processes in microorganisms (Normark and Normark, 2002). Either plasmid or chromosomal conferred resistances, both make the bacteria pay a biological cost through carrying loss in fitness and/ or virulence even in the absence of antibiotic selection pressure. The reasons for that loss are not fully understood (Andersson and Levin, 1999).

Fitness cost is defined as reduced growth rate of resistant bacteria in host and environment as well, also reduced transmission between hosts and increased clearance from infected host. It is measured by laboratory experiments in culture media or animal models.

Virulence is another important characteristic of pathogens associated with their fitness, any decrease in the potential of a microbe to cause disease is considered resistance cost (Andersson and Levin, 1999).

What makes the phenomenon of biological cost not universal (Andersson, 2006) is that microbes are refusing to bear cost for being resistant against the enemy. This paid cost can be