Can Antibiotic Combinations be the solution for Multi Drug Resistant *Pseudomonas*

Thesis

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Abstract

Combination therapy is used to widen the antimicrobial spectrum, minimize toxicity and prevent the emergence of resistant mutants. Antimicrobial synergy resulting from antibiotic combination therapy is often important in the treatment of serious bacterial infections.

The purpose of the present study is to determine the *in-vitro* activities of aminoglycosides (amikacin) in combination with third-generation cephalosporins (cefoperazone), antipseudomonal pencillins (piperacillin – tazobactem), carbapenems(meropenem),fluoroquinolones(ciprofloxacin) and polymixins (colistin) against MDR isolates of *pseudomonas* spp. *And P. aeruginosa* ATCC 27853 that was used as a quality control strain throughout the study.

The effects of these antibiotic combinations were examined by two methods (disk diffusion, and checkerboard).Fractional inhibitory concentration indices were calculated for all isolates with all combinations. Use of the disk diffusion method revealed that amikacin in combination with the β-lactams and ciprofloxacin exhibited synergy against 70% of isolates, whereas the combinations of amikacin and colistin displayed synergic effects against 80% of isolates .Using the checkerboard method , all of the combinations exhibited synergic effects against all the isolates. No antagonism was found with these combinations.

The result of this study indicates that synergism may occur between aminoglycosides, β-lactams and fluoroquinolones although the strains are resistant to the individual antibiotics.

Key Words:

Antimicrobial combinations, Checkerboard titration method, β-lactams Aminoglycosides

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Table of Contents

Abbreviation	Full term
AACs	Aminoglycoside acetyltransferases
AADs	Aminoglycoside adenylyltransferases
ADP	Adenosine diphosphate
ADPRT	ADP-ribosyltransferase
Agl	Aminoglycosides
AK	Amikacin
AHL	Acyl homoserine lactones
AMEs	Aminoglycoside modifying enzymes
ANTs	Aminoglycoside nucleotidyltransferases
APHs	Aminoglycoside phosphoryltransferases
AsialoGM1	Asialoganglioside gangliotetraosylceramide
ATCC	American Type Culture Collection
ATM	Aztreonam;
ATM	Azstreonam
AZL	Azlocillin
c AMP	Cyclic adenosine monophosphate
CAR	Carbenicillin
CAZ	Ceftazidime
CDC	Center for Disease Control
CF	Cystic fibrosis
CFP	Cefoperezone
CFU	colony forming unit
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
CPO	Cefpirome
CT	Colistin
DNA	Deoxyribonucleic acid
EL-2	Elongation factor-2
EDTA	Ethylenediaminetetraacetic acid
ESBLs	Extended spectrum β-lactamases
Exo	Exotoxin
E test	Epsilometer test
E. coli	*Escherichia coli*
FEP	Cefepime
FIC	Fraction inhibitory concentration
Fq	Fluoroquinolones
G+C	Guanine + Cytosine
GAP	GTPase activating protein
GES	Guiana extended spectrum
Gly	Glycine
hrs	Hours
HAIs	Hospital acquired infections
I	Intermediate resistance
IL-8	Interleukin-8
IMP	Imipenem

kDa	Kilodalton
LPS	Lipopolysaccharide
MBC	Minimum bactericidal concentration
MBLs	Metallo-β-lactamases
MDR	Multidrug-resistant
MEM	Meropenem
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NADH	Nicotinamide adenine dinucleotide
NNISS	National Nosocomial Infections Surveillance system
OprD	Outer membrane porin D
OXA	Oxacillinases
PB	Polymyxin
PBP	Penicillin-binding protein
PIP	Piperacillin
PIP/TAZ	Piperacillin/Tazobactam
PSE	*Pseudomonas specific enzyme*
P. aeruginosa	*Pseudomonas aeruginosa*
P. cepacia	*Pseudomonas cepacia*
P. flourescens	*Pseudomonas flourescens*
P. maltophilia	*Pseudomonas maltophilia*
P. putida	*Pseudomonas putida*
P. putrefaciens	*Pseudomonas putrefaciens*
P. stutzeri	*Pseudomonas stutzeri*
QRDR	Quinolone resistant determinative region
QS	Quorum-sensing
r	Reduced susceptibility
rRNA	Ribosomal Ribonucleic acid
R	Resistant
RND	Resistance nodulation division
S	Susceptible
Ser	Serine
spp.	species
SPSS	Statistical package for social sciences
S. aureus	*Staphylococcus aureus*
TIC	Ticarcillin
UK	United kingdom
UTIs	Urinary tract infections
VAP	Ventilator-associated pneumonia
VIM	Verona integron-encoded metallo-β-lactamase

Introduction

Hospital acquired infections are important health care problems all over the world, because of their high morbidity and mortality, and prolonged hospitalization and increased cost of treatment **(*Dundar and Otkun, 2010)***.

Pseudomonas aeruginosa and other *pseudomonas* spp. continue to be a significant cause of morbidity and mortality, especially in intensive care units, and they are frequent isolates causing nosocomial epidemics. The MDR isolates play an important role in colonization or infection of chronically hospitalized patients. They are often resistant to antibacterial agents from different classes, including β-lactams, aminoglycosides, and fluoroquinolones; some strains are only susceptible to polymyxins ***(Mayer and E. Nagy, 1999& Ouderkirk et al., 2003)***.

Combination antibiotic treatment is preferred to provide larger spectrum antimicrobial effect and to prevent the rapid emergence of resistance. Combinations usually comprise a β-lactam and an aminoglycoside or a fluoroquinolone ***(Pier and Ramphal, 2005)***.

Aim of the Work

The aim of this study was to determine the in vitro effects of some antimicrobial drug combinations (aminoglycosides, β-lactams and fluoroquinolones and polymxins on multidrug resistant clinical isolates of *pseudomonas* spp. by two methods (disk diffusion and checkerboard).

Pseudomonas Genus

The genus *pseudomonas* was described by *Mingula* in 1894 and is one of the most diverse bacterial genera whose species have been isolated worldwide in all kinds of environments **(*Peix et al., 2009)*** .They are Gram-negative, aerobic, rod-shaped bacterium with unipolar motility ***(Baron et al., 2003)***.The genus contains more than 140 species, most of which are saprophytic and more than 25 species are associated with humans ***(Ryan and Ray , 2004)***.

Most *pseudomonads*, known to cause disease in humans, are associated with opportunistic infections. These include *pseudomonas (P.) aeruginosa, P. flourescens, P. putida, P. cepacia, P. stutzeri, P. maltophilia, and P. putrefaaciens. P. aeruginosa* and *P. maltophilia* account for approximately 80 % of *pseudomonads* recovered from clinical specimens ***(Bauer et al.,1966 & Baron et al., 2003)***.

Pseudomonas aeruginosa as a human pathogen

Because of the frequency with which it is involved in human disease, *P. aeruginosa* has received the most attention. It is a free-living bacterium and is found in most moist environments ***(Nadeem et al., 2009)***.

Pseudomonas aeruginosa is a commensal bacterium of normal human microflora, which is found on skin surfaces, in nostrils and in upper respiratory tract. It colonizes the intestine of up to 40% of healthy people, and seldom has it caused disease in healthy individuals ***(Gailiene et al., 2007 & Nadeem et al., 2009)***. This percentage increases among hospitalized patients proportionally with increasing duration of

hospitalization ***(Chastre and Trouillet, 2000, Švedienė and Ivaškevičius, 2006)***.

It is major threat to hospitalized and immunocompromised patients, particularly those with serious underlying diseases such as cancer and burns ***(Nadeem et al., 2009)***.

The high mortality associated with these infections is due to combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins ***(Anzai et al., 2000)***.

According to the data of Center for Disease Control (CDC) *P. aeruginosa* is the fifth most common pathogen among hospital microorganisms and causes 10% of all hospital acquired infections (HAIs) ***(Gailiene et al., 2007)***. It accounts for 20% of pneumonia and 16% of urinary tract infections (UTIs) according to data from the National Nosocomial Infections Surveillance system (NNISS) ***(Baron et al., 2003).***

Pseudomonas aeruginosa is a remarkable opportunistic pathogen in that it has uniquely large genome containing genes for many different virulence factors and regulatory mechanisms allowing it to adapt to hostile environments. After being acquired from the environment, it colonizes the respiratory epithelium in patients with predisposing conditions such as cystic fibrosis (CF), mechanical ventilation, immunodeficiency or preexisting respiratory disease ***(Kipnis et al., 2006)***.

Virulence factors:

A-Cell surface virulence factors:

1. Flagella

They are the main motile appendages of Gram negative bacteria; they allow the swimming movement of *P. aeruginosa* through a propeller or screw-like motion. Flagella have a critical role in pathogenesis, by adhering to epithelial cells through binding with a common membrane component, asialoganglioside gangliotetraosylceramide (asialoGM1) ***(Kipnis et al., 2006)***.

2. Pili

Pili or fimbriae are smaller filamentous surface appendages of *P. aeruginosa.* Multiple pili are present on the surface, and are involved in bacterial motility. This motility, called twitching is due to the retractile properties of the pili which allow *P. aeruginosa* to "spread" along hydrated surfaces rather than "swim". This feature facilitates rapid colonization of the airway ***(Mattick, 2002)***. Like flagella, pili are crucial to the adhesion phase of colonization through binding to asialoGM1 of the epithelial cell membrane ***(Kipnis et al., 2006)***.

3. Lipopolysaccharide (LPS)

It plays a role in bacterial adhesion through asialo-GM1 binding **(*Kipnis et al., 2006)***.

4. Alginate

Alginate is a mucoid exopolysaccharide, made up of repeating polymers of mannuronic and glucuronic acid. Alginate, like LPS, functions as an adhesin, anchoring *P. aeruginosa* to the colonized respiratory epithelium. It protects *P. aeruginosa* from phagocytosis and antibiotics; it even attenuates the host response ***(Hentzer et al., 2001 & Cobb et al., 2004)***.

B-Secreted virulence factors:

1. Pyocyanin

Pyocyanin is a blue pigment metabolite having numerous pathogenic effects such as increasing interleukin 8 (IL-8) ***(Denning et al., 1998 & Leidal et al., 2001*)**, depressing host-response ***(Leidal et al., 2001 & Allen et al., 2005),*** and inducing apoptosis in neutrophils ***(Allen et al., 2005)***. Pyocyanin oxidizes glutathione and inactivates catalase in respiratory epithelial cells thus participating in oxidative-stress related damage ***(O'Malley et al., 2003 & O'Malley et al., 2004)***.

2. Pyoverdine

Pyoverdine is a siderophore, a small molecule chelating iron from the environment for use in *P. aeruginosa* metabolism ***(Meyer et al., 1996& Takase et al., 2000)***. Pyoverdine regulates the secretion of other *P. aeruginosa* virulence factors, exotoxin A and an endoprotease ***(Lamont et al., 2002)***.

3. Alkaline protease

Alkaline protease is a fibrin lysing protease secreted by *P. aeruginosa* through a type I secretion system ***(Guzzo et al., 1991)***.

4. Protease IV

It has a role in the pathogenesis of *P. aeruginosa* keratitis ***(Matsumoto, 2004)***.

5. Elastase

Elastase, or lasB, is a metalloproteinase secreted by *P. aeruginosa* into the extracellular space. Elastase causes rupture of the respiratory epithelium through tight-junction destruction, thus increasing epithelial permeability and facilitating neutrophil recruitment ***(Azghani et al.,* 1993; *Azghani 1996 & Azghani et al., 2000)***. Elastase is also pro-inflammatory, increasing IL-8 levels ***(Kon et al., 1999)***.