Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Studies on Persister Cells Emerged From Some Gram Negative Bacterial Cultures

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree
In Pharmaceutical Sciences
(Microbiology and Immunology)

By

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والحمد لله كما ينبغي لجلال وجهه وعظيم سلطانه

Rana Nasser Mohammed

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Abstract

This study involved the collection of 40 clinical bacterial isolates recovered from different specimens. The isolates were identified using the phenotypic characteristics tests including API 20E identification kit. The minimum inhibitory concentration of all the collected isolates against ciprofloxacin were determined using agar dilution technique. From the detected sensitive isolates, two isolates (*Klebsiella pneumoniae* and *Proteus mirabilis*) were selected for further completion of this study.

Persister cell isolation was done by ciprofloxacin dose dependent killing for 24 h; the resultant dose kill curve was biphasic leaving a plateau of survivors. To ensure that these cells were phenotypic variants rather than resistant mutants, MIC of the survivors against ciprofloxacin was redetermined and compared to that of the wild type cell population and it showed no difference. Different environmental factors were investigated for their effects on persister cell recovery of both tested isolates. The effect of ciprofloxacin on the pre-stressed cells showed that ciprofloxacin decreased the percentage of survivors resulting from stress conditions as in the case of *K. pneumoniae*. Such case was not obtained with *P. mirabilis* when similarly treated. Therefore, environmental stressors rendered persisters of *K. pneumoniae* to be more sensitive to ciprofloxacin than those of *P. mirabilis* counterparts. Characterization of persisters of both test isolates were achieved by their slow rate of growth and elongation of cells upon resuscitation in rich medium. In

general, test isolates persisters could be inhibited by applying different approaches such as priming with different sugars before ciprofloxacin exposure, combination of different antimicrobial agents such as silver nitrate with ciprofloxacin or treatment with antimicrobial agent in the presence of sodium salicylate.

Introduction

Bacterial persistence is a phenomenon in which a subpopulation of cells survives antibiotic treatment. In contrast to resistant bacteria, persisters do not grow in the presence of antibiotics and their tolerance arises from physiological processes rather than genetic mutations in a subpopulation of bacteria. The presence of persisters within a population is indicated by killing data that show most cells in a population dying, with a subpopulation (0.1–10%) persisting, even on prolonged exposure or at higher concentrations of the antibiotics. Persisters pre-exist in a population and arise independently of the use of antibiotics. Persisters survive high concentrations of antibiotics by overexpression of genes such as the chromosomal toxin–antitoxin modules that shut down cellular functions and hence the antibiotic targets, resulting in a dormant cell that is tolerant to the lethal action of antibiotics.

Such intrinsic tolerance can cause chronic infections with recurring symptoms after the course of antibiotic therapy, facilitates the development and wide spread of acquired multidrug resistance through genetic mutations and horizontal gene transfer. Thus, targeting persister cells may help improve infection control and prevent the development of multidrug resistant bacteria.

The present study aimed to investigate the prevalence of persistent cells in some Gram negative bacterial cultures under