Ain Shams University
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RECENT METHODS FOR STUDYING THE EFFECT OF SUBLETHAL DOSE OF IONIZING RADIATION ON SOME VIRULENT DETERMINANT PRODUCED BY PATHOGENIC MICROORGANISMS ISOLATED FROM PATIENTS OF URINARY TRACT INFECTION

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APPROVAL SHEET

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Title: Recent Methods for Studying the Effect of Sublethal Dose of Ionizing Radiation on Some Virulent Determinant Produced by Pathogenic Microorganisms Isolated from Patients of Urinary Tract Infection.

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DEDICATION

I would like to dedicate this work to my husband for putting up with me and supporting me all through this work, to my parents for their encouragement and support and to my children wishing them a life overwhelmed with success

Many thanks to all of them

Reham

This thesis has not been submitted for a degree at this or any other University.

Reham El-behery

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ABSTRACT

Plasmid profiles and total protein analysis obtained by gel electrophoresis in conjunction with antibiogram and extracellular slime production as a virulent determinant were used, in studying the role of radiotherapy at dose level 20 Gy gamma irradiation on microorganisms associated with urinary tract infection (UTI). Slime formation is considered a simple, rapid and most effective method of distinguishing true pathogens from simple contaminants. Seventy three out of one hundred cancer bladder patients who fulfilled clinical diagnostic criteria for UTI were retrospectively enrolled into this study. Sixty nine isolates were positive for bacterial infection (34.25% males, 65.75% females) and 4 isolates of yeast infections all of them were isolated from females.

Identification of the isolated organisms to the species level, revealed the isolation of 15 species of pathogenic bacteria and yeasts belonging to 10 genera. All isolated species of pathogenic bacteria were subjected to sensitivity tests before and after *in-vitro* gamma irradiation using 19 different antibiotics with different mode of actions. The changes in antimicrobial susceptibility pattern of all bacterial isolates showed that, the difference in the mean inhibition zones before and after irradiation were of highly statistical significance except in case of cefotriaxone, penicillin G and sulphamethoxazole trimethoprim. P. values were 0.0573, 0.574 and 0.33 respectively. After irradiation the number of resistant isolates increased in case of cefuroxime cefotaxime, imipenem,

amikacin, gentamicin, kanamycin, tobramycin, nalidixic acid, rifampicin, colistin sulphate and ofloxacin. The change in antimicrobial sensitivity to different antibiotics after irradiation leads to emergence of resistant strains. Thirty two out of 73 isolates (43.83%) had the ability to produce extracellular slime material before irradiation whereas 26.03% only were positive after irradiation. Slime production was accompanied by higher incidence of antibiotic resistance.

Although the percentage of slime production was reduced in some isolates after irradiation the antibiotic resistance was higher after irradiation than before. These results indicated the presence of another factor responsible for antibiotic resistance with slime formation.

There are quite clear differences in the number of bands and molecular weight in plasmid profile and total protein analysis after irradiation. All investigated strains had one plasmid band with molecular weight more than 10 Kb after irradiation. Whereas, one extra-band was observed with *Pseudomonas aeruginosa* with M.wt. > 10kb after irradiation. In case of protein analysis for all of the yeast isolates, there was a clear difference in the number of bands (both major and minor bands) before and after irradiation with some bands disappearing and other new bands appearing in the pattern after irradiation. These techniques were valuable in detecting distinct difference before and after irradiation and its use could be helpful in future investigation of infections and multiple drug resistance by other organsims.

INTRODUCTION

In immuno-compromised cancer patients, infection remains the leading cause of morbidity and mortality due to the disease itself and the proposed cancer treatment in the form of chemotherapy and radiotherapy. The treatment leads to more weakening of the body defence mechanisms **(Dionigi *et al.*, 1980).**

It was reported that, cancer patients are particularly susceptible to certain less common types of infections. There has been an increasing incidence of Gram-negative infections **(Altemeier and Wulsin, 1974).**

Antibiotics have served as the main weapon in the medical world's arsenal aganist disease. The ability of bacteria to resist the inhibitory and lethal actions of antibiotics is a major clinical problem and has been observed with every antimicrobial agent **(Shafran, 1990).** The main factor responsible for development and spread of bacterial resistance is the injudicious use of antimicrobial agents which has resulted in most Gram-positive and Gram-negative bacteria continuously developing resistance to the antimicrobials in regular use at different time. Available methods for controling spread of bacterial resistance include rational use of antimicrobial agents, change to newer antimicrobials, and constant surveillance for emerging bacterial resistance **(Urassa *et al.*, 1997).**

Bacterial virulence had in many studies been related to the ability to form extracellular polysaccharide slime and extracellular proteolytic activity of proteinase enzyme by pathogenic microorganisms **(Farrag *et al.*, 2001 b)**.

The ability of bacterial organisms to produce an extracellular polysaccharide matrix known as slime has been

associated with increased virulence and delayed infections in various prosthetic implants. Within a biofilm, this slime may protect the embedded bacteria from host defense mechanisms **(Peters and Schumacher-Pedreau 1994, Heinzelmann *et al.*, 1997 & Farrag, 2001 b).**

Plasmids have been described in almost all analysed bacterial species and proven to be essential genetic tool.

The methods of plasmid profile analysis is a reliable method in the investigation of outbreaks of urinary tract infection **(Suljagic and Cobeljic 2001)**. Plasmids can carry genes that code for functions other than transfer or replication. These genes coding for enzymes that, under certain circumstances are advantageous to the bacterial host. Among the phenotypes conferred by different plasmids are antibiotics resistance, antibiotics production, degradation of complex organic compounds, production of colicins, production of enterotoxins and production of restriction and modification enzymes **(Manniatis *et al.*, 1982).**

An antibiotic resistance gene may transpose from one plasmid to another, may transfer on a plasmid from one bacterial cell to another, or may remain in a bacterial cell which leaves one person and colonizes another**(O'Brien *et al.*, 1980).**

Candida is the most common fungal pathogen of humans and has developed an extensive repertoire of putative virulence mechanisms that allows successful colonization and infection of the host under suitable predisposing conditions. Extracellular proteolytic activity plays a central role in *Candida* pathogencity **(Farrag *et al.*, 2002)**..

The integrity of DNA is essential for the well-being of a cell. Exposure of DNA to high energy radiation such as