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Adherence of Irradiated Slime Producing Bacterial Pathogens to Biomaterial Surface and their Antimicrobial Susceptibility Associated with Catheter Infection in Bladder Cancer Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HAAF and AZAKED designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors ZGMES and MMK managed the literature searches and performed the statistical analysis. All authors managed the analyses of the study, read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To detect the prevalence of biofilm producers among Gram negative bacilli and Gram positive cocci bacterial pathogens along with their antimicrobial susceptibility pattern. Growth and adherence on catheter eluates and in the presence of antibiotics.
Methodology: From laboratory of microbiology, one hundred samples (100 urinary catheters and 100 urine samples from the attached drainage bags) of bladder cancer patients collected in National Cancer Institute in Cairo, Egypt, were identified to species level. Slime production was investigated by the quantitative and qualitative methods. Qualitative method was carried out by tube method. Adherence assay and quantitation of biofilm was performed by spectrophotometric method by measuring the optical densities of stained bacterial films adherent to plastic tissue culture plates. Hydrophobicity was evaluated by adhesion to P-xylene. Identification and minimum inhibitory concentration

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(MICs) of 26 antimicrobial agents against gram negative and 24 against gram positive bacterial isolates were determined using microscan walk away 96 SI system. Plasmid profile analysis was carried out by plasmid isolation kit. Scanning electron microscopy studies for growth, adherence and biofilm formation. Impact of gamma irradiation at a dose level of 24.41Gy was studied.

Results: From the processing of 100 samples, 98 cases were positive. Out of them 110 isolates of gram negative bacilli and 13 of gram positive cocci. They were belonging to 15 and 6 species respectively. Among them, 117 isolates showed positive results for adherence assay and biofilm/slime production. They were identified as; *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Acinetobacter*, *Proteus* spp., *Citrobacter*, *Alcaligenes, Empedobacter (104 strains) Staphylococcus* spp. and *Enterococcus* (13 stains). The results obtained by different methods correlated well with strain to strain variation. Gamma irradiation resulted in changes in slime production and adherence ability for all the tested strains. Cell surface hydrophobicity (CSH) showed a hydrophobic reaction and these with increase in its value after irradiation in case of *Escherichia coli*. On the other hand, *Staphylococcus epidermidis* was moderate hydrophobic before irradiation changed to strictly hydrophilic after irradiation. All the slime producers showed reduced susceptibility to majority of antibiotics. They exhibited highest percentage susceptibility before and after in vitro gamma irradiation at a dose level 24.41Gy for both Amikacin and Imipenem. Scanning electron microscopy (SEM) confirmed growth and biofilm formation in the presence of catheter eluates only with halos surrounding the cells and visible erosion zones on catheter surfaces. The antimicrobial and adherence activity of Amikacin and Imipenem at the MIC level showed marked abnormalities in cells shape and size with significant reduction in adherence ability. Plasmid profile analysis of irradiated strains showed more extra-plasmid bands and / or difference in molecular weight.

Conclusion: The biofilm assay strategy applied in this study may constitute a tool in biomaterial related infection and antimicrobial resistant research for further studies for biomaterial modification. Early detection of biofilm forming organisms can help in appropriate antibiotic choice.

Keywords: Biomaterials; bladder; radiotherapy; hydrophobiciy; antimicrobial; plasmid; adherence; biofilm.

1. INTRODUCTION

Some populations most at risk for immuno-compromise include people with cancer, and patients who are immuno-compromised have an increased risk for infection [1]. A microbial biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface [2,3,4]. The matrix contains polysaccharides, proteins, and extracellular microbial DNA, and the biofilm can consist of one or more microbial (bacterial or fungal) species. The matrix is important because it provides structural stability and protection to the biofilm against adverse environmental conditions, for example, host immunological system and antimicrobial agents [5,6]. Biofilm formation also causes a multitude of problems in the medical field, particularly in association with prosthetic devices such as indwelling catheters and endotracheal tubes [7]. Biofilms can form on inanimate surface materials such as the inert surfaces of medical devices, catheters, and contact lenses or living tissues, as in endocardium, wounds, and the epithelium of the lungs, particularly in cystic fibrosis patients [8]. Bacterial adherence to medical devices has been recognized as an important initial step in the infectious process and the factors affecting adherence were device material, slime production and hydrophobicity [9]. The

hydrophobicity of microorganisms is considered one of the most important parameters which plays a significant role especially in terms of adhesion to surfaces, and the hydrophobic properties of the microorganism cell surface has generally been correlated with enhanced virulence and with increased attachment to the surface of implanted devices. In addition [10], many bacterial, pathogens are drug resistant because they have resistance genes. Lodish et al. [11]. Some bacterial plasmids encode enzymes that inactivate antibiotics. Such drug resistance plasmids have become a major problem in the treatment of number of common bacterial pathogens. As antibiotics using become wide spread, so plasmids containing several drug-resistance gene evolved, making their host cells resistant to a variety of different antibiotics simultaneously. Bacterial pathogens have become increasingly resistant to commonly used antibiotics and antimicrobial resistance has become a major medical and public health problem as bacterial resistance often result in treatment failure, which can have serious consequences, especially in critically ill patients [12,13].

The research work was carried out in an attempt to investigate and elucidate the following points; (1) incidence of bacterial infection associated with urinary catheter in bladder cancer patients,(2) detection of microbial adhesion and biofilm formation on catheter surface and polystyrene tissue culture plates,(3) evaluation of the hydrophobicity, (4) susceptibility to antimicrobial chemotherapy, (5) assessment of catheter eluates and MIC of antimicrobial agents on growth and viability of tested strain, adherence, colonization and biofilm formation to the biomaterials by Scanning Electron Microscopy,(6) plasmid patterns and (7) impact of *In-vitro* gamma irradiation (24.41Gy) on the tested strains.

2. MATERIALS AND METHODS

2.1 Cases

A total of 100 urinary catheters, (about 300 segments) and 100 urine samples from the attached drainage bags from 100 patients, diagnosed as bladder cancer. The patients were attending out-patients and in-patients clinics at the National Cancer Institute (NCI), Cairo, Egypt, for about one year, with an age range of 25–85 years. The base of selection was the fact that none of the patients received any kind of prophylactic antimicrobial chemotherapy prior to the time of samples collection. They were undergoing external pelvic radiotherapy (RT), and the samples were taken prior to the start of the RT.

2.2 Materials and Processing of Samples

Foley urinary catheters (silicone-coated latex, and all-silicone) and their attached drainage bags were brought by medical staff to the laboratory immediately for investigation. Duration of catheterization, was variable, ranged from short-term urinary catheterization to long-term urinary catheterization. In lab., for each catheter, three segments 1cm in length were split longitudinally under sterile conditions, placed in phosphate buffer saline (PBS) pH 7.2±0.2, vortexed vigorously to detach adherent microorganisms and break up clumps. Urine samples from the drainage bags were examined microbiologically without delay.

2.3 Microorganisms and Culture Conditions

All pathogenic bacterial isolates were cultivated on nutrient agar, Tryptone glucose-yeast extract agar, MacConkey agar No. 3 and cystine-lactose electrolyte deficient media (Oxoid). Strains were identified by Microscan Walk Away-96 SI system, Dried Gram negative ID type

2 panels and MicroScan Dried Gram Positive ID Type 2 (Pos. ID Type 2) Panels (Dade Behring, Germany) according to the instructions of the manufacturers at National Cancer Institute, Cairo, Egypt.

2.4 Irradiation Sources

Cobalt 60 (^{60}Co) Gamma cell 220 source and Cesium 137 (^{137}Cs) Gamma cell 40 source product of Canada Co. Ltd. Canada, Located at National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The dose rate at the time of experiments were 1.25rad/sec and 0.90rad/sec respectively. An in-vitro total single dose of 24.41Gy/one fraction was calculated according to the linear quadratic (LQ) formula described by [14], which is biologically equivalent to 70Gy/35 fractions. All the microbiological tests were performed, twice for each isolate, one before irradiation (control) and one after irradiation.

2.4.1 Determination of minimum inhibitory concentrations (MICs)

It was tested by Microscan Walk Away 96 SI system (type I) Dade Behring, Germany at the National Cancer Institute, Cairo, Egypt [15,16] which based on recommendations of CLSI (formerly the National Committee for Clinical Laboratory Standards [17] using 26 and 24 antibiotics (21&20 single antibiotics and 5&4 combined)for gram negative and gram positive respectively. Antibiotics belonged to different groups with different mode of actions.

2.4.2 Slime production

All pathogenic bacterial isolates were subjected to qualitative assessment of slime production by the tube methods [18,19]. In brief, the tested strains were inoculated into a glass tube containing 5 ml of trypticase soy broth (TSB, Oxoid), with minor modifications, containing 0.25% glucose, casemino acid 3% and yeast extract 1% [20] and incubated under static conditions at 35ºC for 48h. After withdrowal of the contents, the tubes were washed twice with distilled water, then, 0.25% safranin was added. Slime production was judged to have occurred and adherent growth to be present if a visible continuous stained film lined the inner walls of the tube. The experiments were repeated three times. The amount of stained biofilm was macroscopically semiquantitated as strong (+++), moderate (++), weak (+) or absent (0). The amount of slime production was estimated by three observers and compared with each others.

2.4.3 Adherence assay and quantification of biofilms

Adherence assay and quantitative determination of biofilm were carried using the microtitration plate assay [21,3]. Briefly, aliquots (200µl) $5x10^5$ to $1x10^6$cfu/ml were added to the wells of sterile tissue culture plates (Nuclon; Danmark) and incubated at 37ºC for 24h.The medium and non-adherent cells were removed by washing three times in PBS (pH 7.3, Sigma). (a)-for gram negative bacteria; slime and adherent organisms were fixed by incubating for 1h at 60ºC, then stained with Hucker crystal violet for 5min. The excess stain were removed by washing with H_2O [22]. The plates were dried for 30min at 37ºC. The extent of biofilm was determined by measuring the absorbance of the stained adherent film with a microplate reader at a wavelength of 492nm. The low cut off was chosen by using the criteria described by [21]. (b)-For Gram-positive bacteria, adherence measurements were carried out by two methods at different wavelengths (490nm and 570nm) and the correlation between the OD readings obtained from both methods was done by using Pearson's correlation index [23,19].

2.4.4 Cell surface hydrophobicity (CSH)

The relative hydrophobicities of *E. coli* and *Staph. epidermidis* selected strain were measured using the p-xylene method described by [24,25,20]. Pre-cultivation of the tested strains on brain heart infusion broth with shaking in a rotary shaker at 120rpm at 37ºC for 18h. Cells were harvested at 5000rpm for 10min, washed three times with PBS (pH 7.4) then resuspended in the same buffer. Different amounts of p-xylene (BDH Laboratory reagents) were mixed with 3ml of the suspensions with vigorous agitation for 60sec. Two phases were allowed to fully separate in 20min. The optical densities at 540nm of the samples taken from the aqueous phase were measured. Then, the relative optical density (ROD) were calculated according to [23].

2.4.5 Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out with *E. coli* and *Staph. epidermidis* the highest slime producers among the positive strains considered to analyze the effect of catheter eluates on growth, adherence and biofilm formation [20,26,3], as well as, the production of extra-cellular matrix material after exposure to Amikacin (Bristol Myers Squibb, Egypt, MIC=16µg/ml), and Imipenem (Tienam-500, Merck sharp and Dohme B.V. Haarlem-Netherlands, (MIC 1µg/ml for control non irradiated strain and 2µg/ml for irradiated strain) were provided by manufactures as standard powders and stock solution prepared [27]. After pre cultivation of the selected strain for 18h, at 37ºC cells were harvested at 2.067g for 30min, washed six times in PBS suspended to conc. of 10^7-10^8cfu/ml in the same buffered for assessment of catheter eluates. Also, The microbial cells (irradiated and non-irradiated) were suspended to a final concentration 10^7-10^8cfu/ml in PBS containing the MIC of the tested antimicrobial agents. Many pieces 1 cm lengths of catheters were immersed in PBS, incubated without shaking at 37ºC for 48hr. Biofilms formed on catheter surfaces were fixed in 4% glutaraldehyde and 0.2M cacodylate (pH 7.4) for 6 to 12hrs at 4ºC, rinsed twice in 0.4M saccharose and 0.2M cacodylate (pH 7.4) for 6 to 12h. at 4ºC. The samples were dehydrated for 5min in increasing degreed ethanol bath (30% to 100%) for 3 times and examined with a tilt angle of 45ºC after gold deposition in vacuum, JEOL JSM-5400-Japan [28].

2.4.6 Plasmid analysis

Plasmid DNA was extracted from cultured cells using the high pure plasmid isolation Kit (Roche, Germany), according to the manufacture's instructions [29]. The DNA was electrophoresed on 1% agarose gel stained with ethidium bromide and visualized by UV-transillumination at 312nm, conducted for 90-120min at constant voltage 75v in Tris-borate buffer [30] and Polaroid film No. 667 was used for photography. Gel was analyzed using (Gel-Pro-Analyzer version 3.1), Biological science and Geology, Faculty of education, Ain Shams University.

2.5 Statistical Analysis

The paired t-test, Wilcoxon singed-Rank test (non-parametric) for antimicrobial activity and Pearson's correlation coefficient (parametric statistical method) for adherence assay was done according to [31,32,33]. The level of significance indicate the normal and abnormal distribution of data (*P* value highly significant ≤0.001, significant <0.05, non-significant >0.05).

3. RESULTS

The question of relationships among malignancy, immuno-compromise, and infectious morbidity and mortality represent one of the most tender areas which imposes itself for urgent discussion.

3.1 Isolation and Identification of Pathogenic Bacteria

Out of 98 positive cases isolated there were 71 males (72.45%) and 27 females (27.55%) with a ratio of 2.63:1. Some of the patients had a polymicrobial infection. There were mixed infections with bacteria and yeasts in some patients (data not shown).The total number of pathogenic gram negative bacilli and gram positive cocci was 123 out of 98 positive infected cases. One hundred and ten isolates belonging to gram negative bacilli and 13 belonging to gram positive cocci. On the basis of morphological and biochemical characteristics, identification was carried out before irradiation. Examination of 100 urinary catheters and 100 urine samples showed same results and revealed the isolation of 23 species of pathogenic Gram negative bacilli and Gram positive cocci belonging to 12 genera (Tables 2, 3). Two or more different types of pathogenic bacteria were isolated from one patient. Each isolate was identified separately and used for further experiments.

In current study, all the tests were carried out twice for each microorganism. In the first group, each one was pre-exposed to a dose level of 24.41Gy of gamma radiation. In the second group, non irradiated strains were considered as control.

3.2 Testing the Susceptibility to Antimicrobial Chemotherapy

Results presented showed that some antibiotics affecting the isolated strains before irradiated may not significantly affect the strain exposed to gamma irradiation. There was difference in the mean of MICs to each type of antibiotics for all isolates between before and after *In-vitro* gamma irradiation, In (Fig. 1), changes in mean MICs for all Gram-negative bacilli after *In-vitro* gamma irradiation was highly statistically significant (*P* value<0.000) with 14 antibiotics While, it was statistically significant with Amikacin, Ceftriaxone, Cefuroxime and Gentamicin (*P* value<0.05), respectively and no significant change with Aztreonam, Cefpodoxime, and Imipenem.

Concerning to Gram positive cocci (Fig. 2), there was an increase in the mean of MICs with the majority of the antibiotics for all isolates after irradiation, and this increase was statistically significant with Chloramphenicol, Clindamycin, Levofloxacin, Nitrofurantoin, Norfloxacin, Penicillin, Rifampin , and Vancomycin (*P* values ranged from 0.027-0.009).

According to post irradiation changes in number and percentage (%) of resistant/sensitive (R/S**)** isolates, the percentage resistance of non irradiated gram negative tested isolates to different antibiotics exceeded 80% in some antibiotics while 50% in others with gram negative bacilli and gram positive cocci. After irradiation, others exhibited variable resistance. The percentage was changed with the majority of the antibiotics, and the tested isolates became more resistant than before irradiation. Statistical significance along with the resistance profile of all tested isolates to the antimicrobial agents were as follows (Table, 1). Furthermore, of all tested isolates, most were uniformly susceptible to Amikacin (78.2% & 71.8%) and Imipenem (82.7%) and remained constant before and after gamma irradiation respectively.

The percentage of resistant isolates against different antibiotics used for *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Acinetobacter*, *Proteus* spp., *Citrobacter*, *Staphylococcus* spp. and *Enterococcus* was increased after *in-vitro* gamma irradiation than before (data not shown).

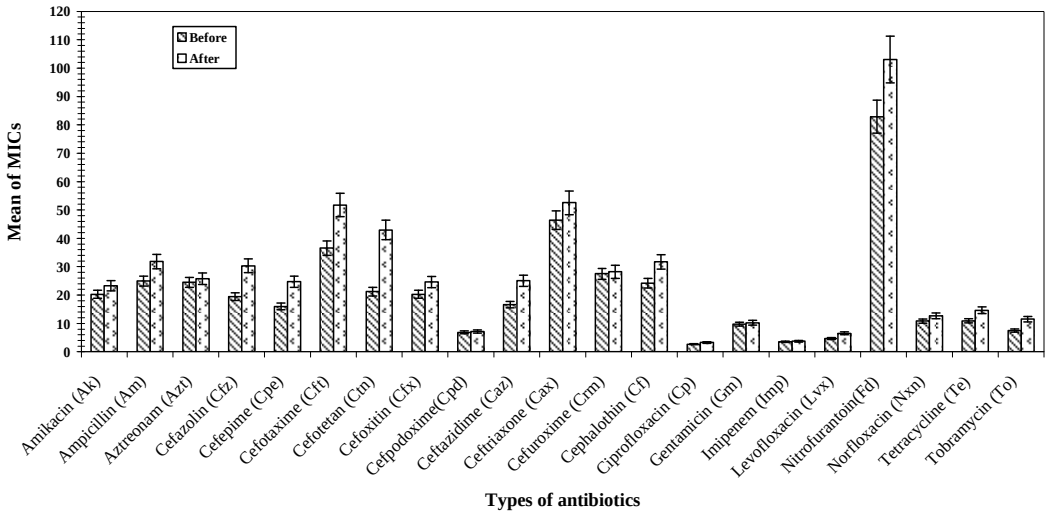


Fig. 1. Changes in mean MICs for all gram-negative bacilli after *In-vitro* gamma irradiation

The MIC was recorded as the last well showing inhibition of growth. When growth occurs in all concentrations of the tested antibiotic, the MIC was recorded as greater than (>) the highest concentration, while, when no growth occurs in any of the concentrations of the antibiotic, the MIC was recorded as less than or equals to (≤) the lowest concentration. Statistical analysis of the results was carried out on single antibiotics only not the combined (Amoxicillin/K Clavulanate (Aug), Ampicillin/Sulbactam (A/S), Ticarcillin/ K Clavulanate (Tim) and Trimethoprim/Sulfamethoxazole (T/S). P value : highly significant (≤) 0.001; Am, Cfz, Cpe, Cft, Ctn, Cfx, Caz ,Cf ,Cp, Lvx, Fd, Nxn, Te and To., Significant (<) 0.05 ; Ak, Cax, Crm and Gm.,Non significant (>) 0.05 ; Azt, Cpd and Imp

3.3 Slime Production

All isolates of pathogenic bacteria, were subjected to a qualitative assessment of slime production by using visual methods. Slime production was detected in 104 out of total 110 Gram-negative bacterial isolates (Table 2). In case of *Escherichia coli*, 26 strains only out of 32 have the ability for slime production. Whereas, the other species for all strains showed positive reaction before and after *In vitro* gamma irradiation and the production of the polysaccharide slime film by the producer strains was changed after irradiation.

From the results in (Table 3) concerning to gram positive cocci, the isolates exhibited variable reaction after exposure to gamma irradiaton

Table 1. Changes in percentage (%) of resistant/sensitive isolates against antibiotics after *In-vitro* gamma irradiation

Types of antibiotics (Gram negative)	Before irradiation	After irradiation	Types of antibiotics (Gram positive)	Before irradiation	After irradiation
	(%) R/S	(%) R/S		(%) R/S	(%) R/S
Amikacin (Ak)*	21.8/78.2	28.2/71.8	Amikacin (Ak)#	15.4/84.6	23.1/76.9
Amoxicillin/K Clavulanate (Aug)**	79.1/20.9	93.6/6.4	Amox.Clav.(Aug)#	30.8/69.2	46.2/53.8
Ampicillin/Sulbactam (A/S)**	69.1/30.9	96.4/3.6	Ampici../Sulb(A/S)#	38.5/61.5	38.5/61.5
Ampicillin (Am)**	74.5/25.5	99.1/0.9	Ampicillin (Am)#	61.5/38.5	61.5/38.5
Aztreonam (Azt)#	75.5/24.5	74.5/25.5	Cefazolin (Cfz)#	53.8/46.2	69.2/30.8
Cefazolin (Cfz)**	57.3/42.7	95.5/4.5	Cefotaxime (Cft)#	46.2/53.8	61.5/38.5
Cefepime (Cpe))**	47.3/52.7	77.3/22.7	Ceftriaxone (Cax)#	53.8/46.2	76.9/23.1
Cefotaxime (Cft))**	60.0/40.0	85.5/14.5	Cefuroxime (Crm)#	46.2/53.8	61.5/38.5
Cefotetan (Ctn))**	30.9/69.1	70.0/30.0	Cephalothin (Cf)#	46.2/53.8	69.2/30.8
Cefoxitin (Cfx))**	60.9/39.1	82.7/17.3	Chloramphen.(C)*	7.7/92.3	53.8/46.2
Cefpodoxime (Cpd)#	83.6/16.4	87.3/12.7	Ciprofloxacin (Cp)*	38.5/61.5	84.6/15.4
Ceftazidime (Caz))**	48.2/51.8	78.2/21.8	Clindamycin (Cd)*	38.5/61.5	76.9/23.1
Ceftriaxone (Cax))**	73.6/26.4	86.4/13.6	Gentamicin (Gm)#	46.2/53.8	69.2/30.8
Cefuroxime (Crm)*	83.6/16.4	90.0/10.0	Imipenem (Imp) #	23.1/76.9	30.8/69.2
Cephalothin (Cf))**	73.6/26.4	100/0	Levofloxacin(Lvx)*	30.8/69.2	76.9/23.1
Ciprofloxacin (Cp))**	58.2/41.8	78.2/21.8	Nitrofurantoin(Fd)*	38.5/61.5	69.2/30.8
Gentamicin (Gm)#	57.3/42.7	58.2/41.8	Norfloxacin (Nxn)*	38.5/61.5	92.3/7.7
Imipenem (Imp)##	17.3/82.7	17.3/82.7	Oxacillin (Ox)#	100/0	100/0
Levofloxacin (Lvx))**	55.5/44.5	78.2/21.8	Penicillin (P)#	61.5/38.5	61.5/38.5
Nitrofurantoin (Fd))**	59.1/40.9	80.0/20.0	Rifampin (Rif)#	46.2/53.8	69.2/30.8
Norfloxacin (Nxn))**	58.2/41.8	73.6/26.4	Tetracycline (Te)#	61.5/38.5	84.6/15.4
Piperacillin / Tazobactam (P/T)#	65.5/34.5	69.1/30.9	----------	----------	----------
Tetracycline (Te))**	61.8/38.2	94.5/5.5	----------	----------	----------
Ticarcillin / K Clavulanate (Tim)#	76.4/23.6	80.9/19.1	Ticar. /Clav.(Tim)#	46.2/53.8	84.6/15.4
Tobramycin (To))**	43.6/56.4	69.1/30.9	Vancomycin (Va)*	38.5/61.5	76.9/23.1
Trimethoprim/sulfamethoxazole (T/S)#	93.6/6.4	92.7/7.3	Trimethopr./Sulf. (T/S)#	61.5/38.5	46.2/53.8

*R/S: Resistant/Sensitive Total number of pathogenic Gram–ve bacterial isolates, n=110 and pathogenic Gram +ve isolates, n=13 **P value highly significant ≤0.001 * P value significant<0.05, # P value non-significant >0.05 Susceptibility breakpoints for the tested antibiotics defined as: AK.≤16µg/ml, Am. ≤0.25µg/ml for Staphylococci & ≤8µg/ml for Enterococci , Cfz.≤8µg/ml, Cft.≤8µg/ml, Cax.≤8µg/ml, Crm.≤8µg/ml, Cf.≤8µg/ml, C≤8µg/ml, Cp.≤1µg/ml, Cd.≤0.5 µg/ml, Gm. ≤ 4µg/ml, Imp.≤4µg/ml, Lvx.≤2µg/ml, Fd.≤32µg/ml, Nxn.≤4µg/ml, Ox.≤0.25µg/ml, P.≤0.12µg/ml for Staphylococci & ≤8µg/ml for Enterococci, Rif.≤1µg/ml, Te.≤4µg/ml and Va.≤4µg/ml*

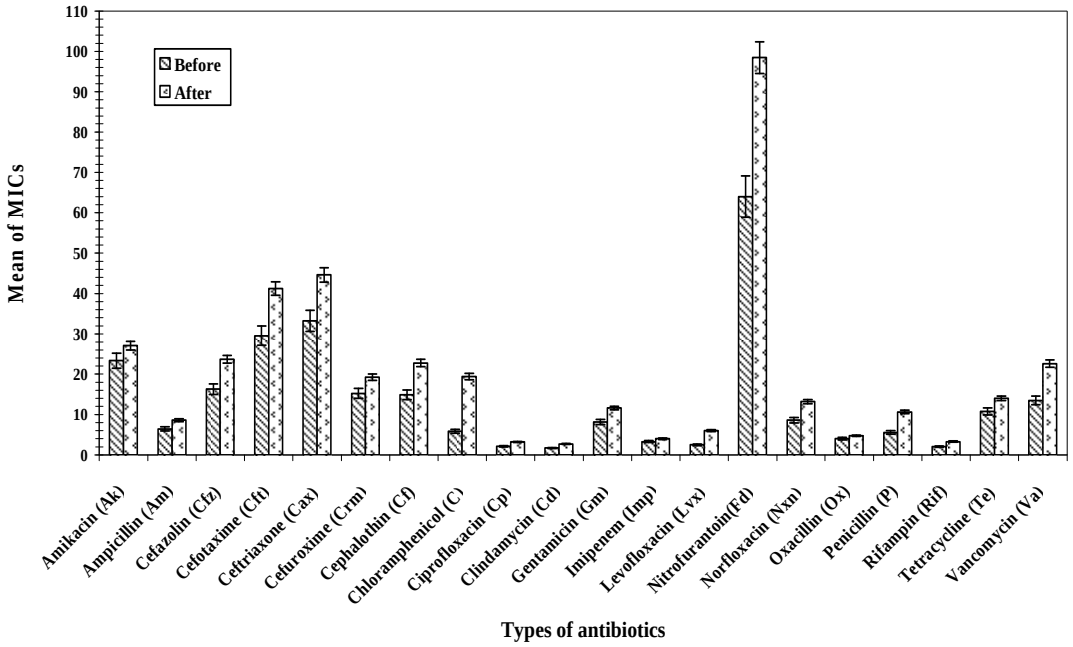


Fig. 2. Changes in mean MICs for all Gram-positive cocci afte *In-vitro* gamma irradiation

P value: Significant (<0.05); C, Cd, Lvx, Fd, Nxn, P, Rif and Va. Non significant (>0.05) ; Ak, Am, Cfz, Cft, Cax, Crm, Cf ,Cp, Gm, Imp, Ox and Te. Lvx, Fd, Nxn, Te and To. Significant (<) 0.05; Ak, Cax, Crm and Gm. Non significant (>) 0.05 ; Azt, Cpd and Imp

3.4 Adherence Assay

3.4.1 Gram–negative bacilli

In general, no major discrepancies occurred between the results of biofilm formation and adherence characteristics obtained by the visual reading methods (qualitative method in tube) and those obtained by the spectrophotometric methods (quantitative micromethod) respectively. The results suggest that both quantifying methods were highly reproducible and the same number of adherent strains was obtained by both methods, and there was a range of variation in adherence ability among the Gram-negative bacilli and gram positive cocci tested strains before and after *In vitro* gamma irradiation.

From the results in (Table 2) it is clear that, in general, the crystal violet (CV) staining method revealed a greater range of variation of adherence ability among the tested strains and striking differences were observed among the adherence-positive strains before and after *In vitro* gamma irradiation. Where, in case of *Escherichia coli* before irradiation the results revealed ODs readings ranging from (0.08) non-adherence to (1.14) strong adherence and *In vitro* gamma irradiation resulted in ODs readings from the range of (0.07) non-adherence to (1.28) strong adherence; there was a strain-to-strain variation in adherence before and after *In vitro* gamma irradiation.

3.4.2 Gram positive bacterial strains

A highly significant strong correlation among the results obtained before irradiation (Pearson r=0.936, *P* value<0.000) and among the results obtained after *In vitro* gamma irradiation (Pearson r=0.976, *P* value<0.000) were observed between OD readings of both methods (Table 3).

3.5 Cell Surface Hydrophobicity of *Escherichia coli* and *Staphylococcus epidermidis* (Hydrophobicity Assay)

In this study, the relative hydrophobicities of two selected different strains (*Escherichia coli* [$E._5$], and *Staphylococcus epidermidis* [$St.ep._{10}$] were determined (Figs. 3 and 4). According to the ρ-xylene method, the percentage of the Gram- negative bacilli adhered to the ρ-xylene phase was significantly higher than was observed for the Gram-positive cocci, and *Escherichia coli* was relatively hydrophobic (surface hydrophobicity>40%) before and after *In vitro* gamma irradiation with an increase in the cell surface hydrophobicity values after irradiation than before, while the coagulase negative *Staphylococcus epidermidis* strain showed intermediate values before irradiation changed to a strictly hydrophilic strain (surface hydrophobicity<20%) after *In vitro* gamma irradiation, which was parallel to the results obtained by the slime production and quantitative adherence measurements.

Table 2. The prevalence (percentage), Slime production and quantitative assessment of adherence of pathogenic Gram-negative bacterial strains before and after in-vitro gamma irradiation

Code no.	*Slime	Adherence (ODs)
	Before/after	Before/after
* 1. *Escherichia coli* (n=32):29.0 %		
E.: 1 ,24	S/S*	0.41 - 0.57 / 0.92 - 1.10
4,15,19 , 26	W/S	0.15 - 0.21 / 0.44 - 0.73
6,25	M/S	0.25 - 0.27 / 0.6 - 0.64
3,7,8,13,14,18,21,23,29-32	S/S	0.3 - 0.65 / 0.33 - 0.96
9, 12, 20,22,27,28	A/A	0.08 - 0.09 / 0.07 - 0.11
2	M/S	0.25 /0.55
5	W/S*	0.15/0.7
10	S/W	0.61/0.19
11	S/M	0.76/0.26
16	S*/W	1.14/0.18
17	W/W	0.16/0.24
*2. *Klebsiella* species (n=21): 19.0 %		
a. *Klebsiella pneumoniae* (n=17)		
K.pn.: 33,34,36,39,48,	W/S	0.13 - 0.23 / 0.32 - 0.63
35,43,46,47	S/W	0.31 - 0.44 / 0.14 - 0.22
37, 41	S/S*	0.69 - 0.72 / 0.93 - 0.95
38,40,44,45,49	S/S	0.30 - 0.51 / 0.38 - 0.89
42	M/S	0.27/0.41
B. *Klebsiella ornithinolytica* (n=2):		
K. or.: 50	S/S	0.63/0.40
51	W/S	0.23/0.34
c. *Klebsiella ozaenae* (n=1):		

Table 2 continued……..

K. oz.: 52	S/M	0.66/0.29
d. *Klebsiella oxytoca* (n=1):		
K. ox.: 53	W/S	0.16/0.38
*3. *Enterobacter* species (n=20): 18.1 %		
a. *Enterobacter cloacae* (n=13):		
En.cl.: 54,55,59,66,	S/S	0.31-0.66/0.43-0.71
56	S/W	0.42/0.13
57, 60,61,65	S/S*	0.34-0.57/0.95 - 1.15
58,62,63,64	W/S	0.16-0. 24/0.36-0.59
b. *Enterobacter aerogenes* (n=7):		
En.ae.: 67,70,73	W/S	0.16 -0.23 / 0.31 - 0.76
68,71,	S/S*	0.56 - 0.63 / 1.06 -1.26
69	S/M	0.41/0.27
72	M/S*	0.27-0.96
*4. *Acinetobacter baumannii*/haemolyticus (n=17): 15.4 %		
Ac.:74,75,79,80,81,83,85,87,90	S/S	0.25-0.72/0.16 -1.01
76,78,88,89	S*/ S*	0.99 -1.1/1.32-1.83
77	W/W	0.22/0.14
82	M/W	0.55/0.71
84	S/M	0.31/0.60
86	S/S*	0.62/0.31
*5. *Proteus* species (n=10): 9.0 %		
a. *Proteus morganii* (*Morganella morganii*) (n=3):		
Pr.mo.: 91	W/S	0.20 /0.45
92,93	S/S*	0.50-0.63/1.37-1.58
b. *Proteus penneri* (n=3):		
Pr.pe.: 94	S/W	0.63/0.21
95	S/M	0.58/0.26
96	W/S	0.19/0.45
c. *Proteus mirabilis* (n=3):		
Pr.mi.: 97,98,99	S/S	0.61-0.72/0.25-0.31
d. *Proteus vulgaris* (n=1):		
Pr. Vu. : 100	W/S	0.23/0.80
6. *Citrobacter freundii* (n=7): 6.3 %		
C.f.: 101,102,103,105,104,106, 107	W/S	0.17-0.22 / 0.42-0.61
	S/S	0.36-0.41 / 0.63-0.78
*7. Alcaligenes xylosoxidans subsp. xylosoxidans (n=2):1.8%		
Al.xy.: 108,109	S/M	0.45-0.53/0.26 - 0.28
*8. Empedobacter (F1.) brevis (n=1): 1.8 %		
Em.110	S/S	0.30/0.59

** Slime production score in three separate experiments ,by three observers ,each performed in-duplicates: The results were registered semiquantitatively using the following estimate grade of slime production: (S*) >3+, (S) strong (3+), (M) moderate (2+), (W) weak (1+) or (A) absent (0). Quantitative assessment of adherence :Adherence measurements were performed at 492 nm in quadruplicate and repeated three times and the ODs values were then averaged. N: non-adherent (ODs ≤ 0.120) W: weakly adherent (0.120 < ODs ≤ 0.240) S: strongly adherent (ODs > 0.240)*

The results seem to clarify some aspects of bacterial adherence to urinary catheters. The difference in behavior of irradiated and non-irradiated strains was confirmed by scanning electron microscopy (SEM) as well as growth in catheter eluates.

Table 3. The prevalence (percentage), Slime production and quantitative adherence assessment of different pathogenic gram positive bacterial strains before and after *In vitro* gamma irradiation

Strain no.	*Slime production adherence (ODs)		
	**Crystal violet		***Safranin
	Before/after *In vitro* gamma irradiation	Before/ after *In vitro* gamma irradiation	Before/after *In vitro* gamma irradiation
*1. *Staphylococcus* species (n=11): 84.6 %			
a. *Staphylococcus haemolyticus* (n=5):			
St.ha.: 1	S/S	0.50/0.89	1.59/1.92
2	S*/S	1.18/0.62	2.55/1.69
3	S*/W	1.76/0.13	2.86/0.68
4	S*/S*	1.30/2.33	2.79/3.16
5	W/S	0.18/0.77	0.75/1.81
b. *Staphylococcus auricularis* (n=2):			
St.au.: 6	S/S*	0.65/1.80	1.57/2.64
7	S/S*	0.79/1.05	1.77/1.97
c. *Staphylococcus warneri* (n=2):			
St.wa.: 8	S/S*	0.57/1.62	1.78/2.53
9	W/S*	0.15/1.41	0.81/2.31
d. *Staphylococcus epidermidis* (n=1):			
St.ep.: 10	S/W	0.96/0.21	1.70/0.79
e. *Staphylococcus cohnii* subsp. cohnii (n=1):			
St.co.: 11	S/S*	1.13/2.68	2.08/3.40
*2. *Enterococcus faecalis* (n=2): 15.4 %			
E.fa.:12	W/W	0.13/0.23	1.24/1.42
13	S/W	0.66/0.16	1.65/1.12

** Slime production score in three separate experiments, by three observers, each performed in-duplicates. S: strong W: weak **Mean optical densities (ODs) of crystal violet stained adherent biofilms at 570nm. W: weakly adherent (0.120<ODs≤0.240) S: strongly adherent (ODs>0.240) ***Mean optical densities (ODs) of 0.25% safranin stained adherent biofilms at 490nm. +: (ODs 0.500-1.500 ++: (ODs>1.500). They were tested in quadruplicate and repeated three times*

3.6 Scanning Electron Microscopy (SEM)

Electron microscopic observations are of great value in showing adhesion of microorganisms to biomaterials. The same strains *Escherichia coli* ($E._5$) and *Staphylococcus epidermidis* ($St.ep._{10}$) were used to study.

3.6.1 Effect of radiation and catheter eluates

On growth, adherence and biofilm formation on inner and outer surfaces to examine the morphology of the biofilm formation. (Fig. 5a-b) showing clean inner and outer catheter surface as a control images.

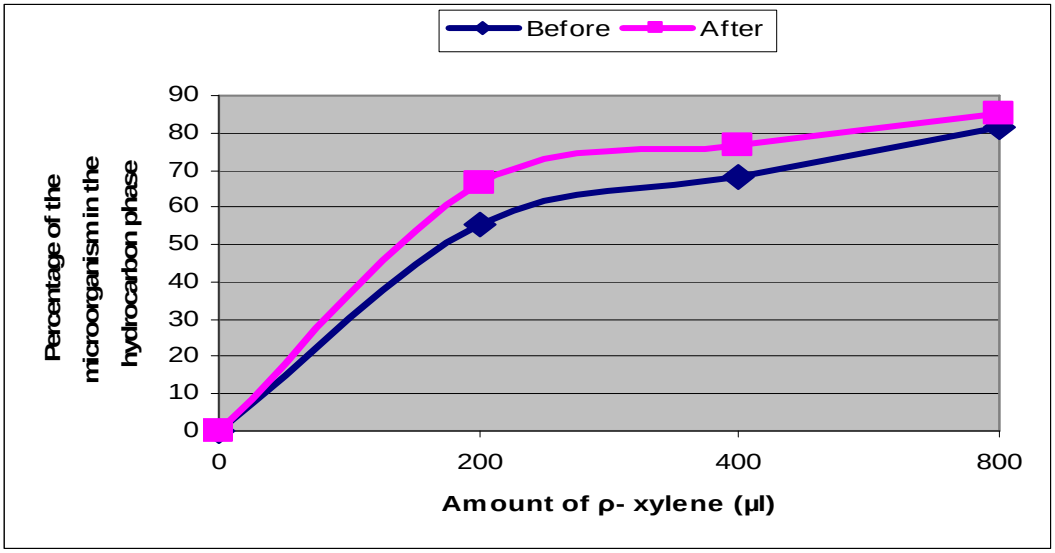


Fig. 3. Adherence of *Escherichia coli* to ρ-xylene before and after *In vitro* gamma irradiation

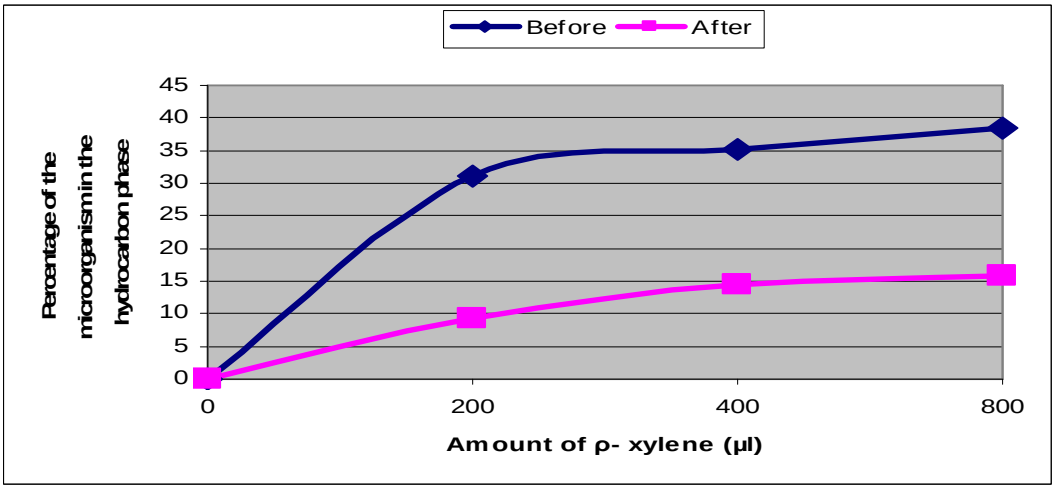
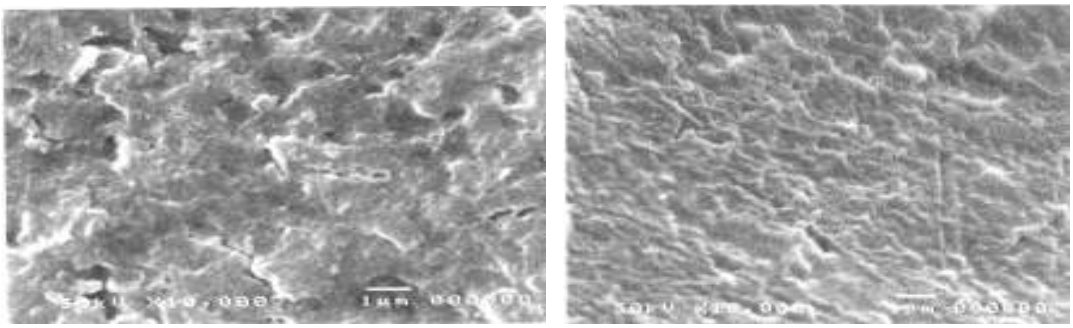


Fig. 4. Adherence of *Staphylococcus epidermidis* to ρ-xylene before and after *in-vitro* gamma irradiation

SEM analysis of the biofilm topography formed on the catheter surfaces of non irradiated microbial cells showed colonization of the catheter surfaces, some of the microbial cells occupied irregularities on the surface at the site of attachment and cells were covered with amorphous material. Parallel to the occurrence of microcolonies, some interactions between non-irradiated cells and the catheter surface appeared as a the presence of extracellular polymeric substances, primarily polysaccharides and the cotton–like material (glycocalyx), surrounding and encasing the cells. These polysaccharides appeared either as thin strands connecting the cells to the surface and one another or as sheets of amorphous material on a

surface. Multiple layers with halos surrounding the cells were detected and clearly visible erosion zones were formed around the border line of the cells (Figs. 6a and 7a).

After irradiation of *E. coli* cells great increase in the number of adherent cells were detected. Most biofilm volume was actually composed of the extracellular polymeric cotton-like substance (glycocalyx) rather than cells. Marked abnormalities in the number of cells adhered to the catheter surfaces were observed in comparing to non-irradiated (Fig. 6b) represent these results.



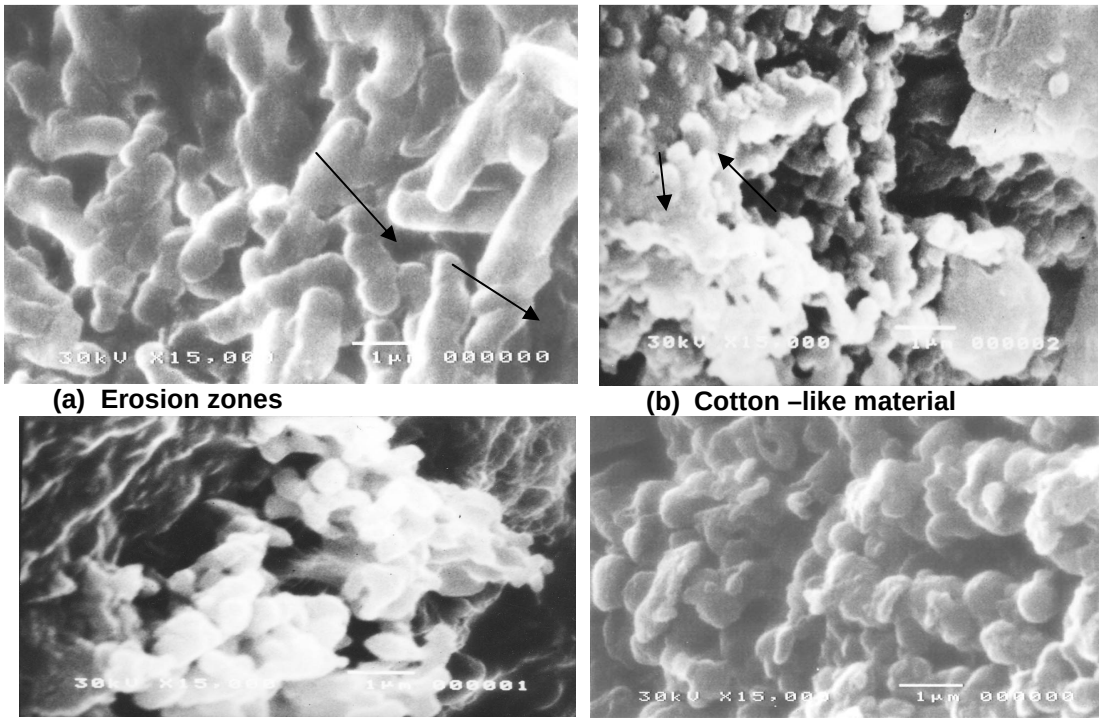
(a) **(b)**

Fig. 5. Scanning electron micrograph of the inner and outer catheter surface
(magnification $10x10^3$)

Concerning the adherence of irradiated *Staphylococcus epidermidis*, cells were had less ability to adhere to catheter surfaces than non- irradiated and abnormalities in shape and size of the adhered cells were observed with production of slimy material (Fig. 7b) depict these results.

3.6.2 Effect of antimicrobial agents on growth, adherence and biofilm formation

It is well known that Amikacin tested against *E. coli* and *Staph. epidermidis* (Figs. 8, 9a and b) and Imipenem against *Staph. epidermidis* (Figs. 10a and b) as antimicrobial agents reduce bacterial adherence at recommended minimum inhibitory concentration (MIC) level, probably due to their mode of actions on cell wall and protein synthesis respectively. Data obtained indicates that treatment with these agents induced a highly significant reduction in the number of cells adhered to catheter surface. With respect to control, where microbial cells were deeply coated with an extracellular amorphous material (Figs. 6a and 7a). Marked abnormalities in cells shape and size and nearly no halos surrounding the microbial cells with no sign of slime formation were detected for both irradiated and non-irradiated cells.



(a) Erosion zones **(b) Cotton –like material**

Fig. 6. Scanning electron micrographs of (a) non-irradiated and (b) irradiated *Escherichia coli* adhered to catheter surfaces
(magnification 15x10^3).

3.7 Plasmid Analysis

As shown previously, the results of susceptibility test showed that, multidrug resistant bacterial strains were isolated before irradiation either slime producer or not. Also, the antibiograms of the majority of the tested strains before irradiation were different from those after irradiation. Because of these differences, their plasmid profiles were studied. The plasmid of highly antibiotic resistant tested strains of *Escherichia coli* (no.5 & 15), *Klebsiella pneumoniae* (no. 29, 31 & 32), *Proteus morganii (Morganella morganii)* (no.95), *Proteus penneri* (no.100), *Proteus mirabilis* (no.101), *Citrobacter freundii* (no.103), *Staphylococcus warneri* (no.9), *Staphylococcus cohnii* subsp. *cohnii* (no.11) and *Staphylococcus epidermidis* (no.10) were extracted and analyzed in agarose gel to determine the degree of similarity and dissimilarity between the plasmid profiles of the different tested isolates before and after irradiation. In some of the tested strains, the plasmid profile analysis after irradiation showed more extra plasmid bands than before irradiation with difference in the molecular weight, Rf., amount and optical density (OD) of the extracted plasmids (Table 4 and plate, 1; A , B ,C, D).

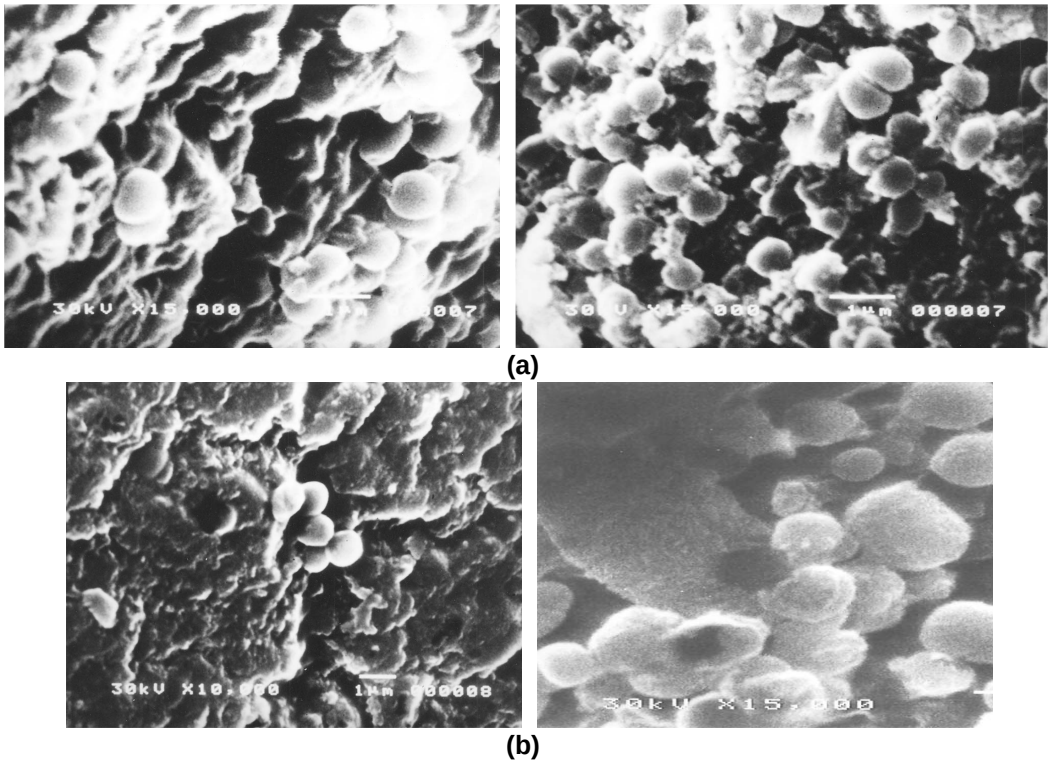


Fig. 7. Scanning electron micrographs of (a) non-irradiated (magnification 15x10^3) and (b) irradiated *Staphylococcus epidermidis* adhered to catheter surfaces
(magnification 10x10^3).

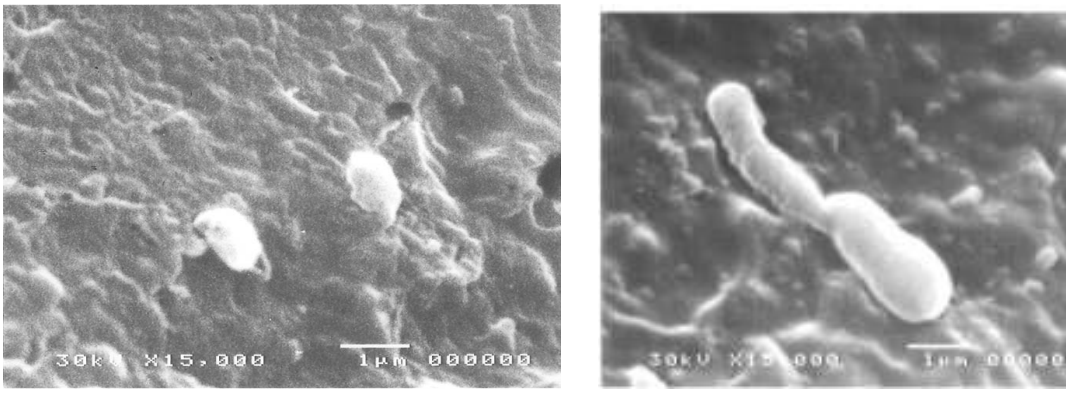
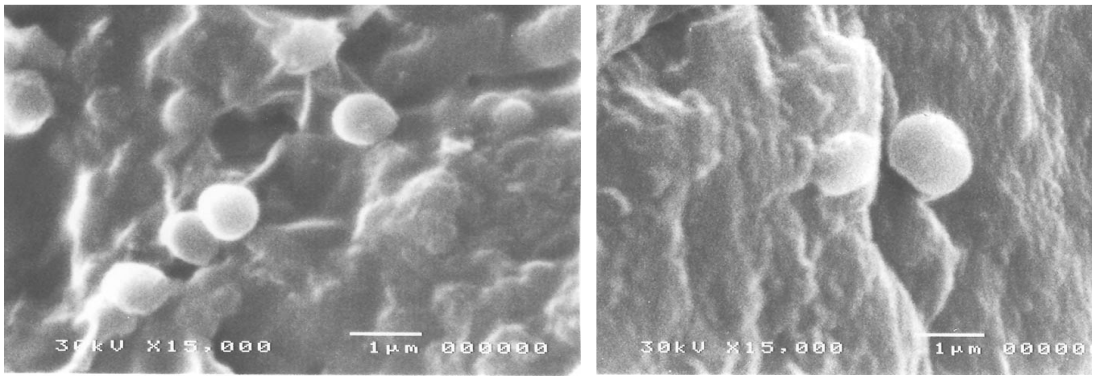


Fig. 8. Scanning electron micrograph showing the effect of MIC 16µg/ml of Amikacin on adherence of (a) non-irradiated and (b) irradiated *Escherichia coli* to catheter surface
(magnification 15x10^3)



(a) **(b)**

Fig. 9. Scanning electron micrographs showing the effect of MIC of Amikacin 16 µg/ml on adherence of (a) non-irradiated and (b) irradiated *Staphylococcus epidermidis* to catheter surface

(magnification 15x10^3)

Table 4. Properties of some selected strains according to their plasmid profile analysis and antibiotics resistance before and after *In vitro* gamma irradiation

Strain	Properties				
	Number of bands B/A	Molecular weight B/A	Rf. B/A	Amount B/A	Number of antibiotics resistant B/A
Pr.pe.100	1/1	4480.3/3980.3	0.14/0.18	7.94/5.61	7/19
Pr.mi.101	1/1	4147/3980.3	0.17/0.18	6.66/5.54	26/25
C.f.103	1/2	4813.7/4647	0.12/0.13	7.07/7.96	3/15
		— /466.67	— /0.66	— /4.17	
K.pn.31	2/2	4480.3/4480.3	0.14/0.14	9.6/10.1	19/19
		1963.4/1963.4	0.36/0.36	3.65/6.16	
St.wa.9	1/1	4813.7/4480.3	0.12/0.14	5.98/14.6	3/14
E.5	4/4	6147/6147	0.087/0.087	13.7/14.1	6/20
		1963/1913	0.38/0.38	4.49/3.78	
		1356/1356	0.45/0.45	5.11/5.08	
		811/811	0.54/0.54	3.88/3.66	
E.15	1/1	6480/6480	0.067/0.067	13.9/11.3	11/19
Pr.mo.95	1/3	6591/6480	0.06/0.067	15.4/11.7	13/19
		— /3147	— /0.26	— /4.52	
		— /2571	— /0.3	— /5.16	
K.pn.29	1/1	6835/6772	0.081/0.085	9.67/9.25	8/15
K.pn.32	2/6	6647/6585	0.093 /0.096	7.03/ 10.1	3/15
		5522/ –	0.16/ –	4.16 / –	
		– /4897	– /0.2	– /2.7	
		– /4397	– /0.23	– /4.3	
		– /3522	– /0.28	– /4.17	
		– /2647	– /0.33	– /4.06	
		– /2292	– /0.38	– /3.38	
St.co.11	1/1	2647/2647	0.21/0.21	6.08/7.78	19/24
St.ep.10	1/1	2813.7/2813.7	0.2/0.2	6.09/8.07	11/12

B: Before In vitro gamma irradiation. A: After In vitro gamma irradiation

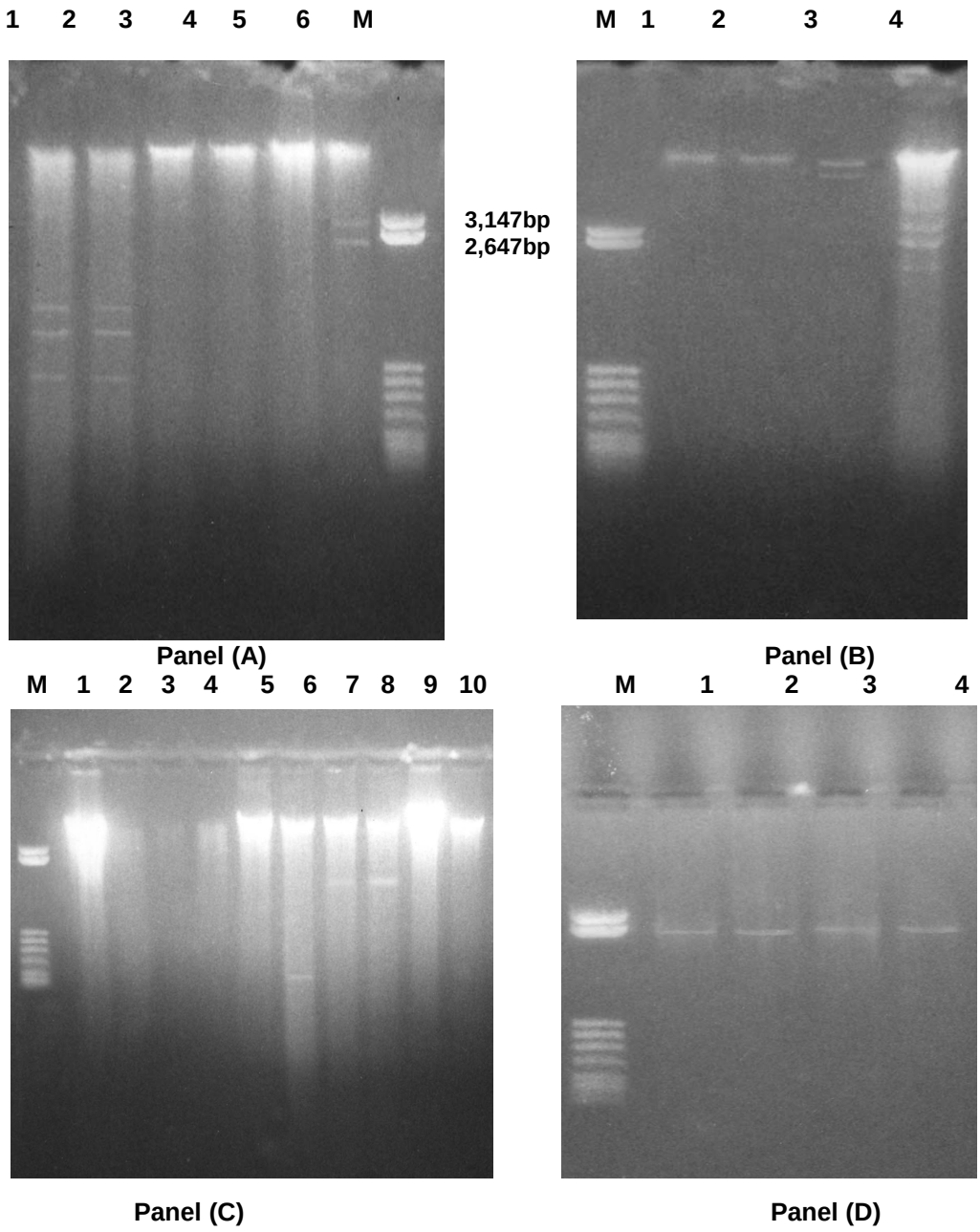
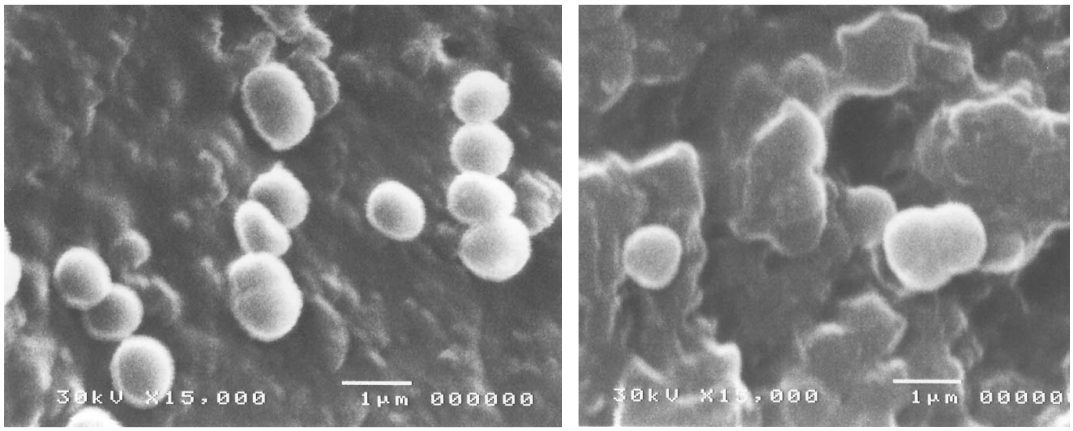


Plate 1. Agarose gel electrophoresis of extracted plasmids DNA

All samples (20 µl of eluted DNA) were run on an ethidium bromide stained agarose gel. Lane M, marker DNA step ladder 50 bp (16 fragments ranged from 50-3147bp, Sigma); Panel (A) Lanes 1&2, 3&4 Escherichia coli [E.$_{5\ \&\ 15}$] and 5&6 Proteus morganii [Pr.mo.95] , Panel (B) 1&2 and 3&4 Klebsiella pneumoniae [K.pn.$_{29\ \&\ 32}$], Panel (C) 1&2 Proteus penneri [Pr.pn.$_{100}$]; 3&4 Proteus mirabilis [Pr.mi.101]; 5&6 Citrobacter freundii [C.f.$_{103}$]; 7&8 Klebsiella pneumoniae [K.pn.31] and 9&10 Staphylococcus warneri [St.wa.9]and Panel (D)1&2, Staphylococcus cohnii subsp. cohnii [St.co.$_{11}$] and 3&4 Staphylococcus epidermidis [St.ep.$_{10}$] before and after irradiation respectively



(a) Erosion zones **(b)**

Fig. 10. Scanning electron micrographs showing the effect of MICµg/ml of Imipenem on adherence of (a) non-irradiated (1µg/ml) and (b) irradiated *(2*µg/ml) *Staphylococcus epidermidis* to catheter surface
(magnification 15x10^3)

4. DISCUSSION

Infections are major causes of morbidity and mortality in patients with cancer [34]. Catheter-associated bacteriuria may lead to bacteremia. Bacteremia, secondary to catheter-acquired bacteriuria (CAB) can develop as a result of mucosal trauma associated with insertion or withdrawal of the catheter [35]. Indwelling urinary catheters are a leading cause of nosocomial infection and have been associated with both morbidity and mortality [36,37].

The results revealed the presence of catheter-associated urinary tract infections (CAUTIs) in immuno-compromised bladder cancer patients with 132 bacterial isolates. Gram-negative bacilli, is much more common than Gram-positive cocci. The result of the microbiologic profile is similar to most reported studies, *Escherichia coli* still being the most common pathogen, followed by other organisms [38,39,40].

Biofilm formation on the surface of indwelling catheters is central to the pathogenesis of infection of urinary catheters. The colonization of uropathogenic bacteria on urinary catheters resulting in biofilm formation frequently leads to the infection of surrounding tissue and often requires removal of the catheter. Within a few hours, adherent bacteria can aggregate, multiply and form biofilm matrices, once surrounded by a dense glycocalyx, may constitute a reservoir of viable microorganisms [41].

In this study, the qualitative method used for biofilm formation was a tube method and a quantitative method for adherence measurements was the microtitre plate assay have been investigated with many different strains which is in a highly good agreement with; [42], they suggested that the most susceptible method for biofilm detection was the microtiter plate assay. Our results show that both the modified tube method with minor modifications [43] and quantitative method are highly reproducible in assessing microbial biofilm production and there was high coincidence in its detection. The adherent growth and slime production required both glucose (0.25% or 1.0% wt./vol.) and casein digests for expression. Glucose was the only carbohydrate source acceptable and in its presence growth was maximal.

Adding yeast extract and casamino acids promoted growth and slime production [43]. Although some variability in values between irradiated and non-irradiated strains was seen, the level of biofilm formation was highly consisted between experiments discriminating strains which produced strong biofilm from which produced weak biofilm. Our data also indicate that these techniques can serve as a reliable quantitative tool for *In vitro* comparing the adherence and biofilm formation of different strains before and after gamma irradiation.

The results obtained are in accordance with the previous findings of [44,45] suggested that, biofilm formation was strongly affected by the presence of an additional carbohydrate source in the medium, or by iron deprivation, indicating a role of slime for survival in stressful conditions.

The ability of the tested organisms to produce slime was changed after *In vitro* gamma irradiation and the production was varied in some of the producer strains from positive to weak positive or negative. The obtained results were in agreement with [46,12]. It was also observed that some isolated strains whose slime production was interpreted as moderate (score 2) by the qualitative method presented an optical density >0.240, corresponding to strong adherence by the quantitative method (i.e. slime production of some strains may be detected by the qualitative method less than that by the quantitative method) which is also in a good agreement with others as [19]. Biofilm formation is an important factor of pathogenicity in all microorganisms. Biofilm protects bacteria against actions of antibiotics and disinfectants and therefore bacteria can survive concentrations of antibiotics and disinfectants even 1000× higher than planktonic forms of the same bacteria [47,48].

The antibiotics were chosen because of their interesting activity against gram negative and gram positive bacterial pathogens further, some of the isolates were resistant to traditional antimicrobial therapies as well as, because the irradiated strains show variability in values than non irradiated. The results obtained in the current study show that antibiotic susceptibility test is different when comparing *E. coli* slime producer (SP) versus non-slime producer (NSP) tested strains. The biofilm producer associated antibiotic resistance, also reported in other studies [49] can be attributed to a decreased antibiotic diffusion through the extensive biofilm matrix

The presence of the catheter gives a solid surface for attachment and an ideal niche for the biofilm to form and flourish. Such organization is typical of biofilms found on medical devices and implants. This provides an opportunity for the microorganisms to accommodate each other.

The results obtained with positive correlation observed between biofilm formation and cell surface hydrophobicity (CSH) which is in a fair agreement with others. *Escherichia coli tested strain* was relatively hydrophobic (surface hydrophobicity >40%) before and after *in-vitro* gamma irradiation. From the results it is clear that, lower values of the ROD, and in contrast, higher values of the percentages of the microorganisms in the ρ-xylene phase correspond to more hydrophobic bacteria. Galliani et al. [50] hydrophobicity (xylene partition) was well correlated with adhesion when testing bacteremic strains of *Staphylococcus epidermidis*. Slime production, adhesion, and hydrophobicity were highly strain dependent among *S. epidermidis* organisms.

The catheters investigated by scanning electron microscopy before and after *In vitro* gamma irradiation of the tested isolates produced more or less different effects. Generally, SEM observations showed the presence of abundant amorphous extracellular material, of

possible polysaccharide nature, embedding cells to form multilayered biofilm with halos surrounding the cells and clearly visible erosion zones were formed around the border line of the cells. Lopez-Lopez et al. [51] many biomaterials, contain several additives to make them flexible enough to be used in catheters. Some of them could be eluted from catheter into the medium and used by different microorganisms. The measurement of bacterial adherence to clean catheters is a simplification of the events that occur *In vivo* because the catheters are rapidly coated with different proteins, fluids and cells, but the initial adherence may depend mainly on the catheter biomaterial. Several properties, including hydrophobicity mediated attachment of many bacterial species to solid surfaces.

In the current study, the effect of catheter components on growth of the tested strains were tested. The eluates obtained from the incubation of catheter segments in PBS as growth media were used. The catheter eluates stimulated the growth of the tested strains before and after *In vitro* gamma irradiation after incubation on the inner and outer surface of catheters in the absence of any other externally supplied nutrients. There was an increase in the number of the irradiated cells attached to catheter surface. However, different results were observed with *Staphylococcus epidermidis*, low numbers of the irradiated cells were attached to catheter surface with formation of slimy materials and the irradiated strains were had less ability to grow than non-irradiated strains. Peters et al. [52] the surface erosion surrounding the colonies of some microorganisms which observed by scanning electron microscopy dose not necessarily imply enzymatic breakdown of the basic plastic material, perhaps other additives used in the production of catheters such as plasticizers, and stabilizers are being degraded. Other explanations for the continuing multiplication could be that the microbial cells contain sufficient nutrients or that the dividing cells get such nutrients from lysed bacterial cells. Cell multiplication and an increase in colony size, in the absence of externally supplied nutrients, would seem possible only if the microbial cells are able to use some catheter components as nutritional source.

There was quite clear difference in the number of bands and/or molecular weight in plasmid profile analysis of the tested strains. The majority of the tested strains had one or more bands with molecular weight more than 3,147bp. As a result of radiation, the plasmid DNA was completely or partially damaged, analysis of irradiated strains showed more extra-plasmid bands than before irradiation and/or difference in the molecular weight, at the same time, the radiation may activated the expression of some other genes, among which are the genes of the antibiotic resistance which was reflected on the increase of such resistance compared to un-irradiated samples. After irradiation the incidence of antibiotic resistance in most of the tested isolates changed and was higher than before irradiation as mentioned previously. The results obtained are in accordance with the previous findings of [53]. It is well known that the effect of ionizing radiation or ultraviolet on living organisms is induced by DNA damage in the cell or cause a change in the molecular structure of the biologically important molecule [54-57].

In future study, the understanding of biofilm dynamics is crucial to develop better control strategies. As the problem of antimicrobial resistance becomes more widespread, the use of narrow-spectrum, antimicrobial agents become less feasible. In addition, catheter associated urinary tract infections, are now becoming therapeutic challenges. Strategies for controlling resistant pathogens need to deal with antibiotic use. Furthermore, the physicians should be familiar with the increased antibiotic resistance associated with bacteria in biofilms and the difficulty of their eradication.

5. CONCLUSION

The next step should be to alter the catheter surface in order to prevent the bacterial adherence and to inhibit biofilm formation which may provide promise for control of catheter associated urinary tract infections. More basic research at the level of pathogenesis and catheter substance is needed to design novel strategies.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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