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In vitro Effect of Low Radiation Doses on Some Pathogenic Bacteria. Its Relation to Surface Hydrophobicity, Adherence and Viability on Plastic Catheter Materials

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Abstract: Adhesion of bacteria differing in cell surface hydrophobicity, growth and viability of single isolates of *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus epidermidis* isolated from catheter associated infections to catheter made of polyurethane, hydrophilic polyurethane, teflon, vialon and siliconised latex was evaluated before and after *In vitro* exposure to test dose of 25 Gy of gamma radiation. The cell surface hydrophobicity of the tested strains was assessed by hexadecane method. Hydrophobic strains adhered more efficiently to the catheter surface than hydrophilic strains. Low number of the hydrophilic *Staphylococcus epidermidis* cells can adhere to the polyurethane catheter surface. Irradiation cause a change in the cell surface hydrophobicity of the tested strains. Colonization of polyurethane and hydrophilic polyurethane in phosphate buffered saline (PBS) to non-irradiated and irradiated *Klebsiella pneumonia* and *Escherichia coli* was followed by scanning electron microscopy (SEM). Regular sampling of specimens at 1h , 3 h, 8 h, 12h,24 h and 48h. demonstrated colonization and adhesion progression with an increase in the exposure interval of the non irradiated cells to the polyurethane catheter surface followed by cell proliferation, possible break down of catheter components and production of a slimy material covering the bacterial colonies. Adherence and colonization of the irradiated cells showed reduction in cells number along the incubation time with abnormalities in the cells shape and size. While, the adherence of the non-irradiated and irradiated cells to the hydrophilic polyurethane surface showed marked decrease in number of cells with abnormalities in the cells shape and size. Bacterial adherence and colonization of catheters in distilled water were the same as in PBS and slightly delayed in the microcolony formation. Bacterial viability and growth was evaluated in eluates obtained from incubation of segments of each catheter in buffer for 24h. Non of the eluates increase the viability and growth of *Staphylococcus epidermidis* along the incubation time. However, all of them, significantly increased the growth of non-irradiated (*Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli*) with the exception of the eluate from siliconised latex, in which the inoculum count was reduced for *E. coli*. The initial growth in different catheter eluates was higher for non-irradiated strains than irradiated ones with all biomaterials tested. So, bacterial adherence to catheter may depend in part on the nature of the biomaterial and that substances eluted from the catheters may affect the viability and growth of different microorganisms . The non irradiated strains can grow in catheter eluates and colonies catheter surface better than the irradiated strains. Although, The irradiation change the hydrophobicity of the tested strains as well as reduce the number of cells with abnormalities in shape and size , the irradiated strains also persist on these biomaterials. The implications of these findings may be important in the pathogenesis of foreign body infections and utilization of new biomaterials to prevent bacterial adherence and colonization in immuno-compromised patients.

Key words: Adherence, growth, hydrophobicity, biomaterials, pathogenic microorganisms, gamma radiation.

Introduction

Medical devices are used extensively in surgical practice. This include devices used on a temporary, intermittent and long-term basis. Transient or permanently implanted plastic devices are frequently the starting point of infection. Chemotherapeutic treatment of these infections is very difficult and usually requires removal of the catheter or new methods to attack the problem. Future remedies will likely involve utilization of new biomaterial designs and application of either highly potent antimicrobials or agents used in combination that penetrate biofilms and eradicate the organisms. (Reid, 1998).

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 defence mechanisms, especially phagocytosis by polymorphonuclear leukocytes (Heinzelmann *et al.*, 1997 and Peters and Schumacher-Pedreau, 1994).

Many medical devices, such as catheters, prosthetic heart valves, and artificial organs are manufactured from biopolymers which are highly hydrophobic. Polymers of high hydrophobicity are adhesive for many bacterial pathogens. Initial adherence is considered to depend mainly on surface properties of bacteria such as surface hydrophobicity and net surface charge (Martinez *et al.*, 1991). Hydrophobicity of bacteria has generally been correlated with enhanced virulence (Rozgonyi, 1994) and with increased attachment to the surface of implanted devices (Christensen, 1993). Adherence permits bacterial persistence and in some cases infection (Speert *et al.*, 1986).

Many biomaterials, contain several additives to make them flexible enough to be used in catheters. Some of these substances could be eluted from catheter into the medium and could be used by different microorganisms (Lopez -Lopez *et al.*, 1991).

Gristina *et al.* (1987) have described bacterial adherence and colonization and coating of host factors on biomaterials as 'race for the surface". Biomaterial surfaces provide a ready site for competitive colonization, because energy sites are available for either bacteria or host cells, however, many polymers have a surface that is anti-adhesive for tissue cells but pro-adhesive for bacterial pathogens. A biomaterial surface that encourages rapid leucocytic cell colonization or is adhesive for host factors may be less susceptible to bacterial colonization.

Antimicrobial coating of medical devices has recently emerged

as a potentially effective method for preventing device related infection (Cook *et al.*, 2000; Darouiche *et al.*, 1998; Nazionale *et al.*, 1998). A photochemical surface modification carried out as a generic means of applying antimicrobial coatings to biomedical device polymers for them to prevent bacterial colonization (Dunkirk *et al.*, 1991). The sterilization of the polystyrene plates with gamma radiation diminish the hydrophobicity of the polystyrene (Christensen *et al.*, 1985). It is well known that the effect of ionizing radiation or ultraviolet on living organisms is induced by DNA damage in the cell. The criterion of radiosensitivity being the cell death or growth inhibition. Hall *et al.* (1988) reported that radiation damage to cellular DNA is initiated by direct energy deposition in the macromolecule and by the attack of free radicals produced in the surrounding medium. Schans Vander *et al.* (1973) described that gamma radiation induced three types of damage in DNA, single strand breaks, double strand breaks and nucleotide damage which include base damage and damage in the sugar moiety. The base damage is a major component of damage induced by ionizing radiation in prokaryotic as well as eukaryotic systems. Thus irradiation produces damage which can cause mutations and disappearance of some or all cell activities. After irradiation, bacterial cells die or loose their ability to divide, some contain abnormal sets of chromosomes or transmit their chromosomes abnormally, while others exhibit heritable changes.

The goals of this study were (a) collection of pathogenic strains from culture of cancer patients with catheter associated infection with identification according to standarded methods; (b) evaluation of the hydrophobicity of four different isolated bacterial strains which play an important role in adherence ; (c) colonization and adherence of *Klebsiella pneumoniae* and *Escherichia coli* over 48 hr. to two different biomaterials (polyurethane and hydrophilic polyurethane) by scanning electron microscope; (d) assessment the effect of eluates with five different biomaterials composed of polyurethane, hydrophilic polyurethane, teflon, vialon and siliconised latex on the growth and viability of *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus epidermidis* in the absence of other nutrient sources and (e) studies the effect of low dose of gamma radiation 25 Gy on hydrophobicity, adherence and colonization of irradiated strains to the biomaterials used and the ability of them to grow on the catheter eluates.

Materials and Methods

Bacterial strains: Out of twenty five samples, one strain each of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus epidermidis* isolated from clinical sources at the National Cancer Institute, Cairo, Egypt were used. They were isolated from urine of catheterized patients and from blood cultures of patients with catheter associated infections. Cultivation were carried out on Blood agar and MacConky agar, strains were identified according to standard methods (Krieg and Holt, 1994): Bergey's Manual of Determinative Bacteriology & Cowan and Steel (1985) and gram negative bacteria were confirmed by API 20 E (API system SA, France).

Heavy suspension of each strain in Tryptic Soy Broth (TSB, BBL) were stored with glycerol (10%) in small volumes at 70°C.

Biomaterials: Catheter made of five different biomaterials were assessed : (a) polyurethane (Pu) Central venous catheter, Becton Dickinson, Singapore. (b) Hydrophilic polyurethane (Hydrocath Pu) Central venous catheter kit, Becton Dickinson, Singapore. (c) Teflon (I.V. Cannula, Helsingborg, Sweden) ; (d) Vialon (I.V. Catheter, Becton Dickinson, Spain and (e) Latex (siliconised) foley catheter, Malaysia).

The catheters examined were split longitudinally, cut into 1-cm lengths under sterile conditions.

Irradiation source: ^{60}Co 220 Gamma cell, was used. A low radiation dose was used @ 0.019 Gy/sec. Twenty five Gys gamma radiation were given. This single dose is biologically equivalent to the fractionated multiple doses given on one daily fraction schedule that is usually used in treatment of some cancer patients (bladder, lung and cervix) according to the type and grade (Barton, 1995) .

Surface hydrophobicity assay: Hydrophobicity assay; Bacterial cell surface hydrophobicities were measured by the hexadecane method (Rosenberg *et al.*, 1980). One to 6-day-old cultured cells were washed once with phosphate buffered saline (PBS) (pH 7.4) before adjusting the cell suspension to an optical density of 0.6 (λ = 550 nm, Beckman 24, USA) for hydrophobicity assay. Two parallel test tubes of the same sample (control and irradiated tube) were used for each measurement and all strains were tested five times, with new cultures being used each time. Three ml of phosphate buffered saline (PBS), pH 7.2-7.4, containing 10^9 cfu/ml (measured by spectrophotometry-initial optical density) was vortex and mixed for 1 min. with 0.35 ml of P-xylene (Prolab.) then left for 30 min. to allow phase separation and the optical density (OD) of the aqueous phase was again measured (final optical density), surface hydrophobicity was expressed as a percentage.

Bacterial growth in catheter eluates: Eluates of the catheters were prepared by incubating 30 segments of each in 10 ml of phosphate buffer saline. Each strain was inoculated into catheter eluates at a final concentration of 10^3 cfu / ml. At timed intervals, 10 μl samples were diluted in ice-cold PBS and pour plates made in Tryptone glucose yeast extract agar (TGY) oxoid, colonies were counted after incubation for 24h. at 37°C.

Scanning electron microscopy (SEM): Two strains were selected for SEM studies. The precultivation of *Klebsiella pneumoniae* and *Escherichia coli* for 18h, at 37°C was performed in N-broth (15 g of tryptone, 4 g of yeast extract, 8 g of NaCl and distilled water to a volume of 1,000 ml), pH 7.5, After centrifugation for 30 min. at 2.067g the supernatant was discarded and the microbial cells (beginning of the stationary phase) were suspended in phosphate buffered saline (PBS) pH 7.0. After six washings with the phosphate buffer, the microbial cells were suspended in the same buffer to a concentration of 10^7–10^8 cfu/ml. Many pieces of the examined catheters were immersed in the previously prepared buffer in glass tubes. These mixture were incubated (without shaking) at 37°C for 48 hr. Catheter pieces

Table 1: Surface hydrophobicity of the tested strains before and after radiation

Strains	Surface hydrophobicity-mean (SD)* of five determinations	
	Before radiation	After radiation
Klebsiella pneumoniae	24.8 (0.1)	11.9 (0.1)
Pseudomonas aeruginosa	45.5 (0.2)	44.6 (0.3)
Escherichia coli	64.2 (0.1)	84.7 (0.1)
Staph. epidermidis	18.2 (0.2)	21.3 (0.1)

*Percentage ± standard

Table 2: Growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in elutes from different catheters before and after radiation

	Means (SD) Number of cfu (10^4/ml)			Means (SD) number of cfu (10^3/ml)		
	Klebsiella pneumoniae			*Klebsiella pneumoniae*		
	Before radiation			After radiation		
	3h	8h	24h	3h	8h	24h
Control	25.3 (0.9)	41.0 (1.4)	52.9 (1.6)	19.9 (0.2)	28.1 (0.4)	35.8 (1.3)
Polyurethane	49.9 (1.13)	105.0 (1.4)	204.9 (1.9)	31.2 (0.4)	69.2 (0.7)	145.8 (1.8)
Hydrophilic polyurethane	26.0 (1.2)	49.9 (1.3)	90.7 (1.9)	16.0 (0.4)	20.1 (0.3)	43.3 (0.4)
Teflon	41.9 (1.7)	101.9 (1.3)	180.4 (1.7)	27.9 (0.3)	45.0 (0.5)	81.9 (0.1)
Vialon	35.9 (1.3)	94.8 (1.6)	160.6 (1.9)	26.0 (1.2)	25.1 (0.2)	76.2 (0.4)
Siliconised Latex	26.9 (1.4)	70.1 (1.3)	145.9 (1.9)	20.2 (0.3)	25.0 (1.9)	70.1 (0.4)
Control	53.3 (0.5)	95.2 (0.5)	142.0 (0.3)	31.2 (0.4)	53.2 (1.6)	98.2 (0.3)
Polyurethane	75.2 (0.4)	164.2 (0.4)	41.0 (0.3)	49.9 (1.3)	101.9 (1.3)	269.0 (0.3)
Hydrophilic polyurethane	61.3 (0.4)	136.1 (0.5)	270.0 (0.4)	29.1 (0.4)	70.1 (1.3)	136.1 (0.5)
Teflon	75.9 (0.3)	186.9 (0.4)	392.2 (0.3)	50.0 (0.1)	105.0 (1.4)	186.9 (0.4)
Vialon	73.1 (0.8)	155.0 (0.3)	415.1 (0.5)	41.0 (1.4)	95.0 (1.6)	205.0 (1.5)
Siliconsided Latex	69.2 (0.7)	169.2 (0.9)	405.8 (0.4)	40.0 (0.3)	95.2 (0.5)	204.9 (1.9)

Table 2: Growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in elutes from different catheters before and after radiation

	Means (SD) Number of cfu (10^4/ml)			Means (SD) number of cfu (10^3/ml)		
	Klebsiella pneumoniae			*Klebsiella pneumoniae*		
	Before radiation			After radiation		
	3h	8h	24h	3h	8h	24h
Control	26.0 (0.5)	45.1 (0.5)	70.2 (0.5)	16.9 (0.9)	19.0 (1.4)	31.2 (0.4)
Polyurethane	31.2 (0.4)	82.1 (0.2)	169.2 (0.9)	21.0 (0.2)	40.0 (0.3)	90.6 (1.9)
Hydrophilic polyurethane	25.0 (0.2)	50.0 (0.1)	96.2 (0.3)	14.1 (0.3)	20.0 (1.1)	61.3 (0.4)
Teflon	25.1 (0.2)	76.2 (0.4)	161.3 (0.5)	19.0 (1.4)	25.9 (0.5)	82.1 (0.2)
Vialon	43.3 (0.4)	98.2 (0.3)	174.9 (0.2)	25.2 (0.9)	50.0 (1.3)	98.0 (0.2)
Siliconised Latex	21.0 (0.2)	41.0 (1.4)	52.9 (1.6)	21.1 (0.2)	53.2 (0.5)	160.6 (2.0)
Control	28.1 (0.4)	11.9 (0.3)	9.2 (0.4)	25.0 (0.2)	9.1 (0.3)	6.3 (0.4)
Polyurethane	16.9 (0.9)	11.2 (1.1)	4.0 (0.3)	13.1 (0.2)	8.3 (0.3)	2.4 (0.4)
Hydrophilic polyurethane	12.1 (0.9)	7.1 (0.3)	3.0 (0.4)	9.0 (0.3)	3.2 (0.2)	2.1 (0.1)
Teflon	20.1 (1.1)	14.0 (0.3)	7.0 (0.2)	21.0 (0.2)	11.9 (0.4)	3.5 (0.3)
Vialon	19.0 (1.4)	11.9 (0.4)	9.1 (0.3)	11.2 (1.9)	9.2 (0.4)	4.0 (0.3)
Siliconsided Latex	13.9 (1.1)	9.0 (0.3)	7.0 (0.2)	9.2 (0.4)	7.0 (0.2)	3.0 (0.4)

were removed at 1 , 3, 8, 12, 24h. and 48 h. of incubation. In each case the inner and the outer surface of the catheter were examined. Similar experiments were carried out with distilled water instead of phosphate buffer.

The removed catheter samples were glued to scanning electron microscopic stubs. They were fixed with 2.5% glutaraldehyde in PBS for 10 min. at room temperature. The tested bacteria fixed on the surfaces were dehydrated in an ethanol-gradeseries (50, 60, 70, 80, 90 and 100%) for 10 min . after each washing with PBS and allowed to dry on a clean hood at room temperature. The bacteria fixed on the surfaces were examined by a scanning electron microscope with a tilt angle of 45° after gold deposition in vacuum (Park *et al.*, 1998).

Statistical analysis: All the experiments were done in replicates five determinants (except for SEM), the mean value and SD were calculated; where appropriate comparison amongst groups were made. Analysis of variance with SAS programme was used to assess statistical significance at $P < 0.05$.

Results

Individual colonies isolated from the growth media were selected according to their morphological characteristics and biochemical tests. They were identified as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa Escherichia coli* and *Staphylococcus epidermidis*. One strain from each was selected for further studies.

Surface hydrophobicity before and after irradiation: Values obtained with the test strains ranged from 64.2% SD 0.1 (*E. coli*) to 18.2% SD 0.2 (*S. epidermidis*) before radiation. While, it ranged from 84.7% SD 0.1(*E. coli*) to 11.9% SD 0.1 (*K. pneumoniae*) after radiation. Two of the strains (*E. coli* and *P. aeruginosa*) were relatively hydrophobic (surface hydrophobicity >40%). *S. epidermidis* (before radiation) and *Klebsiella pneumoniae* (after radiation) were relatively

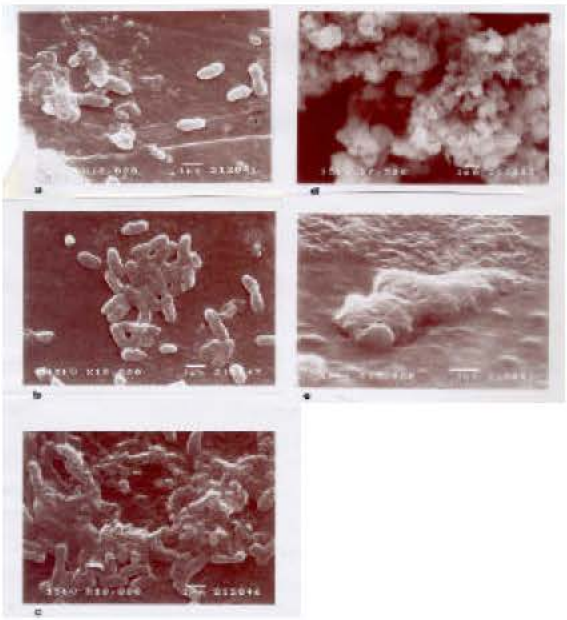

Fig. 1(a-b): Scanning electron micrograhs of non-irradiated *Klebsiella pneumoniae* attached to polyurethan e catheter surface.

I. Few cells appear on the inner and outer surface of the catheter by 8-12 h.

ii. The cathether surface were confluently colonized by adherent bacteria after 24 h.

iii. Heavy colonization were detected after 48h. incubation and dividing cells can be observed.

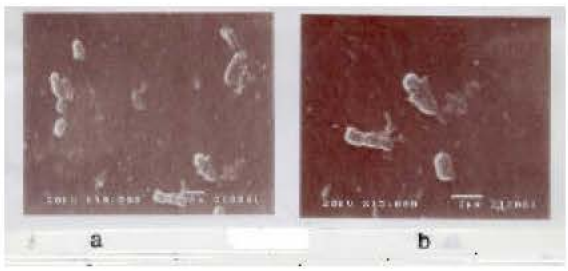

Fig. 2(a-b): Scanning electron micrographs of irradiated *Klebsiella pneumoniae* attached to polyurethan e catheter surface.

I. Single layer of cells were detected after 8-24 h. with no sign of slim formation.

ii. Abnormalities in shape and size wer e observed.

hydrophilic (surface hydrophobicity < 20%). Two strains gave intermediate values 24.8% SD 0.1 *Klebsiella pneumoniae* before radiation) and 21.3% SD 0.1 (*Staph. epidermidis* after radiation) Table 1.

The results seem to clarify some aspects of bacterial adherence and colonization of different catheters. The difference in behaviour of irradiated and non-irradiated strains was confirmed by comparing the adherence to different

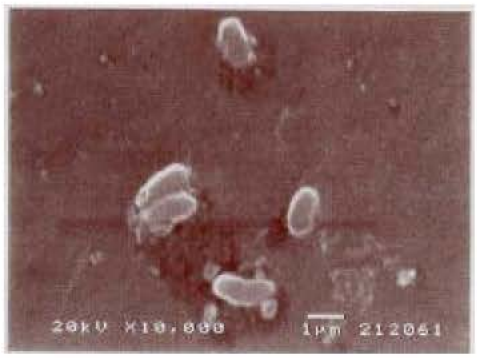

Fig. 3: Scanning electron micrographs of non-irradiated *Klebsiella pneumoniae* attached to hydrophilic polyurethane catheter surface. Marked decrease in number of cells with narrow halos under the cells along the incubation time.

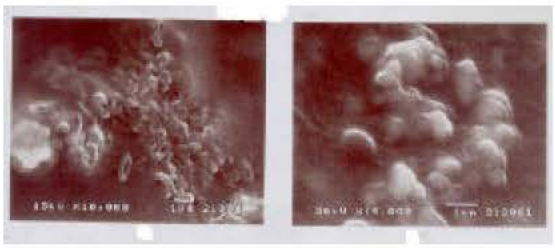

Fig. 4(a-b): Scanning electron micrographs of irradiated *Klebsiella pneumoniae* attached to hydrophilic polyurethane catheter surface.
Marked abnormalities in shape, size and number of cells adhere to catheter surface.

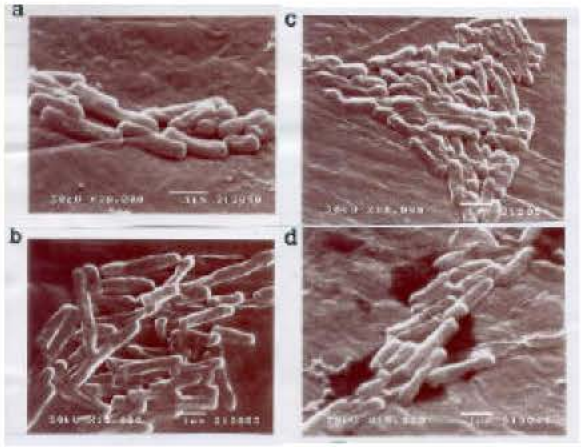

Fig. 5(a-b): Scanning electron micrographs of non-irradiated *Escherichia coli* attached to polyurethane catheter surface.

I. At 1-3h. single layer in close contact to catheter surface.

ii. Multiple layer of cells were detected after 8-12 h. incubation.

c- After 24-48 h., halos surrounding the cells number.

I. Clearly visible erosion zone were formed around the border line of the cells.

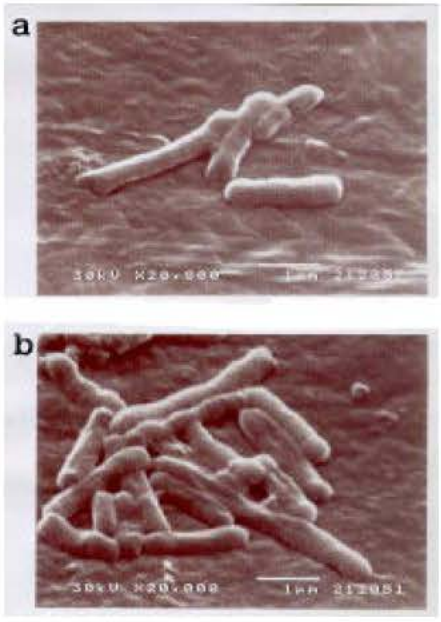

Fig. 6(a-b): Bacterial adherence of *Escherichia coli* and colonization of catheter in distilled water The growth as in phosphate buffer with slightly delayed in microcolony formation along the incubation intervals.

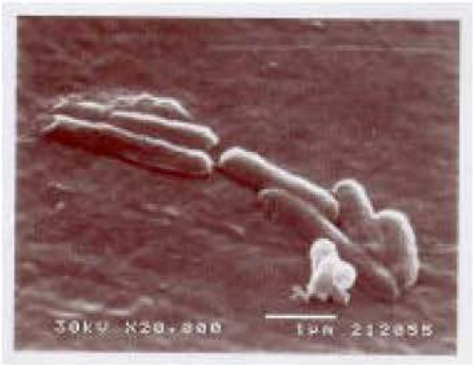

Fig. 7: Scanning electron micrographs of irradiated *Escherichia coli* attached to polyurethane catheter surface.
Light colonization occurs after 8h. with single layer in close contact to catheter surface.

catheter materials by SEM as well as growth in catheter eluates.

Bacterial adherence of some non-irradiated and irradiated tested strains to catheter surface: Examination by scanning electron microscopy of catheter portions removed at intervals from a suspension of *Klebsiella pneumoniae* tested in phosphate buffer demonstrated colonization progressing with an increase in the exposure interval. The initial colonization of different catheters types showed some variation, but the overall dynamics of the phenomenon can be summarized as

Fig. 8(a-b): Scanning electron micrographs of irradiated *Escherichia coli* attached to polyurethane catheter surface.

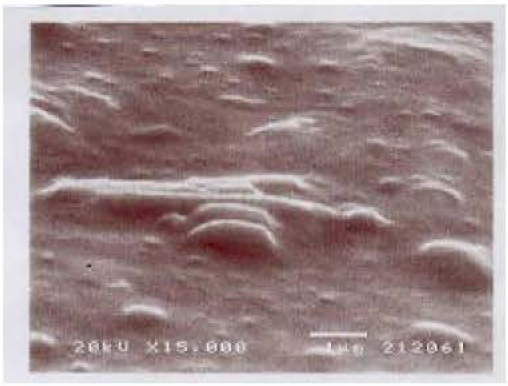

Fig. 9: After 24-84h. reduction in number of cells with some abnormalities in shape and no halos surrounding the micro colonies were detected.
After 24-48h. reduction in number of cells with some abnormalitis in shape and no halos surrounding the microcolonies were detected.

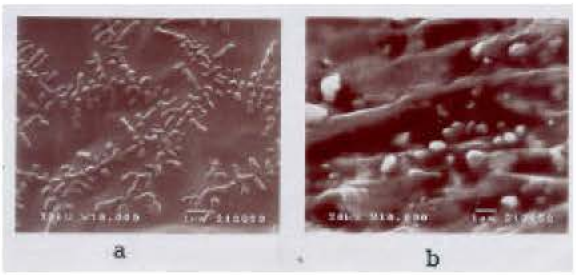

Fig. 10(a-b): Scanning electron micrographs of irradiated *Escherichia coli* attached to hydrophilic polyurethane catheter surface
Marked abnormalitis in the cell shape and size

follows, By 8–12 h. after exposure, a few microorganisms appear on polyurethane (inner and outer surface) some of them occuping irregularities on the surface at the site of attachment, After 24h, the catheter surface were almost confluently colonized by adherent bacteria and the number of adherent bacteria increased. Heavy colonization occurs in 48h, depending on the catheter type investigated. The catheter surface was completely occluded by large numbers of adherent bacteria, which were embedded in large amounts of their own amorphous materials to form a thick adherent

Fig. 11: Scanning electron micrographs of non-irradiated *Staphylococcus epidermidis* attached to polyurethane catheter surface.
Low number of cells were attached to catheter surface with formation of slimy materials

biofilm. Dividing cells can be observed at any time during the 48hr of incubation, (Fig. 1a-e).
Adherence of irradiated *Klebsiella pneumoniae* cells to polyurethane catheter was decreased. It was similar at 8 and 24 hr, the bacteria formed a single layer of cells with no sign of slime production. Abnormalities in shape and size were detected, (Fig. 2a, b).
While, in case of studying the adherence to the hydrophilic polyurethane catheter, marked decrease in number of cells was observed with the non-irradiated *Klebsiella pneumoniae* (Fig. 3) with narrow halos under the cells. While, the irradiated cells (Fig. 4, a-b) after 24h. shows marked abnormalities in the cells shape, size and in the number of cells adhere to the catheter surface. Some cells may be covered with amorphous materials.
Parallel to the occurrence of microcolonies, some interactions between *E. coli* (non-irradiated) and the polyurethane (Pu) catheter surface appeared. Single layer in close contact with the (Pu) catheter surface was observed at 1-3h., with an increase in the exposure interval at 8-12 hr., multiple layer of *E. coli* cells are detected. At 24-48 hr., halos surrounding microcolonies were observed, with increasing the bacterial cells, which sometimes sinking below the catheter surface. The border line of the microcolonies was marked by a clearly visible erosion zone, (Fig. 5a-d).
The dynamics of adherence and colonization of *E. coli* to catheters surface in distilled water were essentially the same as in phosphate buffer, although slightly delayed with regard to the onset of microcolony formation at 8–24 h. and to the other phenomena described above (Fig.6a-b).
After irradiation of *E. coli* light colonization occurs after 8hr. and single layer in close contact with the (Pu) catheter surface was observed (Fig.7). After 24 and 48 hr. reduction in the number of cells were detected in comparing to the non-irradiated cells with some abnormality in the bacterial cells Fig.8 (a, b) and no halos surrounding the microcolonies observed.
Similar pictures as in *Klebsiella pneumoniae* were obtained with non-irradiated and irradiated *E. coli* cells with hydrophilic polyurethane catheter after 24 and 48 hr. incubation. Fig. 9, showed marked decrease in number of non-irradiated cells with abnormalities in cells shape. While, after irradiation (Fig. 10 ; a and b) shows marked abnormalities in the cells shape, size and in the number of cells adhere to the catheter surface. Adherence of the hydrophilic *S. epidermidis* (control cells) to polyurethane catheter surface by SEM revealed that, low number of cells adhere to catheter surface with production of slimy material (Fig. 11).

Growth of non-irradiated tested strains in catheter eluates: The growth rates of bacteria in eluates obtained from the incubation of catheter segments in PBS for 24h. at 37°C are shown in Tables (2–3) and Figs.(1-4). After incubation for 24 h., growth in catheter eluates of the non-irradiated tested gram-negative microorganisms was significantly higher than in the controls. The bacterial count of the tested gram- negative strains increased with time, reaching maximal growth at 24 h. Generally initial count at (3h.) of non-irradiated *Klebsiella pneumoniae* was significantly higher (49.9 X 10^4 cfu / ml, SD 1.3) in polyurethane (P-value 0.0001) and non-significantly lower (26.0 X 10^4 cfu/ml, SD 1.2) in hydrophilic polyurethane than in the controls (P. value = 0.256) while, In case of siliconised latex the P-value was less significant = 0.035, the initial count was (26.9 X 10^4 cfu / ml SD, 1.4) in comparing to the control values (25.3 X 10^4 cfu / ml SD, 0.9) (Table 2, Fig.1).
All the eluates stimulated the growth of non-irradiated *Ps. aeruginosa* along the time of incubation. Moreover, growth of the tested strain in catheter eluates more rapidly than the other tested strain. The maximal growth was observed with vialon followed by polyurethane and siliconised latex than that observed to hydrophilic polyurethane and teflon (Table 2, Fig. 2).
In case of non-irradiated *Escherichia coli*, growth of the tested strain to biomaterials was significantly greater to polyurethane and vialon (P-values 0.0001) at 24h than that observed to hydrop hobic polyurethane (P-value = 0.01) and teflon (P = 0.005). However, in siliconised latex eluates, the number of viable organisms was reduced to (52.9X10^4 cfu / ml SD, 1.6) than in the control (70.2 X10^4 cfu / ml SD, 0.5) after incubation for 24 h. (Table 3 , Fig. 3).
Different results were observed with *Staphylococcus epidermidis*. None of the catheter eluates increased the growth of the non-irradiated tested strains along the incubation time, the biomaterial eluates were significantly decrease the viable number (P-value = 0.0001) after incubation for 24 h. (Table 3, Fig. 4). Only the growth on polyurethane and vialon eluates were non-significant after incubation for 8h. (P-value = 0.059 and 0.77) in comparing to the control values. During incubation the *Staphylococci* cells disappear from the phosphate buffer; only little number of the original inoculum was recovered after 24 h. In control tubes (microbial cells suspended in PBS without catheter pieces), exactly the same reduction in the number of viable cells was observed.
With all tested strains, the lower growth values were obtained with hydrophilic polyurethane (Tables 2, 3, Figs.1-3) for gram negative bacteria, it was ranged from 90.7–270.0 x 10^4 cfu / ml and for gram- positive bacteria, it was 3.0 x 10^4 cfu / ml after incubation for 24h as compare to the growth of all tested strains in the eluates of others biomaterials used.

Effect of gamma irradiation on growth of the tested strains to catheter eluates: Growth on polyurethane eluates ; The number of bacterial cells of non-irradiated strains increased with time, reaching maximal growth at 24h. It was ranged from 169.2–410.0 x 10^4 cfu / ml for non irradiated gram

negative strains. While, it was ranged from 90.6 -269.0 X 10^3 cfu / ml for the same tested strains after irradiation. (Tables 2 and 3, Figs, 1-4). Generally initial growth in different catheter eluates at 3h and 8h was higher for non-irradiated strains than for irradiated strains. Also, similar results were observed with the other tested biomaterials (teflon, vialon and siliconised latex) against all tested gram-negative strains.

Growth on hydrophilic polyurethane eluates; Irradiated strains were initially had less ability to grow (43.3–136.1 x 10^3 cfu /ml) than non-irradiated ones (90.7–270.0 x 10^4 cfu / ml) at 24 h. for gram negative strains. At 3h and 8h., the growth in different catheter eluates was lower with irradiated than with non-irradiated strains (Tables 2, 3, Figs. 1-3) . Similar results were obtained with *S. epidermidis*, the irradiated strains had less ability to grow than non-irradiated on all biomaterial used (Table 3, Fig. 4).

The tested strains cannot multiply in distilled water with less multiplication in pure phosphate buffer without other organic supplements as compare to microbial counts in catheters eluates.

Discussion

Colonisation of medical devices by some pathogenic microorganisms is likely to depend on the ability to adhere the solid surface which then allows micro-organisms to form biofilms in which they are protected from harmful environmental factors. Biomaterials are being used with increasing frequency for tissue substitution. Implantable, prosthetic devices are instrumental in the saving of patient's lives and enhancing the quality of life for many others. However, the greatest barrier to expanding the use of biomedical devices is the high probability of bacterial adherence and proliferations, causing very difficult and often untreatable medical-device centered infections. The difficulty in treating such infections results in great danger to the patient, and usually retrieval of the device with considerable pain and suffering.

Helfgen *et al.* (1995) reported that, plastic materials used for temporary crowns and bridges in prosthetic dentistry were investigated with regard to the possibility of their microbial colonization. They found that, the different intensity of colonization is due to the roughness of certain surfaces. The direct examination of the surface of plastic and metal prostheses removed from patients in whom they had become foci of infection has shown extensive bacterial biofilm development on intravenous, intraperitoneal catheter, cardiac pacemakers and urinary catheters Marrie and Costerton (1984) & Nickel *et al.* (1985). Catheter acquired urinary tract infection account for as much as 35% of the nosocomial infections (Stamm *et al.*, 1977) and are notoriously refractory with respect to antibiotic therapy (Jones *et al.*, 1982).

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 (Lopez -Lopez *et al.*, 1991). Several properties, including hydrophobicity, hydrophilicity and electrostatic forces, mediated by extra-cellular macromolecules mediated attachment of many bacterial species to solid surfaces.

Most studies of microbial factors involved in adherence to inert surface have focused on physical forces such as surface hydrophobicity. The non-irradiated and irradiated tested strains were neither strictly hydrophobic nor strictly hydrophilic. The hydrophobicity was ranged from 18.2-64.2% before radiation and from 11.9 - 84.7 % after irradiation. The obtained results agree with those of Speert *et al.* (1986) who obtained values ranging from 15% to 65% with a polyethylene glycol-dextran system. Many reports have shown that hydrophobic microorganisms adhere more efficiently to hydrophobic substrata than hydrophilic ones (Pascual *et al.*, 1986 and Falkowski *et al.*, 1986). The obtained results confirmed that hydropicity of *E. coli* before and after irradiation was ranged from 64.2– 84.7% which is important during the first 1–3 hr. of interaction with catheters used. *Klebsiella pneumoniae* before irradiation had intermediate surface hydrophopicity values (24.8%), but the initial adherence to the tested catheters used was lower than hydrophobic *E. coli* strains. Irradiation of *Klebsiella pneumoniae* to a dose level of 25Gy cause a decrease in the cell surface hydrophobicity (11.9%) which considered as hydrophilic strain.

Scanning electron microscopic techniques demonstrated a progressive adherence of bacterial cells to catheter surfaces, with reference to bacterial adherence, the various catheter types investigated produced more or less different effects. By comparing attachment of *Klebsiella pneumoniae* and *E. coli* to a variety of biomaterials, the strains of *E. coli* with the greatest hydrophobicity were most adherent, while intermediate and hydrophilic strains of *Klebsiella pneumoniae* were least adherent to polymers (biomaterials). Although the irradiation of *E. coli* increase the hydrophobicity, but the colinization and adherence to the catheter surface were decreased with marked abnormalities in cell shape and size. Therfore the hydrophobicity is not the only factor responcible for microbial adherenc. The cell surface hydrophobicity (CSH) is a non-specific adhesion factor is important in the proliferation of microorganisms on solid surfaces (Mikucka *et al.*, 2000). Hydrophobic interaction is generally considered to play an important role in the adherence of microorganisms to eukaryotic cells and also to certain inert surfaces (Rodrigues *et al.*, 1999). Mutation of cytochrome C, which is one of the subunits of cytochrome O, caused the decrease in outer membrane proteins and the changing fatty acid composition of LPS. These changes in the outer membrane would cause an increase in cell surface hydrophobicity (Kobayashi *et al.*, 1999).

The obtained results clear that, the tested strains were less adherent to the hydrophilic polyurethane catheter surfaces than the hydrophobic polyurethane surfaces. Kiremitci and Pesmen (1996) reported that the uropathogenic strains were poorly adherent to hydrophilic polymer surfaces while showing excellent adherence on hydrophobic polypropylene surfaces. However, a relatively hydrophilic, nonpathogenic *E.coli* strain showed the opposite adhesion behavior to the same surfaces. Vaudaux (1996) and Tebbs and Elliott (1994) reported that, the surface irregularities of standard polyurethane catheter may lead to increase risk of bacterial colonization and the rough surface leads to increase protein adsorption and a proportional increase in *Staphylococcus epidermidis* colonization. On the other hand, they were reported that, the hydrocath hydrophilic coating forms an aqueous sheath on the catheter which reduces thrombus adhesion to the external catheter surface. The hydrocath hydrophilic coating decreases protein adsorption which leads to a proportional reduction infibronectin mediated adhesion of *Staph. epidermidis*.

Peters *et al.* (1982) reported that 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, stabilizers, and antithrombogenic layers-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.

Slime production and multidrug resistance were the two important virulence factors (Nayak and Satpathy, 2000). In the course of catheter infection, *Pseudomonas aerugionsa* produce large amounts of slime-like material. This substance seems to enhance adhesion to the plastic surface furthermore, this material could possibly prevent the recovery of bacteria from catheter samples during routine diagnostic cultures. The presence of this "protective" layer might explain some of the difficulties infections of catheters. This slimy material surely dose not favor the host's natural resistance mechanisms. The present *in vitro* results confirmed by the *in vivo* studies on naturally infected iv catheters by (Peters *et al.*, 1981). The anionic alginate exopolysaccharide produced by cells of *Ps. aeruginosa* is composed of uronic acid molecules and is highly hydrated 99% water (Sutherland, 1977). *Klebsiella* species are opportunistic pathogens which can infect debilitated patients with urinary or respiratory tract complications and are an important cause of bacteraemia in patients compromised by neutropenia, immunosuppression or surgery. They can produce large amounts of slime and capsular materials which may contribute to the virulence of the organisms (Williams *et al.*, 1983). Infections in immuno-compromised patients and in patients with indwelling prosthetic devices are often caused by hospital strains of *Staphylococcu epidermidis* resistant to methicillin (Hedin, 1993). Slime producing coagulase-negative *Staphylococci* are pathogens in vascular surgery by virtue of their ability to adhere and persist on prosthetic graft material (Bergamini *et al.*, 1994 and Levy *et al.*, 1990). Early adhesion of *Staph. epidermidis* to polymer surface appears to depend mainly on hydrophobicity (Galliani *et al.*, 1994).

The survival (*in vitro*) of *Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli* and coagulase-negative *S. epidermidis* adherent to catheters surface in the absence of conventional nutrients has been studied . Franson *et al.*, 1986 and Peters *et al.*, 1982 reported that, some strains of coagulase negative *Staphylococci* could used some of the components of the catheters as nutrients for growth. It may also be possible that adherence to the surface of foreign objects may allow organisms to become metabolically dormant (Franson *et al.*, 1986). To consider this hypothesis, we tested the effect of catheter components on growth of the tested strains . The eluates obtained from the incubation of catheter segments in PBS as growth media were used. Growth of non-irradiated *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E. coli* were significantly higher in teflon, polyurethane, vialon and siliconised latex eluates than in controls. All the catheter eluates stimulated the growth of the tested gram-negative strains after incubation for 24 hr.

Different results were observed with *S. epidermidis*. None of the catheter eluates significantly increased the growth of the tested strain. Lopez-Lopez *et al.* (1991) reported that, *Staphylococci* have complex nutritional requirements and it seems less likely that they would grow in a catheter eluate in PBS. As shown in this studies, the tested gram-negative strains are able to grow and proliferate on the inner and outer surface of catheters in the absence of any other externally supplied nutrients. Peters *et al.* (1982) reported that, 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.

The irradiated strains were had less ability to grow on all biomaterials used than non-irradiated strains. Joiner *et al.* (1996) reported that there is now little doubt of the existence of radio-protective mechanisms or stress responses, that are upregulated in response to exposure of bacteria and yeast cells to small doses of ionizing radiation and other DNA- damaging agents. Changed expression of some genes, only in response to low and not high doses, may occur within a few hours of irradiation and this would be rapid enough to explain the phenomenon of induced radioresistance strains although its specific molecular components have yet to be identified.

Farrag (1991) and Farrag and Saleh (1996) reported that the low doses of gamma radiation had an effect on the antimicrobial activity, DNA content and ploidy pattern. Tested strains became more resistant against different antibiotics after the exposure to low doses of gamma radiation. The difference in DNA content of *Pseudomonas aeruginosa*, other Gram-negative bacteria, Gram-positive bacteria and yeast fungi were highly significant after exposure to 20 Gy gamma radiation. The distribution of DNA content showed a narrow mode between 1n and 2n before radiation. While, an irregular DNA distribution was observed reaching up to 4n and 5n after irradiation. The ploidy pattern showed irregular changes (aneuploidy) after irradiation in case of *Pseudomonas aeruginosa* and other Gram-negative only. Farrag (1996) described that low doses of 20 Gy gamma radiation had an effect on antibacterial substance produced by *Pseudomonas aeruginosa* against *S. aureus* and on pigment production.

The present *in vitro* results confirm the findings in previous study by Farrag (2001) on naturally infected medical device by *Pseudomonas aeruginosa* and the production of slime like materials is an important colonizing and virulence factor which enhance adhesion to the plastic surface and the presence of this protective layer might explain some of the difficulties encountered in antimicrobial treatment of bacterial infections. The *in vitro* exposure of *Pseudomonas aeruginosa* to test dose of 20Gy gamma radiation revealed that, The ability of some slime producer strains was changed after irradiation from positive to weak positive or negative. The sensitivity of the tested strains to different antimicrobial agents were showed resistance after radiation than before.

In conclusion, the hexadecane method is a good measurement of bacterial hydrophobicity and have a predicative value for adhesion. The results indicated that catheter material type may be important in adherence of bacteria to catheter surface. Thus, data may contribute to the explanation of microbial adherence influenced by the nature of the material especially with hydrophilic polyurethane. The hydrophobicity is not the only factor responsible for microbial adherence.

The viability and growth of different microorganisms may depend in part on the nature of the biomaterial and that certain substances eluted from the catheters. These differences may justify in-vitro studies of microorganisms-material interactions when evaluating the application of new materials for clinical use. The next step should be to investigate the possibilities for the prevention of bacterial adhesion to catheter surfaces.

These preliminary data suggest that the effect of new biomaterials on bacterial adherence and viability should be evaluated before clinical trials to obtain catheters that could prevent bacterial growth, adherence and colonization.

In comparing the results of the tested strains before and after irradiation, it is clear that, low doses of gamma radiation have many effects on microbial cell surface hydrophobicity, adherence to catheter surface, shape and size of microbial cells, ability to grow in catheter eluates and formation of amorphous materials. These findings require further characterisation and investigation to define the changes in

microbial cells.

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