8 *Egypt. J.Microbiol*. **47**, pp. 127-140 (2012)

# Studies on Incidence and Prevention of Nosocomial Infection of Urinary Tract Endoscopies by Different Antimicrobial Agents

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THIRTY FIVE bacterial isolates were collected from urine and blood samples of 20 patients, before and after endoscopy examination, in new Surgical Hospitals , Zagazig University Hospital, Zagazig, Egypt. Antibiotic susceptibility profile of purified bacteria revealed four multi-resistant strains identified as *Staphylococcus aureus Zag11*, *Pseudomonas aeruginosa* Zag60, *Escherichia coli Zag126* and *Staphylococcus epidermidis* Zag128. The four selected bacteria were subjected to some disinfectants (glutaraldehyde, hydrogen peroxide, P3-oxonia and Orthophthaladehyde) at different concentrations and different exposure times. It was observed that 10 min were enough to inhibit growth of tested pathogenic bacteria in case of (8% $H_2O_2$ & 0.55% orthophthaladehyde) while completely inhibition was recorded after 15min in case of (2.2% glutaraldehyde, 70% ethanol and 0.45% P3-oxonia). Sterile urinary tract endoscopy was artificially contaminated with mixture (1:1:1:1:1:1) of six clinical pathogenic bacterial strains comparing with the four tested bacterial strains. Upon exposing the contaminated endoscope to different chemical disinfectants; 8% hydrogen peroxide and 0.55% Orthophthalaldehyde inhibited after 30 min exposure while 2.2% Glutaraldehyde, 0.45% P3-oxonia and 70% Ethanol needed 60 min for complete bacterial inhibition. Upon exposure of artificially contaminated endoscope to different physical agents (U.V, γ- rays and dry hot air), Gamma rays showed maximum inhibitory action.

**Keyword:** Nosocomial infection, Urinary tract endoscopies, Antimicrobial agents.

Nosocomial infections called "hospital acquired infections" remain a major worldwide problem, and a lot of people are victims of hospital infections (Edgeworth, 2011). This included infections acquired in the hospital but appearing after discharge and also occupational infections among staff of the facility (Benenson , 1995 and Nguyen, 2004). These infections related to medical care can be devastating and even deadly (Coffin & Zaoutis, 2008); and also cause significant morbidity and mortality and have a considerable impact on healthcare coats (Ramritu *et al*., 2008 and Lee *et al*., 2009). Microorganisms caused hospital infections may be controlled by inhibition or killing by physical or chemical agents as antiseptics, disinfectants, and detergents (Simon *et al*., 2007). Disinfectants are chemicals agents that destroy the gowing forms of bacteria but do not destroy spore forms of microorganism. Disinfectants are applied on

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lifeless things resembling floor and work benches as phenols, chlorhexidine, hypochlorite and alcohol (Zuhlsdorf *et al*., 2004). Deconex is a fluid; alkaline, new production of alcohol based disinfectant and is widely used in hospitals and clinics (Penna *et al*., 2001). Peracetic acid and peroxide hydrogen compounds, and it is a broad spectrum chemical agent that effective against bacteria, fungi, yeasts as well as all known classes of virus. Fort is a chlorhexidine diacetate based disinfectant agent and it may be applied on wet floor, scraper, cotton wipe, wash bucket, or spraying on the inanimate surface (Koburger *et al*., 2010).

The most common causative bacterial strains for UTIs were coagulase negative Staphylococci and Enterobacter spp. Also, antibiotic multiple resistant *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Enterococcus* spp. can be accounted as the most cause of UTI in renal transplant recipients (Shirazi *et al* ., 2005). Among the categories of bacteria most known to infect patients are the category MRSA (resistant strain of S. aureus), member of Gram-positive bacteria and Acinetobacter (A. baumannii), which is Gram-negative. While antibiotic drugs to treat diseases caused by Gram-positive MRSA are available, few effective drugs are available for Acinetobacter. Acinetobacter bacteria are evolving and becoming immune to existing antibiotics, so in many cases, polymyxin-type antibacterials need to be used. "In many respects it's far worse than MRSA," said a specialist at Case Western Reserve University (Pollack, 2010).

Short wave ultraviolet radiation (UV 200-280 nm) has been used to disinfect air and surfaces in operating rooms, patient rooms, laboratories and so on, as well as air in ventilation ducts. Despite the well-documented effect of ultraviolet radiation on air quality, thus reducing the occurrence of infections. One advantage of this method is that the UV sources ensure a continuous reduction in the number of airborne microorganisms that are generated all the time (Banrud & Moan, 1999). The present work was studied the effect of different types of bactericidal agents (physical and chemical agents) on normal and pathogenic isolates which contaminated urinary tract endoscopies.

## Materials and Methods

*Bacterial isolation*

Bacteria were isolated from endoscopy before and after operation from urine and blood of 20 patients before the urinary tract endoscopies operation in new Surgical Hospitals, Zagazig University Hospital, and Zagazig, Egypt.

Samples were collected and streaked on different diagnostic and selective agar plates, Nutrient agar, MacConkey agar and Blood agar. Bacterial isolates were streaked for several consecutive times on nutrient agar medium until pure single colonies.

*Identification of most pathogenic bacterial isolates*

Identification involved examination the bacterial isolates with naked eye, microscopic examination (Gram's stain) and Physiological biochemical tests according to Bergey's manual (Holt *et al.*, 1994).

*Antibiotic susceptibility test*

Antibiotic susceptibility test for the bacterial isolates was carried out by disk diffusion technique according to Baur *et al.* (1966**)**. The tested antibiotic disks were purchased from Oxoid .

*Minimum Inhibitory concentrations (MICs) and Minimum Bactericidal concentrations (MBCs) of tested antibiotics*

Two antibiotics were used Ciprofloxacin 1 gm (raw material) from Epico Company and Ofloxacin 250 mg from Al-Ameria Company in Cairo. MICs were determined using broth dilution method of Washington & Sutter (1980) then MBCs were determined on solid agar media (Moreira *et al.*, 2005).

*Selection of the most resistant isolates to 2% Glutaraldehyde*

The bacterial isolates were tested for antimicrobial sensitivity and susceptibility to 2% glutaraldehyde as the disinfectant.

*Effect of different concentrations of different commercial disinfectants on selected isolates*

Different disinfectants were tested for its effect on the viability of bacterial isolates as follow: Ethanol (97%), Orthophthalaldehyde (0.55%), Hydrogen peroxide (10%), P3-oxonia (30%) and Glutaraldehyde (2.2%).

*Physical disinfection of artificially contaminated endoscopy as, Ultra Violet, dry hot air and Gamma rays*

Short and long wave length were used to study their effect on urological endoscopy contaminated artificially by mixture of most resistant bacterial suspension (*P.aeruginosa Zag60, E.coli Zag126, S.aureus Zag11, S.epidermidis Zag128*) , and other endoscopy was contaminated by mixture of (*Listeria monocytogenus, Proteus, Klebseilla pneumonia, Salmonella typhi, Bacillus cereus* and *Shigella dysenteriae*) with equal cell concentration (cfu/ml) for 5, 10, 20, 30, 40 and 60 min. Also, these endoscopies were disinfected in dry hot oven at different temperature (60, 70, 80, 90 & 120° C) for 15 min. The source used for the irradiation process was cobalt - 60 Gamma cells 220, located at the National Center for Radiation Research and Technology, Nasser City, Cairo, Egypt. The three tested organisms *S. aureus Zag11 , P. aeruginosa Zag60* and *E. coli Zag126* were exposed to gamma radiation at doses of 2, 4, 6, 8 and 10 KGy for *S. aureus* Zag11 and 2, 4, 6 and 8 kGy for *P.aeruginosa Zag60* and *E.coli Zag126*.

## Results and Discussion

*Distribution of collected bacterial isolates from endoscopies before and after operation*

Endoscopic procedures carry a risk of microbial infection. Estimating this risk accurately is difficult because ascertaining that a given infection results from

a contaminated endoscope is not possible. Infection may not be apparent until the patient has been discharged from hospital, and the contaminated endoscope may not be recognized as the source of infection (Weber & Rutala, 2001). One hundred and thirty five bacterial isolates were collected from endoscopy before and after operation, urine and blood of 20 patients in new Surgical Hospitals, Zagazig University Hospital, Zagazig, Egypt. The collected isolates distributed according to the Gram's stain reaction and morphological characteristics and cell shapes & arrangements. The results in Table 1 showed that, the 135 isolates were distributed as 70 Gram positive bacterial isolates (51.85%) and 65 Gram negative bacterial isolates (48.14%).

**TABLE 1. Distribution of collected bacterial isolates according to their Gram's stain reaction and source of isolation.**

| Source of infection | Gram positive isolates | | Gram negative isolates | | Total | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Endoscopy before operation | 22 | 16.29 | 17 | 12.59 | 39 | 28.89 |
| Endoscopy after operation | 22 | 16.29 | 23 | 17.03 | 45 | 33.33 |
| Urine of patients | 10 | 7.4 | 12 | 8.8 | 22 | 16.29 |
| Blood of patients | 16 | 11.85 | 13 | 9.6 | 29 | 21.48 |
| Total | 70 | 51.85 | 65 | 48.14 | 135 | 100 |

Gupta *et al.* (2007) reported that urinary tract infections are among the most common bacterial infections caused by pathogens with an increasing resistance to several classes of antimicrobials including cotrimoxazole, beta-lactams, amino glycosides, and fluoroquinolones. In this study the antibiotic susceptibility pattern of 35 selected isolates to 10 different antibiotics was investigated by using disc diffusion method. Antibiotics include the following: Cip , OFX , CN , Cl, FOX , CEC, IPM, AZM, C and AX. The results revealed that all isolates were susceptible to Imipenem (100%) (the results not shown), so that it represents the most effective antibiotic followed by Ciprofloxacin and Ofloxacin were had (51.4%) , (42.9%) susceptible percentage, respectively. On the other hand, the data showed that (85.7%) of bacterial isolates were resistant to Cefoxitin.

*Cultural, morphological characters and physiological characteristics of the most resistant strain*

The cultural, morphological and physiological characteristics of the selected four isolates which had the higher level of resistance against both different antibiotics and glutraldehyde were studied according to Holt *et al*. (1994). A highly specific and selective media ; Baired-Parker , and MacConky were used for isolation and enumeration the tested isolates. Table 2 indicated that isolate No. 11 was known as *Staphylococcus aureus* Zag11. The isolate No. 60 , 126 and 128 were identified and named; *Pseudomonas aeruginosa* Zag60, *Escherichia coli* Zag126 and *Staphylococcus epidermidis* Zag128, respectively.

**TABLE 2. Morphological and biochemical tests for identification of most resistant bacterial isolates.**

| **Biochemical tests** | **Number of isolates** | | | |
|---|---|---|---|---|
| | **11** | **60** | **126** | **128** |
| Gram stain | + ve | - ve | - ve | + ve |
| Spore forming | - ve | - ve | - ve | - ve |
| Shape | Cocci | Rod | Rod | Cocci |
| Arrangement | Irregular Clusters | Short rods | Short rods | Cells in pair or tetroid |
| Color of colonies | Yellow raised circular colonies 6-8m smooth surface and entire | White/cramycolonies on MacConkey agar medium , produce blue green pigment on nutrient agar | Pink colonies on MacConkey agar medium | Grayish white colonies on nutrient agar medium smooth,mucoid circular entire |
| Catalase | + ve | - ve | - ve | + ve |
| Indole | - ve | - ve | + ve | - ve |
| MR | - ve | - ve | + ve | - ve |
| VP | - ve | - ve | - ve | - ve |
| Nitrate reduction | + ve | + ve | + ve | + ve |
| Citrate Utilization | - ve | - ve | - ve | - ve |
| Urease | + ve | + ve | - ve | + ve |
| Oxidase | - ve | + ve | - ve | - ve |
| $H_2S$ | - ve | - ve | - ve | - ve |
| Coagulase | + ve | - ve | - ve | - ve |
| Gelatin liguification | - ve | + ve | + ve | - ve |
| Motility | Non motile | Motile | Motile | Non motile |
| Carbohydrate utilization | | | | |
| Glucose | + ve | + ve | + ve | + ve |
| Lactose | + ve | + ve | + ve | + ve |
| Sucrose | + ve | - ve | + ve | + ve |
| Maltose | + ve | - ve | + ve | + ve |
| D-sorbitol | - ve | - ve | + ve | - ve |
| D-Mannitol | + ve | + ve | + ve | + ve |
| D-Xylose | - ve | - ve | + ve | - ve |
| D-Mannose | + ve | - ve | + ve | + ve |
| Identification | *S. aureus* | *P. aeruginosa* | *E.coli* | *S.epidermidis* |

*Determination of minimum inhibitory concentration and minimum bactericidal concentration of selected antibiotics*

The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of Ciprofloxacin (Cip) & Ofloxacin (OFX) for *S.aureusZag11, P.aeruginosaZag6, E.coli Zag126* and *S.epidermidis Zag128* were determined. The results in Table (3) showed that all isolates more resistant to OFX than Cip, except *S. epidermidis Zag128* strain which indicate the same MIC. The MICs of Cip were 10 µg / ml for *P. aeruginosa Zag60* and *S .epidermidis Zag128* strains. Nongyao *et al.* (2005) reported that the three most common pathogens isolated surgical site infection were *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which accounted for 15.3%, 8.5%, and 6.8% of infections respectively. Also, the present data showed that the most pathogenic bacterial isolates were *S.aureusZag11, E.coli Zag126, P.aeruginosa Zag60,* and *S.epidermidis Zag128;* which were selected for further studies.

**TABLE 3. MIC and MBC for selected isolates for Cip and OFX**

| Antibiotic / Selected isolates | Ciprofloxacin (Cip) | | Ofloxacin ( OFX ) | |
|---|---|---|---|---|
| | **MIC (µg / ml)** | **MBC (µg / ml)** | **MIC (µg / ml)** | **MBC (µg / ml)** |
| *S.aureus Zag11* | 20 | 30 | 30 | 30 |
| *P.aeruginosa Zag60* | 10 | 40 | 20 | 40 |
| *E.coli Zag126* | 5 | 10 | 10 | 30 |
| *S.epidermidis Zag128* | 10 | 10 | 10 | 20 |

*Effect of different concentrations of different commercial disinfectants on selected isolates*

Microbiocidal activity is affected by age, dilution, and organic stress. Dilution during use is common, and one must ensure that endoscopies or other semi critical items are exposed to an acceptable concentration. Data suggest that 1% to 1.5% glutaraldehyde is the minimum effective concentration when used as a high-level disinfectant (Rutala, 1997). In present study, Fig. 1a revealed that the most effective concentration of glutaraldehyde was 2.2% at 15 min for inhibition vegetative bacteria and 60 min to be effective against all bacterial isolates contaminated the urological endoscopy.

Hydrogen peroxide is an oxidizing agent that now is being used to achieve high-level disinfection. Inactivation of microorganisms is dependent on time, temperature, and concentration. 10% concentration of hydrogen peroxide has been shown to inactivate $10^6$ *Bacillus* species in 60 min, while 3% concentration killed $10^6$ *Bacillus* species in 150 min in 6 of 7 trials (Wardle & Renninger, 1975). The obtained results in Fig. 1b indicated that hydrogen peroxide 10% has effective against all tested bacteria at 30 min Ortho-phthalaldehyde (OPA), a

member of the aldehyde family, has recently been introduced as a liquid chemical disinfectant for medical devices (Hession, 2003) .

The high-level disinfectant label claims for OPA solution at 20$^{o}$C vary: 5 min in Europe, Asia and Latin America; 10 min in Canada; and 12 min in the United States (Rutala & Weber, 1999). Fig. 1c demonstrated that the 0.55% orthophthaldehyde at 30 min was effective against all bacterial isolates. Also, the most effective concentrations of ethanol were 70 % at 15 min for endoscopy disinfection (Fig. 1d). Ali *et al.* (2001) showed that alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Similarly, several new chemical sterilants have been developed recently, including 7.5% hydrogen peroxide, 0.08% peracetic acid plus 1.0% hydrogen peroxide,and 0.55% orthophthalaldehyde,http://hdl.handle. net/ 10755 / 175734 (2011). Similarly, Ghotaslou & Bahrami (2012) showed that the chlorhexidine, peracetic acid and an alcohol based compound (Deconex) agents are able to eradicate the bacteria and they can be used lonely. Also, it was proved that the Gram negative bacteria were more resistance to disinfectant relation to Gram positive bacteria.

Peracetic, or peroxyacetic, acid is characterized by rapid action against all microorganisms. Special advantages of peracetic acid are that it lacks harmful decomposition products (*i.e.*, acetic acid, water, oxygen, hydrogen peroxide), enhances removal of organic material, the combination of peracetic acid and hydrogen peroxide inactivated all microorganisms except bacterial spores within 20 min (Tucker *et al.,* 1996). From the present study, the P3-oxonia which consists of Hydrogen peroxide and Peracetic acid was effective in urological endoscopy disinfection at 0.45% for 60 min to inhibit all bacterial isolates (Fig.1 e).

*Physical disinfection of artificially contaminated endoscopy as ultraviolet, dry hot air and Gamma rays*

The germicidal properties of ultraviolet irradiation are due to the DNA absorption of the UV light, causing cross linking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Miller *et al*., 1999). Due to the mutated base, formation of the hydrogen bonds to urine bases on the opposite strand is impaired. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death. The amount of cross linking is proportional to the amount of UV exposure. The level of mutations that can be reversed depends on the UV repair system present in the target microorganism. Once the threshold of cross linking has been exceeded, the number of crosslink's is beyond repair, and cell death is occurs (Miller *et al*., 1999). Table 4 (a,b) showed that the ultraviolet

(short wave length) has high effect on vegetative cells when it exposed to ultraviolet for 5 min and lethal effect when it exposed to ultraviolet for 10 min while ultraviolet has lethal effect on spore forming bacteria when the exposure time is 30 min.

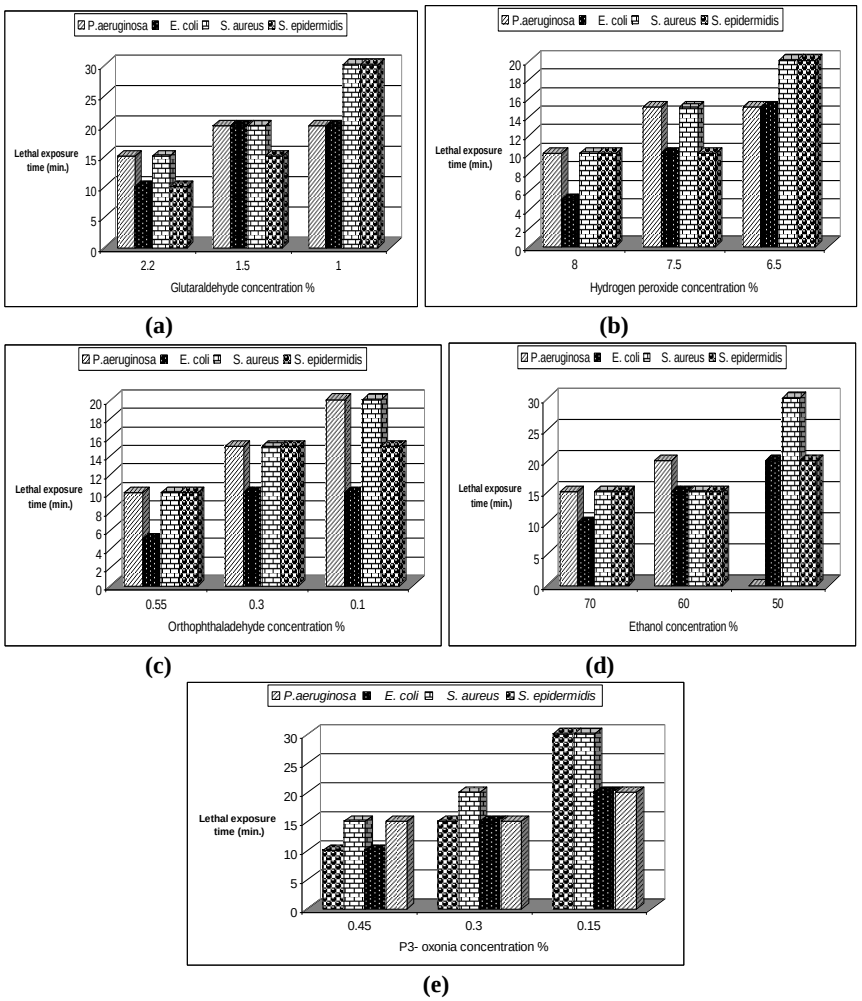


**Fig. 1. Lethal exposure times of different concentrations of (a) glutaraldehyde (b) hydrogen peroxide (c) Orthophthaladehyde (d) ethanol (e) P3-oxonia on *P. aeruginosa, E. coli, S. aureus and S. epidermidis*.**

Dry heat coagulates the proteins in all organisms, causes oxidative free radical damage, drying of cells, and can even burn cells to ashes, as seen in incineration (Campbell & Cripps , 1991). Table (4c) found that the 120°C for 15 min was satisfy to disinfect the contaminated endoscopy which used in study.

**TABLE 4. Effect of U.V on endoscopy contaminated with (a) Selected bacterial isolates (*S.aureus Zag11, E.coli Zag126, P.aeruginosa Zag60* and *S.epidermidis Zag128*) (b) Identified pathogenic bacterial isolates *(Listeria monocytogenus, Proteus, Klebseilla pneumonia, Salmonella typhi, Bacillus cereus and Shigella dysenteriae*) (c) Effect of dry hot air on both contaminated endoscopies:**

**(a)**

| **Type of U.V (at distance 20cm)** | **Exposure time (min)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 40 | 60 |
| Short wave length | ± | — | — | — | — | — | — |
| Long wave length | ++ | + | + | + | ± | ± | — |

**(b)**

| **Type of U.V (at distance 20cm)** | **Exposure time (min)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 40 | 60 |
| Short wave length | ++ | ++ | + | ± | — | — | — |
| Long wave length | ++ | ++ | ++ | + | + | ± | — |

**(c)**

| **Growth of bacterial isolates** | **Temperature** °C | | | | |
|---|---|---|---|---|---|
| | **60** | **70** | **80** | **90** | **120** |
| Selected bacterial isolates in this study | +++ | ++ | ± | — | — |
| identified pathogenic bacteria | +++ | ++ | ++ | ± | — |

where : +++ full field ++ heavy growth + moderate growth
± slightly growth – no growth

Figure 2 (a,b and c) studied the effect of gamma radiation (cobalt-60) at different exposure doses (2, 4, 6, 8 and 10 kGy) for *S.aureus Zag11* and (2, 4, 6 and 8 kGy) for *P. aeruginosa Zag60* and *E.coli Zag126* and their the dose response curves. The result showed that 3 kGy is enough to destroy the *S.aureus Zag11* and 2 kGy to inhibit its toxins production. It was evident that gamma radiation has effect on *S.aureus Zag11* at 4 kGy, while has lethal effect at 6 KGy, gamma radiation has high effect on *P.aeruginosa Zag60* at 2 kGy, while it has lethal effect at 4 kGy. On the other hand, gamma radiation has effect on *E.coli Zag126* at 2 kGy while has lethal effect at 4 kGy. Snyder & Poland (1995) reported that Gram-positive bacteria are more resistant to gamma radiation than gram-negative bacteria, and also it proved that the doses of 1.6 to 2.5 kGy gamma radiation required for killing *P. aeruginosa*. Meanwhile, radiation dose of 8 kGy resulted in the total elimination of *S. aureus* inoculated in pizza samples (Arzina *et al*., 2011).

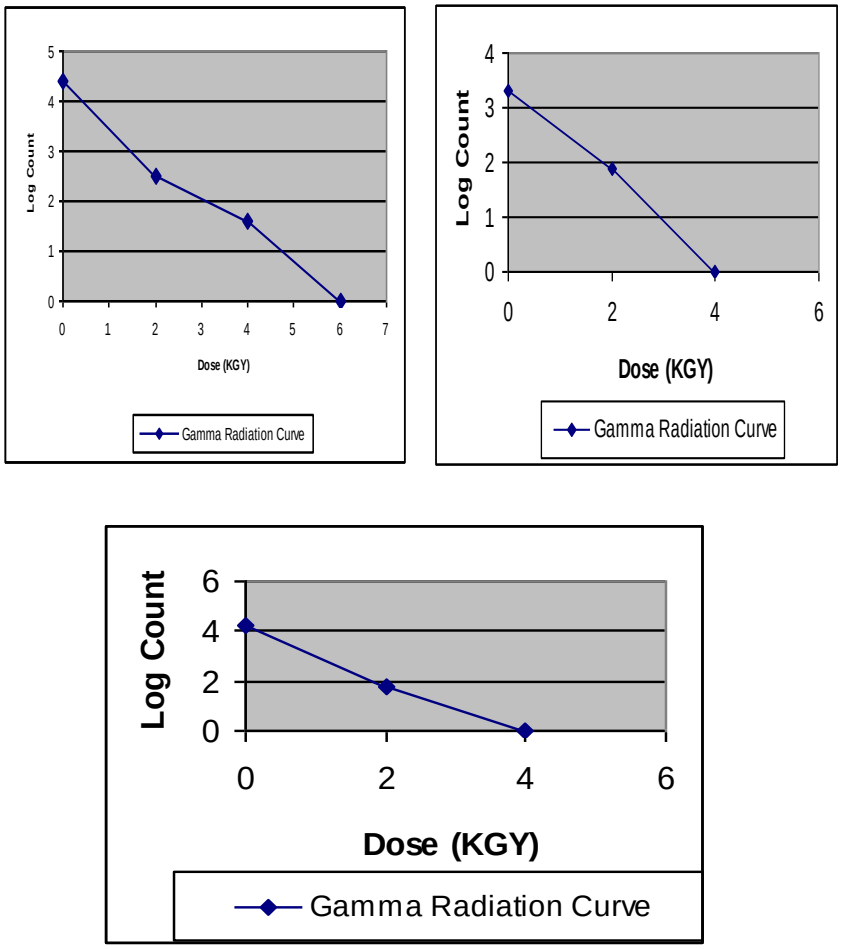


**Fig. 2.Bactericidal effect of Gamma rays on the viability of tested isolates (a) *S.aureus Zag11 (b) P. aeruginosa Zag60 (c) E.coli Zag126.***

## Conclusion

Characteristics of ideal chemical sterilants used as high-level disinfectants which are recommended (Rutala & Weber, 2004): high efficacy, rapid activity, material compatibility, non toxic, odorless, non staining, resistant to organic material, monitoring capability each use, prolonged reuse life, long shelf life, unrestricted disposal and cost effective. The previous characteristics were achieved in the present study with 8% hydrogen peroxide for 30 min and 2.2% glutaraldehyde for 60 min. 70% ethanol and 0.45% P3-oxonia for 60 min.

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(*Received* 6/ 9/ 2012;
*accepted* 22/11/2012)

# دراسات حول حدوث العدوى البكتيرية لمناظير المسالك البولية المكتسبة داخل المستشفيات وطرق القضاء عليها بواسطة مضادات ميكروبية مختلفة

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فى هذا البحث تم جمع خمسة وثلاثين عزلة بكتيرية من عينات البول والدم من ٢٠ مريضا، قبل وبعد الفحص بالمنظار، في المستشفيات الجراحية الجديدة، مستشفى جامعة الزقازيق، الزقازيق، مصر. تم اختبارقدرة الكائنات النقيه المختاره ضد بعض المضادات الحيوية والمعرفه كالاتى
*S. aureus* Zag11 , *P. aeruginosa* Zag60 , *E. coli* Zag126
و *S. epidermidis* Zag128

هذه البكتيريا الأربعة المختارة تم اختتبارها لبعض المطهرات (جلوتارالدهيد وهيدروجين بيروأكسيد و اوكسونيا وأرثوفيثالدهيد) في تركيزات مختلفة وأوقات مختلفة من التعرض. لوحظ أن ١٠ دقائق كانت كافية لمنع نمو البكتيريا المسببة للأمراض التي تم اختبارها في حالة (٨٪ للهيدروجين بيروأكسيد و ٠،٥٥٪ للارثوفيثالدهيد) ، اما ١٥ دقيقه كانت كافيه لتثبيط نمو هذه الكائنات تماما فى حالة ٢،٢٪ جلوتارالدهيد، والإيثانول ٧٠٪ و ٠،٤٥٪ للاوكسينيا.

تم استخدام منظار المسالك البولية بخليط (١:١:١:١:١:١) من ستة سلالات بكتيرية ممرضة مع منظار اخر ملوث بالسلالات البكتيرية الأربعه المختبرة. عند تعريض المناظير الملوثة إلى المطهرات الكيميائية المختلفة؛ ٨٪ بيروكسيد الهيدروجين و٠،٥٥ ٪ Orthophthalaldehyde تحول دون تعرض بعد ٣٠ دقيقة بينما غلوتارالدهيد ٢،٢٪، ٠،٤٥٪ P3-oxoniaوالايثانول ٧٠٪ المطلوبة لتثبيط ٦٠ دقيقة كاملة البكتيرية. وعند تعريض المناظير الملوثة صناعيا لعوامل فيزيائية مختلفة (الأشعة فوق البنفسجية، γ السينية والهواء الحار والجاف)، أظهرت أشعة جاما المثبطة عمل كحد أقصى.