THE HEALTH PROFESSIONAL'S ROLE IN PREVENTING NOSOCOMIAL INFECTIONS

N.M. Awny* and Y.A. Mahmoud
Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, Egypt.

* Corresponding Author

ABSTRACT:

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 S. epidermidis Zag128. The four selected bacteria were subjected to some disinfectants (glutaraldehyde, hydrogen peroxide, P3-oxonia and Orthophthalaldehyde) at different concentrations and exposure times. It was observed that 10 min were enough to inhibit growth of tested pathogenic bacteria in case of (8% H2O2 and 0.55% orthophthalaldehyde) while completely inhibition was recorded after 15 min 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 parallel with the four tested bacterial strains. On 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. The different microbial enzymes activities (hemolysin, lecithinase and proteinase) of selected isolates were determined. The virulence factors for irradiated and chemically treated tested isolates as disinfectants were detected, 2.2% glutaraldehyde and 8% hydrogen peroxide as well as γ-rays were sufficient to reduced virulence factors production by the four tested bacterial strains.

Key words: Nosocomial infection, Urinary tract endoscopies, antimicrobial agents.
INTRODUCTION:

A nosocomial infection called “hospital acquired infection” can be defined as an infection acquired in hospital by a patient who was admitted for a reason other than infection. This included infections acquired in the hospital but appearing after discharge and also occupational infections among staffs of the facility (Benenson, 1995; Nguyen, 2004). These infections related to medical care can be devastating and even deadly (Coffin and Zaoutis, 2008); and also cause significant morbidity and mortality and have a considerable impact on healthcare coats (Ramritu et al., 2008; Lee et al., 2009).

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. The main causative agent is *Escherichia coli*. Although, urine contains a variety of fluids, salts and waste products, it usually does not have bacteria in it. When bacteria get into the bladder or kidney multiple in the urine, it causes UTI (Hudault et al., 2001).

The most common causative bacterial strains for UTIs were coagulated 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).

Endoscopies had been used widely for the diagnosis and therapy of medical disorders and were used increasingly for performing laparoscopic surgery. Endoscopies were contaminated routinely by microorganisms during clinical use. Failure to employ appropriate cleaning, disinfection, or terilization of endoscopes had been responsible for multiple nosocomial outbreaks and series, sometimes life-threatening infections (Spach et al., 1993).

Sterilization results in destruction of all forms of microbial life, whereas disinfection results in destruction of specific pathogenic microorganisms. In medical practice, an object should be disinfected or sterilized depending on its intended use. Items that come in direct contact with mucous membranes, such as endoscopes, require a high level of disinfection (Mohammad et al., 2009).

The characteristics of an ideal chemical sterilant used as a high-level disinfectant should include broad antimicrobial spectrum, rapid activity, material compatibility, lack of toxicity to humans and the environment, odorless, non-staining, unrestricted disposal, prolonged reuse life and shelf life, easy to use, resistant to organic material, ability to be monitored for concentration and cost-effective (William et al., 1999).

The use of disinfectants for decontamination of rigid endoscopies should be discouraged in favor of heat processes that can be more strictly controlled. As an alternative to the use of
liquid chemicals, rigid endoscopes may be disinfected at a high level using various other methods such as slow temperature steam held at 73 to 80°C for a minimum of 10 min (Campbell and Cripps, 1991).

Short wave ultraviolet radiation (UV 200 to 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, there is still the reduction of 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 over time (Banrud and Moan, 1999).

The activity of germicides against microorganisms depends on a number of factors, some of which are intrinsic qualities of the organism, others of which are the chemical and external physical environment. Awareness of these factors should lead to better use of disinfection and sterilization processes such as number and location of microorganisms, innate resistance of microorganisms, concentration and potency of disinfectants, organic and inorganic matter, duration of exposure and biofilms (Favero and Bond, 2001; Block, 2001).

Enzymes that are considered as virulence factors are generally active against host components and contribute to virulence by damaging host tissues. Tissue damage makes the host permissive for microbial infection. Enzyme virulence factors that damage tissue include proteases, neuraminidases and phospholipases. These enzymes damage cells and provide nutrients by digesting substrates into smaller components that can be assimilated by microbes (Cox et al., 2000).

The present work 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 awaiting pure single colonies.
Identification of most pathogenic bacteria isolates

Identification involved examination of 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 the method as described by 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 namely, ciprofloxacin (1 g) (raw material) from Epico Company and ofloxacin (250 mg) from Al-Ameria Company in Cairo. MICs were determined using broth dilution method of (Washington and Sutter, 1980) and MBCs were determined on solid agar media (Moreira et al., 2005).

Selection of the most resistance 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 follows: ethanol (97%), orthophthalaldehyde (0.55%), hydrogen peroxide (10%), P3-oxonia (30%) and glutaraldehyde (2.2%).

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

Short and long wave length were used to study their effect on urological endoscopy contaminated artificially by mixtures of the most resistant bacterial suspension (Pseudomonas aeruginosaZag60, E. coliZag126, Staphylococcus aureusZag11, S. epidermidisZag128),
and other endoscopy was contaminated by mixtures of \(\text{Listeria monocytogenus, Proteus, Klebseilla pneumonia Salmonella typhi, Bacillus cereus and Shigella dysenteriae}\) 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 temperatures (60, 70, 80, 90 and 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 \(S.\text{ aureusZag11, P. aeruginosaZag60 and E. coliZag126}\) were exposed to gamma radiation at doses of 2, 4, 6, 8 and 10 KGY for \(S.\text{ aureus Zag11}\) and 2, 4, 6 and 8 kGY for \(P.\text{ aeruginosa Zag60}\) and \(E.\text{ coliZag126}\).

**Determination of different microbial enzymes activities (Virulence factors) using agar well diffusion assay**

The tested bacterial isolates \(S.\text{ aureus Zag11, P. aeruginosa Zag60 and E. coli Zag126}\) as a control and irradiated strains with gamma rays (2, 4, 6, 8 and 10 KGY) were examined for their capability of producing extracellular degrading enzymes and virulence factors.

**RESULTS AND DISCUSSION**

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 and Rutala, 2001).

One hundred and thirty five bacterial isolates were collected from endoscopy before and after operation and urine and blood of 20 patients in new Surgical Hospitals, Zagazig University Hospital, Zagazig, Egypt. The collected isolates were distributed according to the Gram's stain reaction and orphological characteristics and cell shapes and arrangements. The results in Table 1 showed that the 135 isolates were distributed as 70 gram-positive bacteria isolate (51.85%) and 65 gram-negative bacteria isolate (48.14%).

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: Ciprofloxacin (Cip), Ofloxacin (OFX),
Gentamicin (CN), Cephalexin (Cl), Cefoxitin (FOX), Cefaclor (CEC), Imipenem (IPM), Azithromycin (AZM), Chloramphenicol (C) and Amoxicillin (AX). The results revealed that all isolates were susceptible to Imipenem (100%) (Results not shown), such that it represented the most effective antibiotic followed by Ciprofloxacin and Ofloxacin which had (51.4 and 42.9%) susceptible percentage respectively. On the other hand, the data showed that (85.7%) of bacterial isolates were resistant to Cefoxitin.

The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentrations (MBCs) of Ciprofloxacin (Cip) and Ofloxacin (OFX) for *S. aureus* Zag11, *P. aeruginosa* Zag6, *E. coli* Zag126 and *S. epidermidis* Zag128 were determined. The results in Table 2 showed that isolates were more resistant to OFX than Cip, except isolate no. 128 which indicated the same MIC. The MICs of Cip were 10 μg/ml for isolate no. 60 and 128. Nongyao et al. (2005) reported that the three most common pathogens isolated surgical site infection were *E. coli*, *S. aureus*, and *P. 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. aureus* Zag11, *E. coli* Zag126, *P. aeruginosa* Zag60, and *S. epidermidis* Zag128.

Several investigators have shown that 2% aqueous solutions of glutaraldehyde, buffered to pH 7.5 to 8.5 with sodium bicarbonate, effectively killed vegetative bacteria in less than 2 min, fungi and viruses in less than 10 min, *Mycobacterium tuberculosis* in less than 20 min, and spores of *Bacillus* and *Clostridium* species in 3 h. Microbiocidal activity is affected by age, dilution, and organic stress. Dilution during use is common, and one must ensure that endoscopies or other semicritical 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 the present study, Figure 1 revealed that the most effective concentration of glutaraldehyde was 2.2% at 15 and 60 min for inhibition vegetative bacteria to be effective against all bacterial isolates contaminated with urological endoscopy.
Mycobacterium tuberculosis in less than 20 min, and spores of Bacillus and Clostridium species in 3 h. Microbiocidal activity is affected by age, dilution, and organic stress. Dilution during use is common, and one must ensure that endoscopies or other semicritical 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 the present study, Figure 1 revealed that the most effective concentration of glutaraldehyde was 2.2% at 15 and 60 min for inhibition vegetative bacteria to be effective against all bacterial isolates contaminated with urological endoscopy.
Hydrogen peroxide is an oxidizing agent that is now 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 and Renninger, 1975). The obtained results in Figure 2 indicated that hydrogen peroxide of 10% was 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°C varies: 5 min in Europe, Asia, and Latin America; 10 min in Canada; and 12 min in the United States (Rutala and Weber, 1999). Figure 3 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 (Figure 4). 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.

Peracetic or peroxyacetic acid is characterized by rapid action against all microorganisms. Special advantages of peracetic acid are that it lacks harmful decomposition products (that is, acetic acid, water, oxygen and hydrogen peroxide), enhances removal of organic materials. The combination of peracetic acid and hydrogen peroxide

Figure 3. Lethal exposure times of different concentrations of orthophthaldehyde on P. aeruginosa, E. coli, S. aureus and S. epidermidis.
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 (Figure 5).

Dry heat coagulates the proteins in all organisms, causing oxidative free radical damage, drying of cells, and can even burn cells to ashes as seen in incineration (Campbell and Cripps,
From our study, it was found that the 120°C for 15 min was satisfied to disinfect the contaminated endoscopy which was used in the study.

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 is beyond repair, and cell death is bound to occur (Miller et al., 1999). Table 3 and 4 showed that the ultraviolet (short wave length) has high effect on vegetative cells when exposed to ultraviolet for 5 min and lethal effect when exposed to ultraviolet for 10 min while ultraviolet has lethal effect on spore forming bacteria when the exposure time is 30 min.

From the result of our study, gamma radiation has effect on *S. aureus* Zag1 at 4 KGy, lethal effect at 6 KGy, high effect on *P. aeruginosa* Zag60 at 2 KGy and lethal effect at 4 KGy. On the other hand, gamma radiation has effect on *E. coli* Zag126 at 2 KGy and lethal effect at 4 KGy. Snyder and Poland (1995) reported that gram-positive bacteria are more resistant to gamma radiation than gram-negative bacteria. Amaral et al. (1999) studied the effect of gamma radiation on *S. aureus*. The result showed that 3 KGy is enough to destroy *S. aureus* Zag11 and 2 KGy inhibit its toxins production. Afifi et al. (2001) studied the microbial decontamination by gamma irradiation. Snyder and
Poland (1995) reported that the doses of gamma radiation required killing of *P. aeruginosa* in kGy 1.6 to 2.5. The results demonstrated in Figures 6, 7 and 8 showed 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 dose response curves.

Some bacteria, such as *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, produce a variety of enzymes which cause damage to host tissues. Enzymes which break down the connective tissue component of hyaluronic acid and a range of proteases and lipases include hyaluronidase while DNAses break down DNA, and hemolysins break down a variety of host cells, including the red blood cells (Wikipedia, 2010). In our study, the microbial enzymes activities of control and gamma irradiated selected bacterial isolates were studied. The tested bacterial isolates *S. aureus* Zag11, *P. aeruginosa* Zag60 and *E. coli* Zag126 as a control and irradiated strains with gamma rays were examined for their capability of producing extracellular degrading enzymes (lecithinase, haemolytic and protease).
Conclusion

Characteristics of ideal chemical sterilants used as high-level disinfectants are recommended (Rutala at al., 1999) namely, high efficacy, rapid activity, material compatibility, non toxic, odorless, non staining, resistant to organic material, monitoring capability of 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, 2.2% glutaraldehyde for 60 min, 70% ethanol and 0.45% P3-oxonia for 60 min.

REFERENCES


How to cite this article:
N.M. Awny* and Y.A. Mahmoud, The Health Professional’s Role in Preventing Nosocomial Infections
Vol 2 Issue 2, pp: 203-219