American Journal of Applied Sciences 6 (5): 811-815, 2009
ISSN 1546-9239

Determination of the Antibacterial Efficacy of Common Chemical Agents in Cleaning and Disinfection in Hospitals of North Jordan

[1]Hani A. Masaadeh and [2]Adnan S. Jaran
[1]Faculty of Medicine, Jordan University of Science and Technology,
P.O. Box 3030, Irbid, Jordan 22110
[2]Faculty of Science,
Al al-Bayt University, P.O. BOX 130040, Mafraq 25113, Jordan

Abstract: Problem statement: Hospital cleanleness and hygene are consederd among the most important aspects of clinical success and in preventing nosocomial infections. In this study we will address the problem of effectiveness of commonly used chemical agents in cleaning and disinfection in hospitals of north Jordan. For evaluating the effectiveness of chemical agents. **Approach**: The minimum inhibitory concentration (MIC) was determined by the method of serial broth dilutions. The bacteria used were *Acinetobactev calcoaceticus*, *Enterobacter cloacae* and *Serratia marcescens* were isolated from material collected from Princess Badea and Princess Rahma hospitals in north Jordan, *Bacillus subtilis* ATCC 9372, *Bacillus stearothermophilus* ATCC 7953, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923,were used as controls. **Results:** Chlorhexidine showed no inhibitory activity for *Bacillus subtilis* and *Bacillus stearothermophilus*. *A. calcoaceticus*, showed resistance to the majority of the agents tested, followed by *E. cloacae* and *S. marcescens*. The MIC intervals, which reduced bacteria populations over 08 $\log_{10}$, were: 65-11000 mg L^{-1} of chlorhexidine digluconate, 200-4500 mg L^{-1} of chlorine-releasing-agents (CRAs), 4100-82000 mg L^{-1} of isopropanol or ethanol, 1350-3500 mg L^{-1} of glutaraldehyde, 50-250 mg L^{-1} of formaldehyde, 1200-6000 mg L^{-1} of iodine in polyvinyl-pyrolidone complexes, 450-2400 mg L^{-1} of hydrogen peroxide and 60-160 mg L^{-1} of quaternarium ammonium compounds (QACs). **Conclusion:** Ethanol, Ethanol plus glycerin and Ethanol iodine were found to be the most effective agents against microorganims tested $p<0.05$.

Key words: Antiseptic, disinfectant, bacteriocidal, bacteriostatic, chemical agents, bacteria

INTRODUCTION

Antiseptics and disinfectants are used at length in hospitals and other health care settings for a variety of topical and hard-surface applications. In specific, they play a fundamental role in infection control practices and help in the avoidance of nosocomial infections[1,2]. Mounting concerns over potential microbial contamination and infection risks in food and general consumer markets have led to increased use of antiseptics and disinfectants by the general public. A wide range of active chemical agents (or biocides) is found in these products, many of which have been used for hundreds of years for antisepsis, disinfection and preservation[3].

The word use of antiseptic and disinfectant products has prompted some speculation on the progress of microbial resistance, in particular cross-resistance to antibiotics[4-8].

The aim of this study was to evaluate the bactericidal activity of some commonly used disinfectants against antibiotic-susceptible and antibiotic-resistant bacterial isolates in hospitals.

MATERIALS AND METHODS

Cultures and microorganisms: *Bacillus subtilis* (ATCC 9372), *Bacillus stearothermophilus* (ATCC 7953), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) and microorganisms isolated from clinical materials from Princess Badea and Princess Rahma hospitals in north Jordan. Cultures of bacteria were grown on nutrient, blood, MacConkey and chocolate agr (Difco) at 37°C. The primary identification of bacterial isolates were made based on colonial appearance, pigmentation, Gram reaction, motility test and standard biochemical

Corresponding Author: Adnan S. Jaran, Faculty of Science, Al al-Bayt University, P.O. BOX 130040, Mafraq 25113, Jordan
Tel: 962-2-6297000-Ex 2119 Fax: 962-2-6297034

tests. Working cultures were possessed on Tryptone Soy Agar (TSA, Difco) with weekly transfers. 24 h cultures, grown on TSA, for *S. marcescens*, *E. cloacae*, *A. calcoaceticus*, *E. coli* and *S. aureus*, were grown in Tryptone soy broth (TSB, Difco), centrifuged (1000 g 15 min^{-1} 4°C) and suspended in 10 mL saline. Viable bacterial count was determined by pour plate on TSA using calibrated loop (1 μL) per plate. The plates were incubated at 37°C, atfor 18-24 h, the plates were read the following day but extended to 48 h if there was no bacterial growth within 24 h. Colonies were counted and the viable bacterial count was expressed as cfu mL^{-1}. Spore cultures, developed for 6-days on a sporulation medium (g L^{-1}: D-glucose, Sigma, St. Louis, Missouri, USA), 2.5, L-glutamic acid (Sigma), 0.4, yeast extract (Difco), 4.0, peptone (Difco), 5.0, sodium chloride, 0.01, manganese sulfate, 0.01, bacteriologic (Difco) agar, 20.0), at 37°C for *B. subtilis* and at 62°C for *B. stearothermophilus*, were grown, centrifuged (1935 g for 30 min, four times) and kept suspended in cool 0.02 M calcium acetate solution (pH = 9.7) at 4°C[12].

Chemical agents: The following solutions ethanol (70%, v/v), ethanol (70% v/v) plus glycerin (2% w/v), glutaraldehyde (1.5 pentanedial, 2.0% w/v alkalinized with sodium bicarbonate, pH = 8.3), formaldehyde (monoaldehyde, 37% w/v), sodium hypochlorite (10% w/v), hydrogen peroxide, chlorhexidine digluconate kept at 3-8°C, quaternarium ammonium compounds QACs (10%w/v, benzalkonium chloride, monoquaternary mixture of alkyldimethylbenzylammonium chlorides), aqueous polyvinylpyrolidone-iodine (10% w/v, PVP-I_2, topic and soap) solution, with and without sodium lauryl ether sulfate (25 % w/v), alcoholic polyvinylpyrolidone-iodine (10% w/v, PVP-I_2, alcoholic) in ethanol 70% (v/v). Ethanol at 70% v/v, added with iodine 1% w/v and 10% w/v and sodium hypochlorite at pH 7.0 in Sorensen phosphate-buffered solution were prepared at the laboratory. The concentration of total available chlorine, iodine and hydrogen peroxide was determined by the iodometric[9]. The diluted solutions, prepared with chlorine demand-free glassware, were filtered through a 0.22 m pore size membrane (Millipore).

Minimal inhibitory concentration: The Minimal Inhibitory Concentration (MIC) was determined by using the two-fold broth dilution method[10]. Starting from a chemical agent solution, serial dilutions were prepared in TSB inoculated with the test bacterial populations 10^6 CFU mL^{-1}. The MIC was identified as the lowest concentration of the chemical agent, which resulted in confirmed inhibition of the growth of the tested microorganism, after 24 h of optimal incubation conditions. The chemical agent solutions, started concentrations and pH values are shown in Table 1. The MICs were expressed in percentage are shown in Table 2.

Table 1: The chemical agents and their concentrations used for MIC tests

Chemical agent	pH	Concentration (%)
Ethanol	5.5	70
Ethanol plus glycerin	6.0	70+2
Ethanol iodine	5.5	70+1.0, 70+10.0
Alcoholic polyvinylpyrolidone-iodine	5.6	10.0
Alcoholic polyvinylpyrolidone-iodine in ethanol 70% (v/v)	2.5	10.0
Aqueous polyvinylpyrolidone-iodine-soap with and without sodium lauryl ether sulfate	6.7	10.0
CRAs-chlorine releasing agents-sodium hypochlorite	9.6	1.0
in phosphate-buffered	7.3	0.1
NaDcc-Sodium dichloroisocyanurate	6.8	1.0
Chlorhexidine digluconate	6.5	4.0, 0.1, 1.0
Formaldehyde	7.8	0.5, 2.3
Glutaraldehyde	7.2	2.0, 1.5
Hydrogen peroxide	3.4	3.0
Quaternarium ammonium compounds	6.6	0.5

QACs are not sporocidal, their activity exhibiting inhibition of the outgrowth[11] of germinating spores. The MICs of 0.0120.0160% (120160 mg L^{-1}) for *B. subtilis* and *B. stearothermophilus* were twice those exhibited by vegetative cells of *E. cloacae*, *E. coli*, *S. aureus* and *S. marcescens*, with a MIC ranging from 0.00600-0080% (6080 mg L^{-1}), four times greater than the MIC (12 mg L^{-1}) observed for *A. calcoaceticus*.

Statistical analysis: Two-way ANOVA test was used for comparing data using Minitab programm, the level of significance was set at $p<0.05$.

RESULTS

The MIC of the chemical agents tested to reduce bacterial pobulation both clinical bacterial isolates and ATCC controls over 06 log_{10} are shown in Table 2. Alcohol was most effective agent against *B. stearothermophilus*, *B. subtilis*, *S. aureus*, *E. cloacae* and *E. coli*, with MIC 8.2% (82000 mg L^{-1}) and sensitive to *A. calcoaceticus* at a MIC of 4.1% (4100 mg L^{-1}). The addition of 1% Iodine reduced the alcohol MIC (4.25%) to half for *E. coli* and *S. aureu and* addition of 10% Iodine decreased the MIC of *B. subtilis* to 4.25% and *E. cloacae* and for *S. aureus* to 2.1% .

Table 2: The minimum inhibitory concentrations of the chemical agents to reduce bacterial populations over 6 log_{10}

	Microorganism (%)						
MIC	*Acinetobactev calcoaceticus*	*Bacillus subtilis*	*Bacillu stearothermophilus*	*Enterobacter cloacae*	*Escherichia coli*	*Serratia marcescens*	*Staphylococcus aureus*
Agent							
Ethanol	4.1000	8.200	8.200	8.2000	6.1500	4.1000	8.2000
EtOH (glycerin)	8.5000	-	-	8.5000	8.5000	8.5000	8.5000
EtOH(+I_2 1%)	4.2500	8.500	8.500	8.5000	4.2500	4.2500	4.2500
EtOH(+I_2 10%)	-	4.250	-	2.1000	-	-	2.1000
Alcoholic pvp- I_2 1.25		2.500	1.250	0.3125	0.1500	0.3125	0.3125
Pvp-I_2-soap	0.6250	5.000	0.625	0.6250	0.6250	0.6250	0.6250
Pvp-I_2-topic	1.2500	2.500	1.250	0.3125	0.1500	0.3125	0.3125
CRAs 1%	0.0850	0.450	0.450	0.0400	0.1000	0.0450	0.1000
CRAs 0.1%	0.0200	0.060	0.020	0.0200	0.0200	0.0200	0.0200
NaDcc	0.1000	0.600	0.600	0.0350	0.1000	0.0200	0.0200
Chlorhexidine	0.0065	1.100	-	0.0075	0.0075	0.0150	0.0075
Formaldehyde	0.0050	0.025	0.025	0.0150	0.0150	0.0050	0.0150
Glutaraldehyde	0.3500	0.185	0.350	0.3500	0.3500	0.1350	0.1850
H_20_2	0.0500	0.200	0.200	0.1200	0.2500	0.0500	0.1000
QACs	0.0012	0.012	0.016	0.0080	0.0060	0.0060	0.0060

Quaternarium Ammonium Compounds (QACs) gave the least MIC for Bacteria tested ranging from 0.016-0.0012% (Table 2).

The other chemical agents tested gave varing MIC results for the bacteria used with less effect than Alcohol, Alcohol plus Iodine, but more effective than QACs (Table 2).

DISCUSSION

Alcohol: Several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol, alcohol) and n-propanol (in particular in Europe) are the most widely used[11]. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination[13], but this effect is reversible[12]. To reduce populations over 06 log_{10} of *B. stearothermophilus*, *B. subtilis*, *S. aureus*, *E. cloacae* and *E. coli*, ethanol has shown a similar average MIC of 8.2% (82000 mg L^{-1}). *S. marcescens* and *A. calcoaceticus* were the most sensitive bacteria at a MIC of 4.1% (4100 mg L^{-1}). The presence of 2% glycerin (emollient for hands and forearms) in ethanol at 70% inhibited its activity on germinating spores. The addition of 1% iodine to 70% ethanol reduced the alcohol MIC (4.25%) to half for *E. coli* and *S. aureus* and the addition of 10% (w/v) iodine decreased the alcohol MICs to 4.25% and to 2.1% for *B. subtilis* and *E. cloacae* and for *S. aureus*.

Chlorhexidine digluconate: The application of 4.0% chlorhexidine solution is used for hand washing by the medical staff. In our study, chlorhexidine showed no effectiveness over *B. stearothermophilus* and a MIC of 1.0% for *B. subtilis*. The MIC interval of 0.00650.0075% (65-75 mg L^{-1}) was observed for *E. cloacae*, *E. coli* and *S. aureus* and for *S. marcescens*, the MIC was 0.01300.0150% (130-150 mg L^{-1}), therefore double that of the former.

Chlorhexidine is also used for cleaning contact lenses (0.0050.006%). To reduce populations greater than 5 log_{10} of *S. marcescens*, a MIC of 0.003% was reported to be equivalent to a 10 min exposure at a solution of 0.05%[14]. After 24 h contact time, the ability of *S. marcescens* to grow in chlorhexidine solutions at an interval of 0.001-0.006% was proved[17].

Chlorine-releasing-agents (CRAs): Excellent reviews that deal with the chemical, physical and microbiological properties of Chlorine-Releasing Agents (CRAs) are available[15,16]. The CRAs, as sodium hypochlorite (NaOCl) and sodium dichloroisocyanurate (NaDCC), are at length used for antiseptic and disinfecting purposes and also for decontaminating non-critical surfaces with blood spillage in health care settings[17], despite decreased activity on storage and by organic matter. Similar MIC intervals were observed for the tested bacteria, considering an initial concentration of 8000-9000 mg L^{-1} free chlorine in NaOCl (pH 9) and NADCC (pH 7.0) solutions. The most resistant gram-negative strains, *E. coli* and *A. calcoaceticus*, exhibited a similar MIC (850-1000 mg L^{-1}) and for spores, the MIC was 4500 mg L^{-1}. On adjusting the pH value to 7.0 for NaOCl solutions, the MIC values were reduced to one tenth, due to the higher predominance of HOCI, the formation of which was observed to stabilize at concentrations 0.1% CRAs, providing similar MIC

(200 mg L^{-1}) values for the vegetative bacteria and a MIC of 600 mg L^{-1} for *B. stearothermophilus* and *B. subtilis* (Table 2).

Formaldehyde: Formaldehyde (methanal, CH_2O) is a monoaldehyde that exists as a freely water-soluble gas, formaldehyde solution (formalin) is an aqueous solution containing *ca*. 34 to 38% (wt/wt) CH_2O with methanol to delay polymerization. Its clinical use is generally as a disinfectant and sterilant in liquid or in combination with low-temperature steam, it is bactericidal, sporicidal and virucidal, but it works more slowly than glutaraldehyde[18,19].

Formaldehyde is an extremely reactive chemical[18,20] that interacts with protein, DNA and RNA in vitro[21]. It has long been considered to be sporicidal by virtue of its ability to penetrate into the interior of bacterial spores[22]. Furthermore, it is administered as a urinary antiseptic (dialysis)[23], with concentrations in the bladder of 100200 mg L^{-1}[23].

We observed that, for overnight decontamination of items, the use of a 0.5-1.0% formaldehyde aqueous solution provided a population reduction of 69 log_{10}, by an average MIC of 50 mg L^{-1} for *S. marcescens*, of 150 mg L^{-1} for *E. cloacae*, of 150 mg L^{-1} for *S. aureus* and *E. coli*, of 250 mg L^{-1} for *B. subtilis* and of 250 mg L^{-1} for *B. stearothermophilus* (Table 2).

Glutaraldehyde (pentane dialdehyde): Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron microscopy.

Most vegetative microorganisms other than mycobacteria are destroyed within a few minutes, sporicidal, fungicidal, virucidal effectiveness, is widely used for high level disinfection, at a 2.0% concentration of an activated alkaline (pH = 8.3) solution, for 6-10 h exposure[24]. To reduce populations over 08 log_{10}, the MICs were between 0.350% (3500 mg L^{-1}) for *B. subtilis*, *A. calcoaceticus*, *E. cloacae* and *E. coli*, an interval MIC of 0.1350. 1350% (1850 1850 mg L^{-1}) for *B. stearothermophilus*, *S. marcescens* and *S. aureus* (Table 2).

Hydrogen peroxide (H_2O_2): A 3% solution of hydrogen peroxide is some times used to clean wounds. It is nonirritating to the tissues and has only a brief, mild disinfecting action due to its rapid breakdown to water and oxygen by the enzyme catalase. A H_2O_2 solution was able to reduce populations over 08 log_{10} and showed a MIC of 0.12-0.24% for *B. subtilis* and *B. stearothermophilus*, a MIC of 0.12-0.25% for *E. coli*, *E. cloacae*, a MIC of 0.05-0.1000% for *S. marcescens*, *S. aureus* and MICs of 0.05% for *A. calcoaceticus* (Table 2).

Iodine in polyvinyl-pyrolidone complex (PVP-I_2): Iodine is among the most effective skin antiseptics used widely as a skin antiseptic and minor wound cleaner. Iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal[24]. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable, in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I_2) being primarily responsible for antimictrobial efficacy[24]. For aqueous (topic and soap) PVP-I_2 solutions, the MIC varied from 0.6-1.2%. For the alcoholic PVP-I_2 complex, the MIC varied from 0.15% for *E. coli* to 1.25% for *A. calcoaceticus* and *B. stearothermophilus*. The highest MIC was observed for *B. subtilis* at 2.5-5.0%. (Table 2).

Quaternarium ammonium compounds QACs: Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other water attracting (hydrophilic or polar) group. Hopeful on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are grouped into cationic, anionic, nonionic and ampholytic (amphoteric) compounds. Of these, the cationic agents, as prescribed by Quaternary Ammonium Compounds (QACs), are the most beneficial antiseptics and disinfectants[12]. The MICs of 0.0120-0.0160% (120-160 mg L^{-1}) for *B. subtilis* and *B. stearothermophilus* were twice those exhibited by vegetative cells of *E. cloacae*, *E. coli*, *S. aureus* and *S. marcescens*, with an MIC ranging from 0.0060-0.0080% (60-80 mg L^{-1}), four times greater than the MIC (12 mg L^{-1}) observed for *A. calcoaceticus*. (Table 2).

CONCLUSION

In conclusion, the findings of the present study showed that among the antiseptic and chemical agents used in hospitals of north Jordan, Ethanol, Ethanol plus glycerol and Ethanol Iodine (1%) gave the most antibacterial activity against the microorganisms tested ($p<0.05$). No significant difference was detected among the chemical agents effectiveness against the different microrganisms used.

REFERENCES

1. Larson, E.L., 1996. Antiseptics. In: APIC Infection Control and Applied Epidemiology: Principles and Practices, Olmstad, R.N. (Ed.). Mosby-Year Book, Inc., St. Louis, Mo, ISBN: 0815102437, pp: 19-1-19-7, G1-G17.
2. Rutala, W.A., 1996. APIC guidelines for selection and use of disinfectants. Am. J. Infect. Control, 24: 313-342. http://www.ncbi.nlm.nih.gov/pubmed/8870916.
3. Block, S.S., 1991. Historical Review. In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.). Lea and Febiger, Philadelphia, Pa, ISBN: 0812113640, pp: 3-17.
4. Denyer, S.P., W.B. Hugo and V.D. Harding, 1985. Synergy in preservative combinations. Int. J. Pharm., 25: 245-253. DOI: 10.1016/0378-5173(85)90166-8.
5. Russell, A.D., 1998. Mechanisms of bacterial resistance to antibiotics and biocides. Prog. Med. Chem., 35: 133-97. DOI: 10.1016/S0079-6468(08)70036-5.
6. Russell, A.D. and W.B. hugo, 1987. Chemical Disinfectants. In: Disinfection in Veterinary and Farm Animal Practice, Linton, A.H., W.B. Hugo and A.D. Russell (Eds.). Blackwell Scientific Publications, USA., ISBN: 10: 0632018178, pp: 12-42.
7. Russell, A.D., W.B. Hugo and G.A.J. Ayliffe, 2003. Principle and Practices of Disinfection, Preservation and Sterilization. 4th Edn. Blackwell Scientific Publications, USA., ISBN: 1405101997.
8. Russell, A.D., 2002. Antibiotic and biocide resistance in bacteria: Introduction**.** J. Applied Microbiol., 92: 1s-3s. doi:10.1046/j.1365-2672.92.5s1.14.x.
9. Vessoni Penna, T.C., D. Schaffner, L.E. Abe and I.A. Machoshvili, 1996. Inactivation of Brazilian wild type and enterotoxigenic *Escherichia coli* by chlorine. J. Ind. Microbiol., 16: 57-61. DOI: 10.1007/BF01569922.
10. Wiegand, I., K. Hilpert and R.EW. Hancock, 2008. Agar and broth dilution methods to determine the Minimal Inhibitory Concentration (MIC) of antimicrobial substances. Nature Protocol., 3: 163-175. DOI: 10.1038/nprot.2007.521.
11. McDonnell, G. and A.D. Russell, 1999. Antiseptics and disinfectants: Activity, action and resistance. Clin. Microbiol. Rev., 12: 147-179. http://cmr.asm.org/cgi/content/abstract/12/1/147.
12. Penna, T.C.V., P.G. Mazzola and A.M.S. Martins, 2001. The efficacy of chemical agents in cleaning and disinfection programs. BMC Infect. Dis., 1: 16. DOI: 10.1186/1471-2334-1-16.
13. Cortezzo, D.E., B. Setlow and P. Setlow, 2004. Analysis of the action of compounds that inhibit the germination of spores of *Bacillus* species. J. Applied Microbiol., 96: 725-741. DOI: 10.1111/j.1365-2672.2004.02196.x.
14. Ayres, H., J.R. Furr and A.D. Russell, 1993. A rapid method of evaluating permeabilizing activity against *Pseudomonas aeruginosa*. Lett. Applied Microbiol., 17: 149-151. DOI: 10.1111/j.1472-765X.1993.tb00381.x.
15. Bloomfield, S.F., 1996. Chlorine and Iodine Formulations. In: Handbook of Disinfectants and Antiseptics, Ascenzi, J.M. (Ed.). Marcel Dekker, Inc., New York, ISBN: 0824795245, pp: 133-158.
16. Dychdala, G.R., 1991. Chlorine and Chlorine Compounds In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.). Lea and Febiger, Philadelphia, Pa, ISBN: 0812113640, pp: 131-151.
17. Gavin, J., N.F. Button, I.A. Watson-Craik and N.A. Logan 1995. Efficacy of standard disinfectant test methods for contact lens-care solutions. Int. Biodeteriorat. Biodegradat., 36: 431-440. DOI: 10.1016/0964-8305(95)00102-6.
18. Fraise, A.P., 1999. Choosing disinfectants. J. Hosp. Infect., 43: 255-264. DOI: 10.1016/S0195-6701(99)90421-8.
19. Scott, E.M. and S.P. Gorman, 1991. Glutaraldehyde. In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.). Lea and Febiger, Philadelphia, Pa, ISBN: 0812113640, pp: 596-614.
20. Thomas, S. and A.D. Russell, 1974. Studies on the Mechanism of the sporicidal action of glutaraldehyde. J. Applied Bact. 37: 83-92. DOI: 10.1111/j.1365-2672.1974.tb00417.x.
21. Kiernan, J.A., 2000. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. Microscop. Today, 00-1: 8-12. http://publish.uwo.ca/~jkiernan/formglut.htm.
22. Sykes, G., 1970. The sporicidal properties of chemical disinfectants. J. Applied Bacteriol., 33: 147-156. DOI: 10.1111/j.13652672.1970.tb05240.x.
23. Rodríguez-Benot, A., R. Santamaría, A. Martín-Malo and P. Aljama, 2002. Sterilization procedures and biocompatibility. Contrib. Nephrol., 137: 138-145. DOI: 10.1159/000060240. http://www.ncbi.nlm.nih.gov/pubmed/12101947.
24. Gottardi, W., 1991. Iodine and Iodine Compounds. In: Disinfection, Sterilization and Preservation, Block, S.S. (Ed.). Lea and Febiger, Philadelphia, Pa, ISBN: 0812113640, pp: 152-167.