A STUDY OF SOME ANTIBIOTICS; DISINFECTANTS AND ANTISEPTICS EFFICACY AGAINST SOME SPECIES OF PATHOGENIC BACTERIA

S.Y.A. AL-DABBAGH; H.H. ALI; I.I. KHALIL and M.A. HAMAD

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Email: narkikuk@yahoo.com

ABSTRACT

Received at: 13/10/2015
Accepted: 30/10/2015

In this study the efficiency of four types of routinely used commercial disinfectant and antiseptics (Ethanol 70%, Dettol –Chloroxylenol- 5%, Hibitine - Chlorohexidine gluconate- 6% and Bleach (Sodium hypochlorite 10%) which used in the laboratory of microbiology in College of veterinary medicine, Mosul, Iraq were tested against four different bacterial strains which isolated from clinical specimens of infected animals (E.coli, Pseudomonas aeruginosa, Staphylococcus arueus and Corynebacterium renale). Antibiotic sensitivity tests were applied for different nine antibiotics (Ampicilin, Ciprofloxacin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Polymyxin-B, Trimethoprim Sulphamethaxazoll and Penicillin), all the tested bacteria showed resistence for (Ampiciin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Trimethoprim Sulphamethaxazoll and Penicillin). Broth dilution method used for determination of minimum inhibitory concentration (MIC) and disc diffusion method, the results of MIC method after 5 minutes of the exposure to the different concentration of Ethanol, Dettol, Hibitine and Bleach showed that Dettol has no effecincy, followed by Hibitine and Ethanol which showed lower activity while Bleach was the most effective disinfectant on the all tested bacteria. The Gram positive bacteria tested in this study were more sensitive than Gram negative bacteria to all used disinfectant and antiseptics, Disc and diffusion methods had similar effective ness for the tested bacteria against the disinfectants and antiseptics that used in this study.

Key word: disinfectants, Antiseptics, Pathogenic bacteria, Antibiotic sensitivity test.

INTRODUCTION

Disinfectants and Antiseptics are widely used as agents for killing or eliminate bacteria especially in microbiological laboratory, hospitals, other humans and animals care centers (MacDonnell and Russell, 1999). The extensively use of disinfectant and antiseptic to control and prevent the growth of microbes in both living tissue and inanimate objects lead to a common problem in the selection of disinfectant and antiseptic against pathogenic microorganisms (Russell and Russell, 1995; Al-Masaudi et al., 1991).

The widespread use of disinfectant and antiseptic products have prompted some speculation on the development of microbial resistance, in particular cross resistance to antibiotics (Russell, 1998). Many chemical agents are now available commercially as disinfectants and antiseptics, these preparations could be halogen compounds, phenols, alcohols, peroxides, quaternary ammonium compounds, chlorohexidine and sodium hypochlorite (Fraise, 1999; Russell et al., 1987).

The most commonly used disinfectant in microbiology laboratory are Ethanol, Dettol, Chlorohexidin and soap (Ho-Hyuk Jang et al., 2008). Ethanol, as a dehydrating agent causes cell membrane damage, denaturation of protein and cell lyses (Larson and Morton 1991). Dettol, effect by denaturation of protein and also act on the cytoplasmic membrane of microorganisms, Bleach with a main constituent of Sodium hypochlorite effect by oxidizing of the cell of microorganism of attaching essential cell component including protein, lipid and DNA, while Hibitin (chlorohexidine) act by disruption of membranes, precipitation of proteins and inactivation of enzymes (Manivannan, 2008).
The antimicrobial properties of the disinfectant agent against some of the pathogenic bacteria have been reported. Moreover, microorganisms are continuously acquiring resistance to new disinfectant and antiseptic (Wisplinghoff et al., 2007). Therefore, it is necessary to evaluate the effectiveness of disinfectant or antiseptic against a specific pathogen so appropriate agent easily selected (Tortora et al., 2013, Brown, 2005).

Antibiotic resistance by various mechanisms has increased worldwide in pathogenic bacteria leading to treatment failures in human and animal infections (WHO, 2007). Bacteria are able to adapt rapidly to new environmental condition include the presence of antimicrobial molecules (Quinn et al., 2004). So that a consequence resistance increases with the antimicrobial uses for pathogenic bacteria (Falagas and Bliziotis, 2007). The successful eradication of these pathogens with antibiotics has been complicated by the development of highly resistant strains as well as the appearance of new virulent pathogens. Some non antibiotic agents to various preparations have been developed and introduced with the aim of breaking the chain of infections in homes, industries and hospitals (Jansen et al., 2006).

MATERIALS and METHODS

Disinfectants and antiseptics:
Four different types of disinfectants and antiseptics as showed in table 1 were used to test susceptibility of the bacterial isolates:

Table 1: Disinfectants and antiseptics used in this study:

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 70%</td>
<td>Baghdad CO. / Iraq</td>
</tr>
<tr>
<td>Dettol (Chloroxylenol) 5%</td>
<td>Ekal industrial CO.</td>
</tr>
<tr>
<td></td>
<td>Amman/ Jordan</td>
</tr>
<tr>
<td>Hibitane (Chlorohexidine gluconate) 6%</td>
<td>Zaid CO. for antiseptic and disinfectant</td>
</tr>
<tr>
<td></td>
<td>Baghdad / Iraq</td>
</tr>
<tr>
<td>Bleach (Sodium hypochlorite) 10%</td>
<td>Sehat. CO./ Iran</td>
</tr>
</tbody>
</table>

Bacterial Strains:
Bacterial strains used in this study were Gram positive (Staphylococcus aureus and Corynebacterium renale), and Gram negative (Escherichia coli and Pseudomonas aeruginosa), all clinical bacterial isolated from infected animals, were properly collected and stored in the Microbiological laboratory, Department of Microbiology, College of Veterinary Medicine, Mosul University, Iraq.

Antibiotic sensitivity test:
All isolates were tested for nine different antibiotics (Bioanalyse) by the standard disc diffusion method according to (Vandepitte et al., 1991) on Muller Hinton agar and incubated for 24 hour at 37°C, those antibiotics included: Ampicillin (Amp)10 μg, Ciproflaxacin (Cip) 5μg, Gentamycin (CN) 10μg, Cefotaxim (Czc)30μg, Cephalothin (KF)30μg, Lincomycin (L) 10μg, Polymyxin-B (pB) 300 U, Trimethoprim sulphamethaxazoll (Tpx) 25μg and Pencillin (P) 10 U.

Sterilization test of used disinfectants and antiseptics:
The four different disinfectants and antiseptics being used in this study were tested for their sterility from microorganisms for accurate sensitivity test as follow, serial dilution (100%, 75%, 50%, 25%). As a negative control one inoculated test tube left without addition of disinfectants and antiseptics while the first dilution (stock) of each disinfectant and antiseptic used in this study considered as positive control. The antimicrobial activity of used disinfectants and antiseptics were tested against 4 types of bacteria (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Corynebacterium renale) and were isolated from pathogenic animal cases.

Viable Bacterial count:
0.1 ml of each diluted disinfectant and antiseptic were inoculated into plate count agar after 5 minutes of the bacterial inoculation and incubated for 24 hours at 37°C.

Minimum Inhibitory Concentration (MIC) method:
The MIC test was determined according to the method suggested by Baron and Feingo (1990). Depending on the turbidity of the bacterial growth.
**Disc diffusion method:**
All bacterial strains were cultured on nutrient broth for 24 hr. at 37°C, the bacterial inoculums were (5x 10^8 CFU/ml) according to (Masri et al., 2013). The disc prepared through this study from the same disinfectant and antiseptic used in MIC test and were done according to the method (Wage and Hedin, 1985), the concentration of used disinfectants and antiseptic were (100%, 75%, 50%, 25%). for each, Ethanol 70%, Dettol (Chloroxylenol) 5%, HibiTane (chlorohexidine gluconate) 6% and Bleach (Sodium hypochlorite) 10%. The sensitivity test of used disinfectants and antiseptics discs were determined according to (Vandepitte et al., 1991).

**RESULTS**
Antibiotic sensitivity test were applied for different nine antibiotics, all the tested bacteria showed resistance to (Ampicillin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Trimethoprim Sulphamethaxazoll and Penicillin) but sensitive to (Ciprofloxacin, and Polymyxin-B), as listed in Table 2.

**Table 2: Antibiotic sensitivity results for bacterial strains:**

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Amp</th>
<th>Cip</th>
<th>CN</th>
<th>Czc</th>
<th>KF</th>
<th>L</th>
<th>pB</th>
<th>Tpz</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em></td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td><em>Corynebacterium renale</em></td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

R: Resistant, S: Sensitive, I: Intermediate

The results showed that different types of bacteria varied in their response for different types of disinfectants and antiseptics, after 5 minutes of exposure to different concentrations of disinfectants and antiseptics with the comparative of control negative and control positive. Dettol was the least affective against all the tested bacteria in this study followed by the HibiTane (Chlorohexedixn 6%) and Ethanol (70%). On the other hand Bleach (Sodium hypochlorite 10%) was the most affected against the tested bacteria (*E.coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Corynebacterium renale*), as shown in Figures 1,2,3 and 4.

**Fig. 1:** Viable plate count of different concentrations of Dettol (chloroxylenol) on the tested bacteria.
Fig. 2: Viable plate count of different concentrations of Hbitane (chlorhexidine gluconate) on the tested bacteria.

Fig. 3: Viable plate count of different concentrations of Ethanol on the tested bacteria.

Fig. 4: Viable plate count of different concentrations of Bleach (Sodium hypochlorite) on the tested bacteria.
The zone of inhibition results were differed in their ranges, Dettol was the least effective against all the tested bacteria, the range was varied from 6 to 14 mm for all tested bacteria, none of the four tested bacteria were sensitive to the different concentrations of Dettol, as shown in figure 5.

The different Chlorohexdinie gluconate concentrations showed different effect on the tested bacteria ranged from 8 to 24 mm. *E.coli* was more resistant for most concentrations than the other bacteria, as shown in figure 6. The effect of different concentrations of ethanol on the tested bacteria ranged from 6 to 25 mm, *Staphylococcus aureus* was the most sensitive bacteria to all concentrations than the others (figure 7).

Bleach has the best efficiency against the four tested bacteria in all concentrations, the range of the inhibition zones were ranged from 12 to 26 (figure 8).

**Fig. 5:** Inhibition zones of different concentrations of Dettol (chloroxylenol) on the tested bacteria.

**Fig. 6:** Inhibition zones of different concentrations of Hibitane (chlorohexidine gluconate) on the tested bacteria.
Fig. 7: Inhibition zones of different concentrations of Ethanol on the tested bacteria.

Fig. 8: Inhibition zones of different concentrations of Bleach (Sodium hypochlorite) on the tested bacteria.

DISCUSSION

Disinfectants and antiseptics as antimicrobial products contain approximately 300 different active ingredients, they are marketed in different formation including sprays, liquids, gels, concentrated powders and gases (Mnivannan, 2008 and Bloomfield, 1978).

The extensive use of these disinfectant and antiseptics against the pathogenic bacteria have not only developed resistant but they also grow on the solution of these biocides, all the tested bacteria show resistant to Ampicillin, Cefotaxim, Cephalothin, lincomycin, Trimethoprim sulphamethaxazoll and Penicillin. These results were agreed with (Ayliffe, 1987; El-Mahmood and Doughari, 2009).

In this study the tested E.coli, Pseudomonas aeruginosa, Staphylococcus aureus and Corynebacterium renali isolates showed resistance to Dettol and Hibilit. Ayliffe (1987) reported that bacteria isolated from contaminated disinfectant solutions and antiseptics exhibit increased resistance to commonly used antibiotics that given a fact that bacteria have the ability to share resistant markers and once the resistance develops for one agent, cross-resistance to other agents can occur. Dettol was more effective against Staphylococcus aureus than the other tested bacteria, this result agreed with (Saha et al., 2009).
Bleach and ethanol showed high efficiency against the four tested bacteria used in this study, and the obtained results were supported by (Gaonkar et al., 2006). The immediate efficiency of bleach and ethanol was revealed by the high reduction rate in the 30S reaction (Stephen et al., 2004). The immediate killing of bleach can be explained by its oxidizing mechanism, (Fraise, 1999 and Barendra et al., 2006) who found similar result, that bleach was rapidly bactericidal for vegetative organisms. The concentration of 10% bleach kill all tested bacteria after 5 minutes of the exposure of this disinfectant. The reason for that results mainly for the mechanism of bleach sterilizing due to oxidation reactions when the bleach is dissolved in water lead to destroy the organisms.

Ethanol was less effective than bleach against the four tested bacteria, as the ethanol sterilization action is mainly due to dehydration of protein and the enzymes to deactivate and prevent bacterial growth (Tortora et al., 2013 and James et al., 1999). The results of inhibition zones were similary to the viable plate count for the effectiveness on the tested bacteria that agreed with (Saleh et al., 2012; Masri et al., 2013).

The result showed that Gram negative bacteria were less susceptible to disinfectants and antiseptics. This achieved result agreed with (Saleh et al., 2012) in which the complex cell wall and the outer membrane of these bacteria act as a permeability barrier in limiting or prevention the entry of many chemically types of antibacterial compounds (Russell et al., 1997; Sheldon, 2005).

The wide spread of disinfectant and antiseptic agents have promoted some speculation on the development of microbial resistant (Denyer et al., 1985) and this resistance to those agents are mainly of intrinsic nature as the antimicrobial resistant is frequently conferred by plasmid or transposons which have allowed raped and extensive spread through the globe. Development of resistance to antimicrobial agents and biocides is considered as a problem which is compounded by cross-resistance mechanisms between antibiotics and between antibiotics and biocides (Russell, 1986 and Saurina et al., 1997).

As a conclusion, the efficacy of the four disinfectants and antiseptics (Ethanol 70%, Dettol (Chloroxylenol) 5%, Hibitane (Chlorohexidine gluconate) 6% and Bleach (Sodium hypochlorit) 10%) on the four tested bacteria (E.coli, Pseudomonas aeruginosa, Staphylococcus aureus and Corynebacterium renale) had different efficiency of sterilizing pattern and from the obtained result 10% Bleach had the best efficiency against the tested bacteria followed by Ethanol 70%, while Dettol and Hibitane had less efficiency against the tested bacteria.

REFERENCES


Dr. سمية ياسين عبد الله الدباغ، هيئة حسن على، اسرايرا ابراهيم كليل، محمد علي حمد

Email: narkikuk@yahoo.com
Assiut University web-site: www.aun.edu.eg

(1) تم في هذه الدراسة اختيار كفاءة اربعة انواع من المعقمات والمطهرات التجارية والمستعملة روتينيا – Ethanol 70%, Dettol في مختبر Chloroxylenol- 5%, Hibitine -Chlorohexidine gluconate- 6% and Bleach -Sodium (hypochlorite- 10%)

(2) الأحياء المجهرية لكلب الاطفال الباطني في جامعة الموصل، العراق لتحليتها ودراسة اربعة انواع من الجراثيم المزعولة من حالات (E.coli, Pseudomonas aeruginosa, Staphylococcus aureus and Corynebacterium renale)

(3) كما تم اجراء اختبارات فصاعل حيوية مختلة:

Trimethoprin (Ampicillin, Ciproflaxacin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Polymyxin-B (Sulphamethaxazoll and Penicillin)

Ampicillin, Gentamycin, Cefotaxim, Cephalothin, وانتشرت النتائج معاً المجرات المحروقة للمضادات الحيوية

Ampicillin, Trimepronin Sulphamethaxazoll and Penicillin

Ampicillin, Gentamycin, Cefotaxim, Cephalothin، والمطهرات. وقد ظهرت طريقة تحاليف وانتشار بالإفراص تناول مقارنة في تحديد كفاءة المعقمات والمطهرات التي استخدمت

217