EVALUATION OF GLUTARALDEHYDE AS DISINFECTANT OF LAPAROSCOPE

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ABSTRACT

The study was conducted to evaluate the effect of glutaraldehyde as disinfectant to laparoscope. 30 dogs under going laparoscopy for different surgical intervention, the laparoscope was soaked for 20 minutes in 2% glutaraldehyde before the procedure. After standard skin preparation with 5% tincture iodine. Cultures of the umbilical area, the laparoscope and the peritoneum were taken to document the type of organisms commonly encountered under clinical condition. Cultures for aerobic and anaerobic bacteria as well as for fungi were taken. The result demonstrated growth of common skin organisms even after skin preparation with tincture iodine. The organisms cultured from the skin and peritoneum were similar but the organisms of laparoscope cultures were different. The study proofed that glutaraldehyde successfully can be used as disinfectant for laparoscope.

Tقييم استخدام الكلوتراليدين كمطهر لجهاز اللابروسكوب

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الخلاصة

تهدف الدراسة إلى تقييم استخدام الكلوتراليدين كمطهر لجهاز اللابروسكوب حيث تم في هذه الدراسة استخدام 30 كلب أثناء إجراء مختلفة العمليات الجراحية بطريقة الجراحة المنظارية. لتقديم استخدام الكلوتراليدين كمطهر لجهاز اللابروسكوب تم نقع الأدوات 20 دقيقة قبل إجراء العمليات. وبعد أن تم تطهير جلد الحيوانات المستخدمه في العمليات الجراحية بواسطة 5% محلول اليود. أخذت منسجات من منطقة السره، والمنظر وكذلك باقي البدن لفحص أنواع الكائنات الحية من خلال نمو الأحياء المجهرية اليوانية واللاهوائية إضافة إلى نمو الفطريات. أثبتت الدراسة نمو العديد من الكائنات الحية من الجلد حتى بعد مسح المنطقة بحلول اليوود. وكانت هذه الكائنات مشابهة للكائنات التي عزلت من اليواد وتختلفت مع الكائنات التي عزلت من اللابروسكوب. أثبتت الدراسة فاعلية استخدام الكلوتراليدين كمطهر لجهاز اللابروسكوب.
INTRODUCTION

Basically stated personal responsible for operating rooms sterility insisted that gas sterilization of laparoscope with ethylene oxide was the only safe method when problems of spore forming bacteria and viruses were considered. Most of surgeons noted that the cost in time and additional instruments necessitated by this policy, balanced against the rarity of spore forming bacteria actually encountered in Operating Room Theater was such disinfectant as 10-20 minutes sacked with glutaraldehyde was quite safe and sufficient.

Sterilization means destroy or removes all living organisms from an article. Effective disinfection can destroy viruses and all common vegetative pathogens but not bacterial spores (1,2).

Most laparoscopic equipment, such as graspers, scissors, and trocars are safety sterilized by using steam autoclave. Since this is relatively inexpensive, fast and effective, it is the preferred method for achieving sterilization. Laparoscopic camera will be damaged by heat. Gas sterilization by ethylene oxide can be used, but is impractical because of the long turnover time required 12-24 hours. So, the best choice of reprocessing these types of laparoscopes is chemical germicides (3). Most of chemical germicide can produce sterile instruments if the instruments are exposed to germicide for prolonged period of time. This would lead to long turn over times. However, and in some instances damage the laparoscopes. This is impractical, most hospitals that used liquid germicides for processing the laparoscopes use shorter exposure time with the aim of achieving high-level disinfection.

Most manufactures of glutaraldehyde currently recommended a minimum of 20 minutes of exposure to provided high level disinfection. Glutaraldehyde formulation is the most popular chemical sterilant used for disinfection of medical equipment in the United States (4). It become popular because of the advantages of excellent biocidal activity in the presence of organic contamination, non corrosive action on endoscopes or equipment and non coagulation of protein aqueous material (2).

MATERIALS AND METHODS

The instrument of laparoscope was soaked for 20 minutes in 2% glutaraldehyde before the procedure.

1- Swabbing method: 30 dogs under going laparoscopy for different surgical intervention. Dogs prepare for aseptic procedure as routine work. Abdomens at umbilical region were washed with 5% tincture iodine using gauze sponges held in ring forceps. A small sponge away in one minute, the abdomen was then draped with a mini-laparotory sheet with a central aperture.

a- Umbilical culture: Using a sterile cotton swab an umbilical culture was taken and the swab immediately placed in a tube of brain heart infusion broth, Figure 1.

b- Telescope culture: The telescope was immersed in brain heart infusion broth and rotated for a few second to promote transfer of any bacteria present, Figure 2.

c- Peritoneum culture: Culture of peritoneum were taken as follows, through incision, a trocar and sheath were inserted. The trocar was removed and blunt, 5 m cannula inserted and aimed toward the peritoneum under laparoscopic guidance, the cannula was placed in the peritoneal cavity.
distended with CO₂ pneumoperitoneum, 20 ml of sterile water was injected through cannula and try to wash pelvic organs. The liquid, mixed with peritoneum fluid and often with a little blood from the trocar wound was aspirated in to syringe via the cannula, then 5 ml of this liquid was inoculated in 5ml of brain heart infusion broth, Figure 3.

2- Bacteriological culture, isolation and identification of microorganisms.
Specimen a- Umbilical: The swab in brain heart infusion broth was distributed on two blood agar plates, chocolate agar plate, MacConkey agar plate and sabouraud dextrose agar plate. The first blood agar plate was incubated anaerobically by using anaerobic jar, gas Pak generating kit (Al-Razi diagnostic, Baghdad, Iraq) at 37 °C in Gas pak jar and examined after 48hr. Sabouraud dextrose agar plate was incubated at room temperature for one week, the second blood agar plate, chocolate agar plate and MacConkey agar plate incubated aerobically at 37 °C for 24-48 hrs.
Specimen b- telescope: This culture was treated exactly the same as the umbilical culture.
Specimen c- saline from peritoneal cavity: Brain heart broth containing 5 ml of saline from peritoneal cavity also treated the same as the umbilical culture (Figure 3). For all culture, whenever growth appeared in plates, it was gram stained and subculture appropriately after the time of culturing. Aerobic and anaerobic culture plates were examined and checked under light microscope. The morphology of different types of colonies was smeared to study the isolated type. Then subcultured to get isolated colonies and made biochemical test on each microorganisms which included all organisms isolated were identified at least to genus level (5).

3- Biochemical tests and stains, Included the following:
1- Gram stain, 2- Catalase test, 3- Goagulase test, 4-Oxidase test,
5- IMVIC tests, 6- Carbohydrates fermentation test, 7- Hydrolysis of gelatin.
These tests were performed according to (6).

RESULTS AND DISCUSSION

Data on isolates according to site aerobic and anaerobic species appear in table 1 and 2.
All organisms cultured from the umbilical area were commonly encountered on normal skin surfaces. Included in the umbilical isolates were one fungi Aspergillus spp. Spore forms were found in 3 Bacillus spp.
SPECIMEN A

5 ml BH broth

0.2 ml Blood Agar Plate
0.2 ml Blood Agar Plate
1.0 ml Chocolate Agar Plate
1.0 ml MacConkey Agar plate

Figure 1: culture protocol from umbilical site

SPECIMEN B

5 ml BH broth

0.2 ml Blood Agar Plate
0.2 ml Blood Agar Plate
1.0 ml Chocolate Agar Plate
1.0 ml Sab Dextrose Plate
1.0 ml MacConkey Agar plate

Figure 2: culture protocol from telescope

SPECIMEN C

20 ml Aspirate Saline

5 ml Aspirate saline + 5 ml BH broth

0.2 ml Blood Agar Plate
0.2 ml Blood Agar Plate
1.0 ml Chocolate Agar Plate
1.0 ml Sab Dextrose Plate
1.0 ml MacConkey Agar plate

Figure 3: culture protocol from peritoneal site
Table 1: Organisms isolated during laparoscope procedure showing aerobic microorganisms.

<table>
<thead>
<tr>
<th>Aerobic Organism</th>
<th>Source</th>
<th>Umbilicus</th>
<th>Telescope</th>
<th>Peritoneal</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis (Fig 4)</td>
<td></td>
<td>23</td>
<td>2</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>S. aureus (Fig 5)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus subtilis.</td>
<td></td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Corynebacterium sp. (diphtheriods)(Fig6)</td>
<td></td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Streptococcus faecilis (Fig 7)</td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus Sp.(Fig 8)</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>36 (85%)</td>
<td>4 (7%)</td>
<td>8 (16%)</td>
<td>48</td>
</tr>
</tbody>
</table>

Staphylococcus epidermidis (was the most common umbilical isolate. While, propionibacterium spp., was most common in the telescope anaerobic culture.

In the case with positive peritoneal cultures, three organisms had isolates of the same species those found on the telescope, and six has isolates of the same species cultured from the umbilical area.

Table 2: Showing anaerobic microorganisms isolated during laparoscopic procedure.

<table>
<thead>
<tr>
<th>Anaerobic Organism</th>
<th>Source</th>
<th>Umbilicus</th>
<th>Telescope</th>
<th>Peritoneal</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionibacterium sp.</td>
<td></td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Bacteroides sp</td>
<td></td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Peptococcus sp</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>25</td>
</tr>
</tbody>
</table>

It is important to remember that while (Sterilization) is an absolute term (within experimental limit of verification), disinfection is a relative one.

Glutaraldehyde has many advantages as a chemical sterilizing agent: Full antimicrobial activity lasts for 14 days. A ten–hour soak is in deed (sterilization) while the commonly employed 10 to 20 minute soak is (disinfecting); the real difference resides in the time necessary to kill spores. First, the agent has no deleterious effect on the glass lens and cements used in most endoscope instruments (7). Stone et al., (7) further noted that it is non corrosive dose not injure rubber and dose not coagulate blood, making instrument cleaning easier. Ethylene oxide sterilizing procedure take 2-8 hours and require aeration time after wards to remove as mach gas as possible Costs are high, lenses may be fogged, and the agent is particularly adherent to plastics and rubber. Further the agent is under surveillance as a suspected carcinogen (8).

The crux of the discussion has centered around the spore problem. Aerobic, gram–positive spore forms such as Bacillus cereus and the anthrax bacillus-
certainly not, common operating room in habitants. Anaerobic Clostridium spp such as (tetanus, botulinum and perfringens) are more common, and infact Cl. perfringens was cultured from the telescope in our study.

The sporocidal activity of glutaraldehyde follows the expected logarithmic time curve. Stone et al. (7) used Bacillus megaterum as a test organism and showed that with an initial count of 3980; only 250 were surviving at 30 minutes, 4 at 6 minutes and none by two hours. In general they concluded that spores of various organisms were all killed by three hours, and Mycobacterium tuberculosis was killed after ten minutes of contact.

Klein and Deforest (9) reported on inactivation of viruses by various germicides. Gluteraldehyde was clearly superior to the other tested. Against polio viruses. I., coxsackie B1, echo, adenovirus 2, herpes simplex, vaccine and Asian influenza virus, Total inactivation was achieved within One minute.

A review of umbilical cultures demonstrates the difficulty of sterilizing the particular area of the body. The organisms grown are normal skin flora, But Maki has stated (Most wound) contamination come either from the patients own skin or from deep organs (10) Maki points out that count of air borne microorganisms from two to ten per cubic foot are common in the operating room site near the patient and are directly proportional to the number of the people in the room and the amount of traffic.

In case of peritoneal culture, the specific organisms' cultures were similar to the skin Findings. These organisms are probably carried in to the peritoneal cavity by the insufflating gas needle and or trocars rather than by the telescope it self, which was inserted through the sheath after removal of the trocar. This suggested that the peritoneum was being contaminated by skin flora and not by the laparoscope.

Figure 4 Staphylococcus epidermidis on blood agar isolated from umbilicus, telescope and peritoneum
Figure 5: *Staphylococcus aureus* on blood agar isolated from peritoneum

Figure 6: Colonies of *Coryne bacterium spp* (*diphtheroids*) on blood agar medium isolated from umbilicus, telescope and peritoneum.
Figure 7: *Streptococcus faecalis* on MacConkey Agar isolated from umbilicus area.

Figure 8: *Aspergillus niger* on sabouraud glucose agar medium isolated
In Conclusions, the study proved that source of infection mostly occurred through the skin and less important from telescope. Glutaraldehyde destroys most of microorganisms in telescope and safely can be used as disinfection material for laparoscope.

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REFERENCES