Chlorhexidine Lavage in the Treatment of Experimental Intra-abdominal Infection

Victor M. Bondar, MD; Carlo Rago, BS; F. John Cottone, MD; Donald K. Wilkerson, MD; Jim Riggs, PhD

Hypothesis: Closed postoperative peritoneal lavage (CPPL) with chlorhexidine gluconate reduces the number of intraperitoneal bacteria and improves the outcome of intra-abdominal infection.

Design: Laboratory animal trial.

Interventions: Intra-abdominal infection was produced in mice by the cecal ligation and puncture technique. After 16 to 18 hours, the animals underwent re-laparotomy and placement of an intra-abdominal catheter for CPPL. In the first experiment animals were randomly divided into 4 groups: no lavage (served as a control), CPPL with chlorhexidine, CPPL with cefoxitin, and CPPL with lactated Ringer solution (LR). Lavage was continued intermittently every 8 hours for 24 hours. All animals received systemic cefoxitin every 8 hours for 7 days. Mortality was recorded every 8 hours for 10 days. In the second experiment, animals were divided into 3 groups: no lavage (served as a control), CPPL with chlorhexidine, and CPPL with LR. Lavage was continued intermittently every 8 hours for 24 hours. The animals were killed 48 hours after reoperation. Bacterial counts from peritoneal fluid and biopsy specimens, as well as peritoneal white blood cell counts, were measured before and after lavage.

Results: Closed postoperative peritoneal lavage with chlorhexidine reduced mortality from 71% in a control group to 37% (P = .003). There was no survival benefit in either the CPPL with cefoxitin (91% mortality) (P = .14) or CPPL with LR groups (90% mortality) (P = .17). The statistically significant findings of analysis of variance evaluation demonstrated a decrease in bacterial counts after cecal excision in all 3 groups. There was a greater reduction in bacterial counts in the chlorhexidine group compared with the control group (P<.05). Bacterial counts decreased in peritoneal fluid, as well as in tissue biopsy specimens, after cecal excision. White blood cell counts significantly decreased after cecal excision in all 3 groups. There was no difference in white blood cell counts between the groups. Correlation analyses demonstrated weak interaction between bacterial and white blood cell counts before or after treatment in all the groups. Pearson r ranged from −0.37 to +0.35, none of which were statistically significant.

Conclusions: In our experiments chlorhexidine lavage resulted in a 50% reduction in mortality and a significant reduction in bacterial counts compared with the control group. There was no survival benefit from lavage with either cefoxitin or LR. There was no reduction in bacterial counts in the LR group relative to the control group. Thus, the survival benefit and the reduction in bacterial numbers are attributed to the antibacterial properties of chlorhexidine rather than to the mechanical washing of the abdominal cavity. Closed postoperative peritoneal lavage with 0.05% chlorhexidine gluconate might be useful in the multimodal treatment of intra-abdominal infection.


Intra-abdominal infection (IAI) as a result of a defect in abdominal hollow viscus is a life-threatening condition. Mortality from IAI ranges from 17% to 38%. The current treatment of IAI consists of (1) surgical control of the source of infection, (2) supportive measures, and (3) mechanical reduction of intra-abdominal bacterial inoculum. While the first 2 measures are the mainstay of treatment for IAI, the third is still widely debated.

Peritoneal lavage is used to decrease bacterial load, facilitating bacterial clearance by the peritoneal defense mechanism. Intraoperative peritoneal lavage and closed postoperative peritoneal lavage (CPPL), using physiologic solutions with or without antibiotics, have been studied extensively in both experimental and clinical settings.

Few prospective randomized clinical trials have been done to evaluate these modalities. Only one of the trials produced favorable results.
MATERIALS AND METHODS

EXPERIMENTAL MODEL

Two hundred thirty male CBA/CaJ mice were obtained from the Jackson Laboratory, Bar Harbor, Me. The mice weighed 22 to 26 g at 5 to 6 weeks of age. The care of these animals was in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 80-23, 1985). All animals were group-housed and fed water and chow ad libitum before and after operations. A 12-hour light/dark cycle and a constant temperature of 24°C were maintained. The mice were acclimatized to these laboratory conditions for at least 7 days prior to the experiments.

SURGICAL PROCEDURE

All interventions were performed under aseptic conditions. The cecal ligation and perforation (CLP) model of IAI initially described by Wichterman et al18 was used. All animals were anesthetized with an intraperitoneal injection of 40 mg/kg of thiopental (Pentothal; Abbott Laboratories, North Chicago, Ill) and 40 to 120 mg/kg of ketamine. The anterior abdominal wall was shaved and cleansed with 4% chlorhexidine gluconate. One milliliter of 0.004% lidocaine was administered subcutaneously for skin incision. A 1-cm midline incision was made. The cecum was mobilized, ligated distal to the ileocecal valve with a silk suture, and punctured twice with a 27-gauge needle along the antimesenteric border. The cecum was gently squeezed to extrude feces and ensure that the puncture holes did not close. The cecum was placed back in the left upper quadrant of the abdomen. The abdomen was closed in 2 layers. Sixteen to 18 hours after initial surgery, all animals underwent relaparotomy. Peritoneal fluid and biopsy samples were obtained. Representative blood cultures were obtained from the retroorbital venous plexus. A 20-gauge silicone catheter was introduced percutaneously in the right lower quadrant of the abdomen. The catheter was secured in position with polypropylene to the muscles and nylon to the skin. The abdomen was closed in 2 layers. Lavage was done with 2 mL of the appropriate solution administered through the intraperitoneal catheter. The fluid was drained through the catheter for 5 to 10 minutes for complete recovery. Lavage was conducted every 8 hours for 24 hours. Mice were observed and their mortality was recorded every 8 hours. All animals that died had a necropsy. Surviving mice were later killed and also underwent a necropsy. At necropsy, peritoneal fluid and biopsy specimens, as well as peritoneal cell samples, were obtained.

BACTERIOLOGIC ANALYSES

The samples were thawed, diluted in 10-fold steps in isotonic sodium chloride solutions, and inoculated on blood agar medium. The tissue specimens were macerated prior to dilutions. Identical sets of culture plates were incubated aerobically and anaerobically in sterile jars (Gas Pak; Bacto Laboratories, Liverpool, England). The anaerobic plates were stored at −70°C until analyzed.

BACTERIOLOGIC SAMPLING

Prior to relaparotomy or necropsy, the peritoneal cavity was filled with 1 mL of isotonic sodium chloride solution. Animals were agitated to ensure adequate mixing of fluid. Peritoneal fluid samples (0.1 mL) and biopsy specimens (0.07-0.13 mg) were collected for bacterial quantification. Three to 4 biopsy specimens were obtained from the left and right sides of the parietal peritoneum. Biopsy specimens were weighed on a microbalance immediately on collection. All specimens were mixed with 1 mL of tryptic soy broth and stored at −70°C until analyzed.

PERITONEAL CELL COUNT

Peritoneal fluid samples (50 µL) were collected at the time of relaparotomy and necropsy. The specimens were stained with aliquots (50 µL) of vital blue dye. White blood cells were counted in a cell counter. Flow cytometry was negative bacteria and fungi. Chlorhexidine gluconate also has a low toxic profile at therapeutic concentrations of 0.05%, both in animal models of peritonitis and in the clinical setting. This study was undertaken to test the hypothesis that CPPL with chlorhexidine reduces the number of intraabdominal bacteria and improves the outcome of IAI.

RESULTS

Pilot studies demonstrated that CLP with a 27-gauge needle produces 70% mortality (Table 1). Toxicity studies showed that a 0.05% concentration of chlorhexidine gluconate is well tolerated by mice; all 10 mice survived. Higher concentrations of chlorhexidine gluconate produced significant mortality: 3 (30%) of 10 mice...
PILOT STUDIES

Forty mice underwent CLP with 19-, 21-, 25-, and 27-gauge needles to determine the degree of contamination and duration of infection that produces significant, but not 100%, mortality in the presence of systemic antibiotic cefoxitin (160 mg/kg every 8 hours subcutaneously).

TOXICITY STUDIES

Thirty mice underwent operation with placement of intra-peritoneal catheter for CPPL. Two milliliters of chlorhexidine gluconate in concentrations of 0.5%, 0.1%, and 0.05% was administered through the intra-abdominal catheter. The fluid was drained for 5 to 10 minutes. Lavage was conducted every 8 hours for 24 hours. All animals that died had a necropsy. Surviving mice were killed 48 hours after lavage and also underwent a necropsy.

EFFECT OF ANTIMICROBIAL LAVAGE ON ANIMAL SURVIVAL

One hundred mice were prepared with CLP. Sixteen to 18 hours later the animals underwent relaparotomy and placement of an intra-abdominal catheter. The cecum was not excised. The animals were randomly divided into the following groups: no lavage (served as a control), CPPL with 0.05% chlorhexidine gluconate, CPPL with 0.4% cefoxitin, and CPPL with lactated Ringer solution (LR). Ten mice were prepared on each day: 4 mice were assigned to the control group, 4 mice to the chlorhexidine group, and 2 mice to either the cefoxitin or the LR group. Prior to reoperation, all animals received cefoxitin (160 mg/kg in 1 mL of LR subcutaneously). Cefoxitin was continued every 8 hours for 7 days. Representative blood cultures were obtained from the retro-orbital venous plexus prior to the onset of antibiotic therapy. The control group had no catheter and did not receive lavage. The treatment groups received lavage with 2 mL of the appropriate solution administered through the intra-abdominal catheter. Lavage was conducted every 8 hours for 24 hours.

Mice were observed and their mortality was recorded every 8 hours. All animals that died had a necropsy. Surviving mice were killed after 10 days and also underwent a necropsy. The presence of diffuse peritonitis or intra-abdominal abscesses was determined visually and confirmed by routine bacteriologic methods.

EFFECT OF ANTIMICROBIAL LAVAGE ON BACTERIAL AND CELL COUNTS

Sixty mice were prepared with CLP. The animals were randomly divided into the following treatment groups: no lavage (served as a control), CPPL with 0.05% chlorhexidine gluconate, and CPPL with LR. In addition, 10 mice underwent sham operations. Sixteen to 18 hours after initial surgery, all animals underwent relaparotomy. Peritoneal fluid samples, biopsy specimens, and peritoneal cell samples were obtained. The cecum was excised. The treatment groups received lavage with 2 mL of the appropriate solution. Lavage was conducted every 8 hours for 24 hours. Mice were observed and their mortality was recorded. All animals that died had a necropsy. Surviving mice were killed 48 hours after relaparotomy and also underwent a necropsy. At necropsy, biopsy specimens, peritoneal fluid, and cell samples were obtained.

STATISTICAL ANALYSES

The Fisher exact test was used to assess the significance of difference in mortality. Bacterial and white blood cell counts were transformed logarithmically. Three-way analysis of variance (ANOVA) allowed comparison between bacterial counts in treatment groups over time and in different media (tissue and fluid). Two-way ANOVA was used to compare white blood cell counts in treatment groups during 2 time intervals. The post hoc Scheffe test was done to determine which measurement was statistically significant. Correlation analysis between bacteria and white blood cell counts was performed using the Pearson product moment correlation, followed by the Fisher z transformation. Statistical significance was determined by a P value of .05 or less.

EFFECT OF ANTIMICROBIAL LAVAGE ON ANIMAL SURVIVAL

One hundred mice entered the experiment. Seven mice were excluded from the study: 2 had anesthesia death, 3 had complications of surgical technique, and 2 died before the treatment began. Thus, 93 mice were left for statistical analysis. Of 34 mice that served as a control group, 24 (71%) died compared with 14 (37%) of 38 mice that received CPPL with chlorhexidine (P = .003), 10 (91%) of 11 mice that received CPPL with cefoxitin (P = .14), and 9 (90%) of 10 mice that received CPPL with LR (P = .17). There were no significant intergroup variations (Table 2). Fifty-two (91%) of 57 deaths occurred within 48 hours after relaparotomy. Mortality in the chlorhexidine group was less than that in the control group at any time point (Figure 1).

Thirty-three of the 57 mice that died had signs of localization of infection in the right upper quadrant of the abdomen. This varied between mice that died in the first 24 hours and those that died afterwards. Eleven (36%) of 31 mice that died in the first 24 hours had signs of localizing infection compared with 22 (85%) of 26 mice that died after 24 hours. All surviving mice had a walled-off abscess in the right upper quadrant of their abdominal cavities. All representative blood cultures were positive. The most common organisms cultured were Escherichia coli, Klebsiella pneumonia, and Enterococci species. Peritoneal cultures were obtained from 70 mice at necropsy. All peritoneal cultures had bacterial growth except for 2 negative cultures in survivors in the chlorhexidine group.

in the 0.1% chlorhexidine gluconate group and 9 (90%) of 10 mice in the 0.5% chlorhexidine gluconate group. There was no evidence of local toxicity at necropsy.

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EFFECT OF ANTIMICROBIAL LAVAGE ON BACTERIAL AND CELL COUNTS

Five mice died prior to reoperation and were excluded from analysis. The statistically significant findings of ANOVA evaluation demonstrated a decrease in bacterial counts after cecal excision in all 3 groups (Table 3). Bacterial counts decreased in peritoneal fluid, as well as in tissue biopsy specimens after cecal excision. There was a greater reduction in bacterial counts in the chlorhexidine group compared with either the control or LR groups (Figure 2). According to the Scheffe test, only one specific comparison was significant: control group vs chlorhexidine group (P < .05). The most common organisms cultured were *E coli*, *K pneumonia*, and *Enterococcus* species. There was no consistent anaerobic growth, probably as a result of flaws in specimen processing. The sham-operated mice had no bacterial growth.

White blood cell counts significantly decreased from 7.91 ± 0.16 (mean ± SD) to 7.26 ± 0.35 log10 cells/mL after cecal excision in all 3 groups. There was no difference in white blood cell counts between the groups. Flow cytometry demonstrated white blood cell differentials of up to 90% polymorphonuclear leukocytes with the remaining 10% divided between monocytes, T lymphocytes, and B lymphocytes. There was no difference in white blood cell differentials between the groups.

Correlation analyses demonstrated weak interaction between bacterial and white blood cell counts before or after treatment in all the groups. Pearson rs ranged from −0.37 to +0.35, none of which was statistically significant.

**COMMENT**

Peritoneal lavage is a routine practice for many surgeons. Lavage is used to facilitate removal of pus, necrotic tissue, feces, bile, or any other adventitious substances from the peritoneal cavity. Physiologic solutions, antibiotics, or an-

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**Table 1. Selection of the Needle Size for Cecal Ligation and Perforation**

<table>
<thead>
<tr>
<th>Needle Gauge</th>
<th>No. of Mice</th>
<th>No. Dead</th>
<th>Mortality Rate, %</th>
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<tbody>
<tr>
<td>19</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>10</td>
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<td>25</td>
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<td>9</td>
<td>90</td>
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<tr>
<td>27</td>
<td>10</td>
<td>7</td>
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**Table 2. Mortality in Different Treatment Groups**

<table>
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<tr>
<th>Day</th>
<th>Control Group</th>
<th>CPPL With 0.05% Chlorhexidine Gluconate</th>
<th>CPPL With Cefoxitin</th>
<th>CPPL With LR</th>
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<tr>
<td></td>
<td>No. Dead/Total No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3/4</td>
<td>1/3</td>
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<tr>
<td>2</td>
<td>3/3</td>
<td>2/3</td>
<td>...</td>
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<td>10</td>
<td>3/4</td>
<td>1/4</td>
<td>2/2</td>
<td>...</td>
</tr>
<tr>
<td>Total (%)</td>
<td>24/34 (71%)</td>
<td>14/38 (37%)</td>
<td>10/11 (91%)</td>
<td>9/10 (90%)</td>
</tr>
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</table>

*CPPL indicates closed postoperative peritoneal lavage; LR, lactated Ringer solution; and ellipses, not applicable. Mortality in the chlorhexidine group was significantly lower than in the control group (P < .003). There were no significant intergroup variations.

**Table 3. Bacterial Counts in Different Treatment Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Fluid Before</th>
<th>Fluid After</th>
<th>Tissue Before</th>
<th>Tissue After</th>
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<tbody>
<tr>
<td>Control</td>
<td>9.64 ± 1.20</td>
<td>6.29 ± 1.31</td>
<td>9.22 ± 1.20</td>
<td>6.85 ± 1.43</td>
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<tr>
<td>Chlorhexidine gluconate</td>
<td>9.55 ± 0.99</td>
<td>4.92 ± 1.08</td>
<td>9.04 ± 0.91</td>
<td>5.52 ± 1.19</td>
</tr>
<tr>
<td>LR</td>
<td>9.30 ± 1.01</td>
<td>6.15 ± 1.41</td>
<td>9.42 ± 0.96</td>
<td>6.63 ± 1.21</td>
</tr>
</tbody>
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*All data are expressed as mean log10 colony-forming units per gram of tissue or milliliter of fluid ± SD. There was a significant decrease in bacterial counts after cecal excision in all 3 groups. There was a greater reduction in bacterial counts in the chlorhexidine group compared with either the control (P < .05) or LR groups. LR indicates lactated Ringer solution.

**Figure 1.** Effect of antimicrobial lavage on animal survival. Mortality in the chlorhexidine gluconate group was less than in the control group at any time point (P < .003). LR indicates lactated Ringer solution.

**Figure 2.** Effect of antimicrobial lavage on bacteria. There was a greater reduction in bacterial counts in the chlorhexidine gluconate group compared with either the control (P < .05) or lactated Ringer solution (LR) groups.
tiseptics can be used for lavage. Despite multiple experimental and clinical trials, the effectiveness of any kind of peritoneal lavage had not been established.4

Although lavage is generally considered to be a safe procedure, there are several objections to its use. First, the ability of any kind of peritoneal lavage to remove bacteria from the peritoneal cavity is questionable. Even if bacterial counts decrease immediately after lavage, they return to prelavage levels in 24 hours.15,20 It has been demonstrated that during IAI there is a stable population of adherent microorganisms that cannot be removed by lavage with either physiologic solutions or antibiotics.20

Second, there is a concern that peritoneal fluid may itself serve as an adjuvant substance and adversely affect the abdominal defense mechanism during the course of IAI.21 This view is supported by an observation of spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. Patients with an ascitic fluid protein concentration of less than 10 g/L are 10 times more likely to develop spontaneous bacterial peritonitis than those with a protein concentration greater than 10 g/L.22 The dilution of opsonins is thought to be responsible for these findings. The importance of opsonins for phagocytosis receives further support from experimental trials.23,24

The effect of peritoneal fluid in experimental IAI was studied by Dunn et al.21 Bacteria were inoculated into the peritoneal cavity of rats in small (1 mL) and large (30 mL) amounts of fluid. The study demonstrated bacterial proliferation with decreased peritoneal clearance, decreased incidence of positive blood cultures, and increased mortality in the animals receiving large volumes of fluid to compare with the group receiving small volumes of fluid. Bacterial phagocytosis in the large-volume irrigation group was impaired during the first 2 hours, but later was uninhibited. Early in the course of IAI, there are 3 major mechanisms available to clear bacteria from the peritoneal cavity: the abdominal lymphatics, the resident peritoneal macrophages, and the influx of polymorphonuclear leukocytes that starts at 2 hours and peaks at 6 to 8 hours.25 Thus, the decreased peritoneal bacterial clearance observed by Dunn et al should be attributed to impaired absorption by the abdominal lymphatics and/or decreased clearance by the resident peritoneal macrophages. Still, the increase in mortality in the group receiving large volumes of fluid may be difficult to explain in view of another study by Dumont et al.26 In that study, blocking of the diaphragmatic lymphatics resulted in decreased bacterial clearance, decreased incidence of positive blood cultures, and improved animal survival. Clearly, more studies are needed to elucidate the role of intraperitoneal fluid in IAI.

Peritoneal lavage with antiseptics is a different approach to the treatment of IAI. It relies on the antimicrobial properties of any given antiseptic rather than on the mechanical washing of the abdominal cavity. Chlorhexidine gluconate is an antiseptic that is widely used in different concentrations for many purposes. Hibiclens (Stuart Pharmaceuticals, Wilmington, Del) and Excide (Baxter Healthcare Corp, Glendale, Calif) are 4% chlorhexidine gluconate formulations used in a sudsing base for surgical scrubbing, hand washing, and skin cleansing. External chlorhexidine has extremely low toxicity, even when used on intact skin of newborns.27

Periex (Procter & Gamble, Cincinnati, Ohio) contains 0.12% chlorhexidine gluconate in a mouth rinse formulation. It is used in dental practice to control mouth flora in periodontitis and gingivitis. Toxicity of Periex is limited to teeth discoloration and alterations in taste perception.

Vorherr et al28 studied effect of 4% chlorhexidine and 10% povidone iodine on vaginal bacteria of nonpregnant women. Vaginal cleansing with either solution killed almost 99% of bacteria. Chlorhexidine was significantly more effective than povidone iodine. Chlorhexidine was not absorbed in measurable amounts into the bloodstream.

Several studies used 0.2% to 0.4% chlorhexidine vs physiologic solutions for a one-time vaginal irrigation to prevent maternal peripartum infection.29,30 Chlorhexidine was not effective as tested; there was no significant toxicity from the treatment.

Adesanya et al31 tested 0.05% chlorhexidine gluconate for perioperative intermittent bladder irrigation in prostatectomy patients. The authors observed a decrease in the rate of intraoperative bacteremia and severe wound infection. There was no evidence of systemic or local toxicity.

A single clinical study with 0.05% chlorhexidine gluconate in IAI studied intraoperative peritoneal lavage with saline vs chlorhexidine vs povidone iodine.17 Fifty-three patients entered the trial. Overall mortality was 23%. The authors did not specify mortality figures for the treatment groups. There was no difference in wound complications between the groups. There was no significant toxicity caused by the antiseptics used in the trial.

To our knowledge, there are no experimental or clinical studies addressing the use of CPPL with chlorhexidine in the treatment of IAI.

For our experiments we used the CLP model of IAI. This model enabled us to regulate the volume and the duration of intra-abdominal contamination and to simulate the clinical situation in which treatment is started after IAI is established and systemic manifestations of the disease are present. Previous animal studies with intra-peritoneal chlorhexidine used lavage immediately after inoculation of bacteria into the peritoneal cavity.14,16 Those models studied prevention rather than treatment of IAI and have little clinical application.

In our experiments, chlorhexidine lavage resulted in a 50% reduction in mortality and a significant reduction in bacterial counts compared with the control group. Most important, there was a reduction in the number of serosally adherent bacteria. The bacterial reduction was observed 24 hours after lavage. There was no survival benefit from lavage with either cefoxitin or LR. There was no reduction in bacterial counts in the LR group relative to the control group. Thus, the survival benefit and the reduction in bacterial numbers are attributed to the antibacterial properties of chlorhexidine rather than to the mechanical washing of the abdominal cavity.

Chlorhexidine does not seem to be toxic for peritoneal polymorphonuclear leukocytes as demonstrated...
by white blood cell counts and flow cytometry. The lack of correlation between bacterial and white blood cell counts probably reflects the difference in individual responses to IAI.

Closed postoperative peritoneal lavage with 0.05% chlorhexidine gluconate might be useful in the multimodality treatment of IAI.

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