

Research Article

Antibacterial Activity of Various Antiseptics While The Experiment and Laparoscopy in Patients With Acute Pancreatitis

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ABSTRACT

The aim of this study is to examine the effectiveness of different sanitation mediums, combined with physical factors of impact. Reference strains of nosocomial flora *Escherichia coli* (ATCC) with a concentration of 10⁷-10⁸ CFU/ml were used to study the effectiveness of various antiseptics, on the basis of the Surgery Department of State Budget Educational Institution of Continuing Professional Education of Kazan State Medical Academy. The treatment of bacterial suspension was carried out during 5, 15, and 45 seconds by flushing of fresh growing cultures from agar plates by the jet of HCH (carbonated aqueous chlorhexidine 0.05%), CHX (aqueous chlorhexidine 0.05%), OSS (ozonated saline solution) and by natural saline solution as a control. Treated by solutions bacterial suspension was taken into tests tubes aseptically, and was tested on initial concentration at above-mentioned exposure. The number of microorganisms was determined by seeding on Petri dishes with nutrient agar, using a calibrated loop. After 20-22 hours of incubation at 37⁰C the numbers of grown bacteria were counted, the degree of microbial seeding was determined by the table and was expressed in CFU/ml. According to data, obtained in the course of the study, the most effective means for the sanitation was an aqueous solution of carbonated chlorhexidine 0,05%, which at 45 second exposure inhibited growth of *E.Coli*- (5· 10¹ -10² CFU/ml). This antiseptic preceded the aqueous solution of chlorhexidine 0,05% - inhibited growth of *E.Coli* (5· 10⁵ CFU/ml), and ozonated saline solution and natural saline solution wherein the concentration of *E.Coli* was (5· 10⁷ -1·10⁸ CFU/ml) at 45 second exposure. So, the combination of antiseptics with physical factors of impact – saturation with gas components, in particular carbon dioxide, significantly inhibits the growth of *E.Coli*-(5· 10¹ - 10² CFU/ml) compared to antiseptics without combining with the physical impact factors.

Keywords: Acute pancreatitis, surgical infection, antiseptic, chlorhexidine, carbon dioxide.

INTRODUCTION

A significant role in the development of mortality in case of abdominal surgical infection, infected pancreatic necrosis, per pancreatitis, belongs to the pathogenic microflora, especially for nosocomial infections, emerging due to inadequate antibiotic therapy [1, 5, 6, 7, 8, 9]. One of the important surgical stages of the treatment of pancreatic necrosis is

adequate sanitation of the abdominal cavity, retroperitoneal space for the prevention and treatment of surgical infections. Taking this information into account, the efforts of clinical surgeons are directed to the development of new methods for the prevention and treatment of surgical infections, in particular, the use of physical factors when sanitizing abdominal

cavity, retroperitoneal space [2, 10]. The effectiveness of physical factors related to the fact that the adaptation ability of microorganisms to them is very limited [3]. It is difficult enough to study the impact of physical factors on the tissues of patient due to the difficulty of detecting the prevalence of a particular absorption or energy migration in complex multi-molecular structures of living cells [5], but the effectiveness of the latter is indicated in the current literature [4]. Nowadays, the microorganisms are resistant for the used sanitation environments: francolin, chlorhexidine. The combination of gas components (oxygen, ozone, nitrogen and carbon dioxide), ultrasound [3] for the treatment of the abdominal cavity in case of abdominal infections is path genetically justified and requires further researches [5, 6]. In connection with the above, the following goal was set: to study the effectiveness of different sanitation environments, combined with the physical factors of impact.

MATERIAL AND METHODS

To determine the effectiveness of sanitation mediums, the following items were selected: natural saline solution, aqueous solution of chlorhexidine (CHX -chlorhexidine) 0.05%, carbonated chlorhexidine 0.05%, (HCH-carbonated chlorhexidine) and ozonized saline solution (OSS) with the concentration of residual ozone 1 -3 mg/l. Natural saline solution treatment was conducted for comparison. Ozone at a concentration of 50 mg/l was synthesized by generator "Medozon" (Nizhniy Novgorod) and it was bubbling through the solution. The ozone concentration in saline solution was determined by iodometric method and amounted 1.0 mg/1000ml. All studies with medical ozone were conducted in accordance with the guidelines of the Ministry of Health of Russian Federation "The technique of ozone therapy", 1991. In the clinical setting, in one patient, it is impossible to quantify the microbial content in the process of treatment by HCH, CHX and OSS (HCH - carbonated chlorhexidine, CHX - chlorhexidine or OSS - ozonized saline solution) as microbial content of the wound immediately

changed after processing by the first solution, that makes impossible determining the content for another solution. Consequently, the modeling of microbial content was carried out in vitro: the same microbial content was created before treating by HCH, CHX and OSS, then they were treated with compared in parallel solutions and after a regular intervals of exposure, the number of remaining microorganisms was calculated. The reference strains of nosocomial flora Escherichia coli (ATCC) with a concentration of 10^7 - 10^8 CFU/ml were used for the research of the effectiveness of different in composition solutions, practicing for the sanitation of the infectious center. The treatment of bacterial suspension was carried out during 5, 15, and 45 seconds by flushing of fresh growing cultures from agar plates by the jet of HCH, CHX and OSS. Treated by solutions bacterial suspension was taken into tests tubes aseptically, and was tested on initial concentration at abovementioned exposure. The number of microorganisms was determined by seeding on Petri dishes with nutrient agar, using a calibrated loop. After 20-22 hours of incubation at 37°C the numbers of grown bacteria were counted, the degree of microbial seeding was determined by the table and was expressed in CFU/ml.

RESULTS

The results of the comparative tests of HCH, CHX and OSS showed, that the growth of bacteria during the treatment by HCH was absent at all stages of the exposure (5-15-45 seconds), having initial concentration of $5 \cdot 10^7$ E.Coli. In the process of treatment by distilled water, the original concentration of microbes $5 \cdot 10^7$ E.Coli reduced to $5 \cdot 10^6$ CFU/ml after 15 seconds of exposure and then slightly increased to $5 \cdot 10^7$ - $1 \cdot 10^8$ CFU/ml at increasing exposure to 45 seconds. In the process of treatment by ozonized saline solution the initial concentration of microbes decreased to $5 \cdot 10^5$ CFU/ml at 15 seconds of exposure and then slightly increased to $5 \cdot 10^7$ - $1 \cdot 10^8$ CFU/ml at 45 seconds of exposure after the treatment of initial concentration. In the process of treatment by chlorhexidine, the initial concentration of

microbes decreased to $5 \cdot 10^7$ CFU/ml at 15 seconds of exposure, and then reduced to $5 \cdot 10^5$ CFU/ml at 45 seconds of exposure. In the process of treatment by carbonated chlorhexidine, bacterial growth was inhibited at

all stages (15-45 seconds) at an initial concentration E.Coli $5 \cdot 10^7$, decreasing to $5 \cdot 10^1 - 10^2$ CFU/ml. Bacterial growth after treatment by various antiseptics is shown in Table 1.

Table 1. The change of Escherichia coli concentration after its treatment by various antiseptics at exposure of 15, 45 seconds.

	NSS (CFU/ml)	OSS (CFU/ml)	CHX (CFU/ml)	HCH (CFU/ml)
Before treatment	$5 \cdot 10^7 - 1 \cdot 10^8$	$5 \cdot 10^7 - 1 \cdot 10^8$	$5 \cdot 10^7 - 1 \cdot 10^8$	$5 \cdot 10^7 - 1 \cdot 10^8$
15 sec	$5 \cdot 10^6$	$5 \cdot 10^5$	$5 \cdot 10^7$	$5 \cdot 10^5$
45 sec	$5 \cdot 10^7 - 1 \cdot 10^8$	$5 \cdot 10^7 - 1 \cdot 10^8$	$5 \cdot 10^5$	$5 \cdot 10^1 - 5 \cdot 10^2$

According to data, obtained in the course of the study, the most effective means for the sanitation was an aqueous solution of carbonated chlorhexidine 0,05%, which at 45 second exposure inhibited growth of E.Coli- ($5 \cdot 10^1 - 10^2$ CFU/ml). This antiseptic preceded the aqueous solution of chlorhexidine 0,05% - inhibited growth of E.Coli ($5 \cdot 10^5$ CFU/ml), and ozonated saline solution and natural saline solution wherein the concentration of E.Coli was ($5 \cdot 10^7 - 1 \cdot 10^8$ CFU/ml) at 45 second exposure. So, the combination of antiseptics with physical factors of impact – saturation by gas components, in particular carbon dioxide, significantly inhibits the growth of E.Coli- ($5 \cdot 10^1 - 10^2$ CFU/ml) compared to antiseptics without combining with the physical impact factors. Among 21 patients with acute pancreatitis, complicated with enzymatic peritonitis, in the process of programmed sanitations, there was used 0.05% aqueous chlorhexidine gluconate solution, saturated with carbon dioxide, showing effectiveness in comparison with usual antiseptics (0.05% aqueous chlorhexidine gluconate solution, ozonized solution of NaCl 0,9 %) for nosocomial E.Coli strains in the experiment. Carbonated chlorhexidine was prepared from aqueous chlorhexidine solution at a concentration of 0,05%, saturated with carbon dioxide by sparging for 5 minutes in 500 ml of hermetically sealed 0.05% aqueous solution of chlorhexidine, to form a fine fraction of antiseptic solution, increasing its productivity. The operation ended with the determination of exudate bacteriological control and drainage of the abdominal cavity, if necessary,

retroperitoneal space, sac lesser omental sac, by domestic drains and drainage of the original design. Programmed sanitation of abdominal cavity in 21 patients with acute pancreatitis was performed while an increase of endotoxemia and enzymatic peritonitis, despite an initial diagnostic and therapeutic laparoscopy. The indications for laparoscopy in our hospital, is progressing pancreatogenic peritonitis, combination of acute pancreatitis with destructive cholecystitis, combination of acute pancreatitis with obstructive jaundice, the need for differential diagnosis with other acute surgical diseases. An absolute contraindication is a terminal condition. As relative, the following contraindications may be considered: unstable hemodynamics, surgeries on the stomach and pancreas, large postoperative ventral hernias. The tasks of laparoscopy on the diagnosis stage were the following: the confirmation of the diagnosis and the morphological form of the disease (the presence of edema, exudate with high activity of amylase, locus of steatonecrosis), revealing of severe pancreatitis signs (hemorrhagic effusion, generalized steatonecrosis, extensive hemorrhagic impregnation), at the stage of treatment - removal of peritoneal exudate, decompression of the retroperitoneal, bile ducts. The criteria for severity of pancreatic necrosis, according to laparoscopy, are the nature of the exudate, retroperitoneal damage, the damaged area. Bacteriological analysis, conducted before and after laparoscopic sanitation showed reduction of bacterial contamination: E.Coli, St. aureus. Only in 38% of cases (8 patients), there was the need to carry out additional surgical

interventions (punctures, drainages, laparotomy, lobotomy) compared with the group of patients who underwent the sanitation with the natural saline solution (63% of patients required repeating surgery). Improving of antibacterial properties of antiseptic is observed not only due to the additional use of physical factors – by saturation of gas, but also due to mechanical impact. Saturation of the solution with gas forms a sufficient amount of bubbles, which allows to increase the area of contact of the solution with an area of the treated surface.

CONCLUSIONS

Thus, the saturation of 0.05% aqueous solution of chlorhexidine by carbon dioxide and its treatment the bacterial suspension for 45 seconds significantly reduces bacterial contamination of E.Coli from $5 \cdot 10^7$ to $5 \cdot 10^1$ - 10^2 CFU/ml, compared to other antiseptics (0,05% aqueous chlorhexidine solution, ozonated saline solution, natural saline solution). The use of this solution among 21 patients, in the process of occasional sanitation of the abdominal cavity in case of progressive enzymatic peritonitis, acute pancreatitis, showed a reduction of bacterial contamination E.Coli, St. Aureus and reduced the need for re-treatment.

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