BIO Web of Conferences **63**, 05006 (2023) https://doi.org/10.1051/bioconf/20236305006
ASE-2023

Study of the Effect of Some Chlorine-Containing Liquids on Bacteria, in Particular E. Coli

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Abstract. The widespread use of chlorine as a disinfectant has led to some research into its effect on bacterial growth and development. It is highly effective against suspended vegetative bacteria as well as some enteric viruses and has minimal effect on the nutritional quality of the product. Chlorine-active substances, which are highly sensitive to pollutants of an organic nature, are destroyed by proteins to a greater extent. It is widely accepted that the use of chlorinated fluids does not lead to the development of antimicrobial resistance. Chlorhexidine is one of the most widely used antiseptics. Chlorhexidine is bacteriostatic at low concentrations and bactericidal at high concentrations, causing cell death by cytolysis. Chlorhexidine has strong biocidal activity against Gram-positive bacteria and weaker activity against Gram-negative bacteria.

1 Introduction

Chlorine is the most used disinfectant. It is highly effective against suspended vegetative bacteria, as well as some intestinal viruses, and has a minimal effect on the food quality of the product [17]. Cl2 is an oxidizing gas that is sparingly soluble in water [8, 12].

Chlorine-containing substances with water form hypochlorous acid:

1) a chlorine molecule in water forms hydrochloric acid: Cl2 + H2O -> HCl + HOCl;

2) hypochlorites decompose in water solutions. NaClO + H2O -> NaOH + HOC1;

3) chloramines decompose in water. Hydrolysis of chloramine B (sodium salt of benzochlorosulfamide C6H5SO2 NClNa) is expressed by the following equation: C6H5SO2 NClNa + H2O -> C6H5SO2NHNa + HOCl;

The following chemical reactions will go with the formation of oxidizing agents such as O2 and Cl2, the same oxidizing agents form disinfectant properties. Chlorine-active compounds have a number of disadvantages: chlorine-active compounds have a toxic effect, they also have a negative effect on the mucous membranes of the eyes and upper respiratory tract, and therefore it is necessary to use a respirator [3]. Chlorine-containing substances decompose and lose their activity even in dry form if stored properly [1]. Chlorine-active substances, which are highly sensitive to pollutants of an organic nature, are destroyed by proteins to a greater extent. Metal tools and equipment widely used in medicine are susceptible to corrosion when treated with preparations containing chlorine [4]. Chlorine gas is highly reactive and causes respiratory toxicity if inhaled. Human

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exposure to chlorine can occur as a result of accidents at work and at home. Large accidental releases of chlorine were the result of a train derailment, resulting in loss of life. Chlorine damages the cells that line the airways, and inhalation of high concentrations of chlorine can lead to acute lung injury characterized by disruption of the epithelial-endothelial barrier, pulmonary edema, pneumonitis, and airway obstruction. Clinical symptoms include dyspnea, cough, hypoxemia, and bilateral infiltrates on chest x-ray [21].

2 Research Methodology

Sodium hypochlorite is a clear yellowish solution with a characteristic chlorine odor and is commonly used as a disinfectant and bleaching agent. It is used in various medical settings due to its fast-acting broad-spectrum antimicrobial activity. It is a known irritant and there are some reports that it can also cause type IV hypersensitivity allergic contact dermatitis [11]. Sodium hypochlorite (NaOCl) is the active ingredient in household bleach and is a very common chemical. It has been used for medical and commercial purposes since the 18th century for its disinfectant properties, including topical use in medicine as an antiseptic. NaOCl is a proven and safe chemical. However, exposure to NaOCl outside of topical applications, whether intentional or accidental, carries significant risks due to its strong oxidizing properties. Potentially damaging scenarios include ingestion, inhalation, tissue deposition, or injection into the bloodstream. All of these scenarios can lead to significant morbidity and even mortality [9].

Sodium hypochlorite has been used in clinical practice for over 70 years for a variety of infectious indications due to its bactericidal, antiviral, and sporicidal properties. It is widely accepted that the use of sodium hypochlorite does not lead to the development of antimicrobial resistance. Unlike other antiseptic preparations, dilute sodium hypochlorite has been found in human and animal studies to be non-toxic to mucosal tissues and surfaces [10].

Chloramine is a colorless to yellow liquid with a strong pungent odor. When chloramine is used as a disinfectant, managing acceptable “residue” in all water distribution systems, especially once nitrification has begun, is challenging. Management of chloramine breakdown prior to nitrification with effective control strategies is essential, and to date strategies developed for nitrification have been ineffective [14].

3 Results and Discussions

Chlorhexidine is one of the most widely used antiseptics. Chlorhexidine is bacteriostatic at low concentrations and bactericidal at high concentrations, causing cell death by cytolysis. Chlorhexidine has strong biocidal activity against Gram-positive bacteria and weaker activity against Gram-negative bacteria. It is also active against yeasts, some dermatophytes and some lipophilic viruses. The most widely used chlorhexidine found in dentistry and antiseptics. However, it can also cause side effects, limiting the time it can be taken. Unfortunately, chlorhexidine exhibits cytotoxic activity against human cells, can cause staining of teeth and fillings, and its activity depends on the pH of the medium and the presence of organic substances [19].

Chlorhexidine digluconate is used as a disinfectant. Chlorhexidine digluconate is considered the “gold standard” biocide with a broad spectrum of activity and is used as a disinfectant and antiseptic. Both gram-positive and gram-negative bacteria are sensitive to chlorhexidine, and the biocide exhibits both bactericidal and bacteriostatic activity depending on the concentration [7]. Minimum inhibitory concentrations (MICs) are commonly used to study or compare the susceptibility of individual microbes to a particular antimicrobial agent [15]. Determining the MIC involves using a dilution range of the biocide to determine the concentration that does not allow microbial growth for an initial inoculation of 1 x 10 6 CFU/mL. A high MIC value indicates that a high concentration of a given antimicrobial agent is required to inhibit microbial growth, so the test isolate is highly resistant to this particular antimicrobial agent [18]. Due to the development of resistance of some microorganisms to biocides, nosocomial pathogens may not be completely eradicated, despite the use of antimicrobials in the disinfection of hospital surfaces and equipment [9].

Materials and methods of research

10 strains of Escherichia coli were studied, distributed as follows:

Objects from which isolated strains of E. coli	Number of strains
Feces of patients	5
Feces of healthy people	3
Soil contaminated with faeces	2

The studied strains were kindly provided by the bacteriological laboratory of the Central Collective Use Center of the Chechen State University named after A.A. Kadyrov. This research work was carried out in the educational and research laboratory of the Chechen State University named after A.A. Kadyrov.

Meat-peptone agar and meat-peptone broth were used as a nutrient medium to obtain a pure culture of Escherichia coli. Incubation was carried out at 370C during the day.

The study used disinfectants belonging to the group of chlorine-containing agents:

1. “Whiteness” (DV: sodium hypochlorite - more than 5%, but less than 15%); manufacturer: Blago LLC, Russia)
2. “Sulfochlorantin D” (DV: dichloranthin - 14% AH; manufacturer: AOA “Orgsteklo”, Russia)
3. “Brilliant” (DV: 0.9 ± 0.1% alkyldimethylbenzylammonium chloride; manufacturer: LLC TsP “Hygiena-Med”, Russia)
4. Chloramine (DV: Chloramine; manufacturer: China)
5. Chlorhexidine (DV: Chlorhexidine bigluconate 0.05%; manufacturer: YuzhFarm LLC, Russia)

According to the methodological recommendations of Fedorov R.V. (1995) experiments were carried out and pure cultures of Escherichia coli were also obtained.

A bacterial loop was prepared for inoculation of E. coli by calcining it over the flame of an alcohol lamp and cooling it in advance. E. coli from the test tube with a bacterial loop was applied to a Petri dish with MPA and evenly distributed. The Petri dish was immersed upside down in a thermostat for 24 hours at a temperature of 37 °C.

To obtain a culture in liquid form, meat-peptone broth (MPB) was prepared. In Petri dishes on meat-peptone agar, E. coli colonies grew - transparent, with a grayish-blue tint. The resulting culture was inoculated into MPB, incubated in a thermostat for 6-18 hours at a temperature of 37°C. In the BCH, the microorganisms gave abundant growth, with a significant turbidity of the medium, a parietal ring was formed, and there was no film on the surface of the broth.

Next, molten MPA was poured into a sterile clean Petri dish. The Petri dish was divided into sectors (1,2,3,4,5). Liquid culture with MPB was evenly distributed over the entire surface of the dish and sent to a thermostat.

The effect of disinfectants on the sensitivity of bacteria was carried out as follows. Working concentrations of disinfectants were prepared, then they were applied drop by drop onto Petri dishes, divided into sectors: (1- “Whiteness”; 2- “Sulfochlorantin D”; 3 -. “Brilliant”; 4- “Chloramine B”; 5- “Chlorhexidine”).

The sensitivity of E. coli to chlorine-containing disinfectants revealed that E. coli obtained from the body of patients are less sensitive to all disinfectants studied (36%), those obtained from the body of healthy people showed medium sensitivity (53%), soil strains were more sensitive (90%).

Table 1. Susceptibility of E. coli strains obtained from the faeces of sick people

Strains (n=5)	"Whiteness" DV: sodium hypochlorite - < 5 %, но > 15%	"Sulfachlorantin" D 1% solution DV: dichloranthin – 14%	"Diamond" DV: 0.9±0.1% alkyldimethylbenzylammonium chloride	"Chloramine B" 1% solution DV: Chloramine	Chlorhexidine DV: Chlorhexidine bigluconate 0.05%	% %
E. coli 1	+	+	-	-	+	60
E. coli 2	-	-	+	-	-	20
E. coli 3	+	-	-	+	-	40
E. coli 4	+	-	-	-	-	20
E. coli 5	-	+	+	-	-	40
Total						**36**

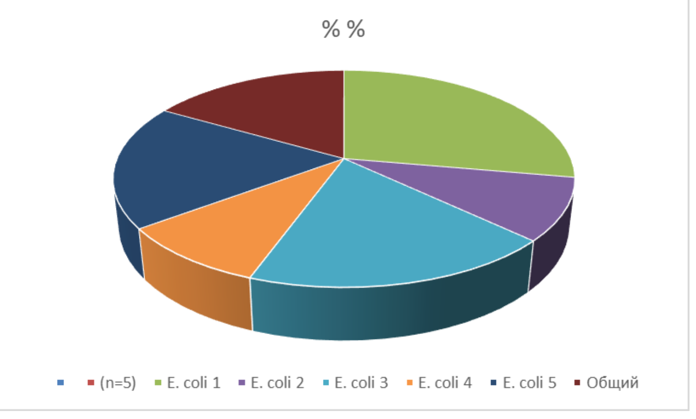

Fig.1. Susceptibility of E. coli strains obtained from the faeces of sick people.

Table 1 and Fig. 1 show that among the strains obtained from the faeces of sick people, 2 strains (E. Coli 2 and E. Coli 4) are sensitive to 20% of the 5 disinfectants presented: E. Coli 2 to the solution "Diamond"; E. Coli 4 to the "Whiteness" solution. 1 strain (E. Coli 1) is sensitive to 60% disinfectants (Whiteness, Sulfachlorantin D, Chlorhexidine). 2 strains (E. Coli 3, E. Coli 5) to 40% disinfectants: E. Coli 3 - to solutions "Whiteness" and "Chloramine B"; E. Coli 5 - Sulfachlorantin D and Brilliant.

Thus, strains obtained from the faeces of sick people showed low sensitivity to all disinfectants studied (36%).

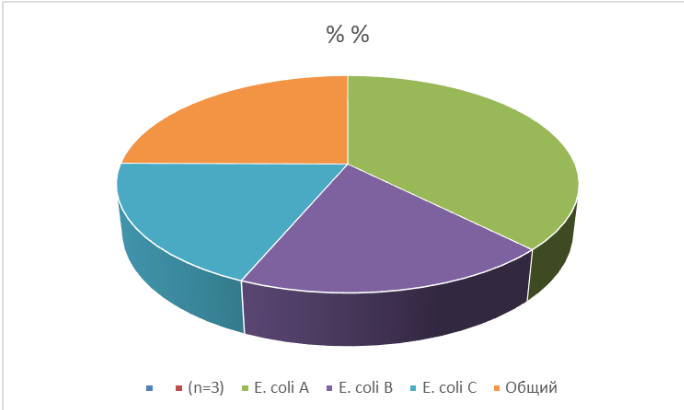

Fig. 2. Susceptibility of Escherichia coli strains obtained from the faeces of healthy people

Table 2. Susceptibility of Escherichia coli strains obtained from soil contaminated with human and animal faeces.

Strains (n=2)	“Whiteness” DV: sodium hypochlorite - < 5 %, но > 15%	“Sulfachlorantin” D 1% solution DV: dichloranthin – 14%	“Diamond” DV: 0.9±0.1% alkyldimethylbenzylammonium chloride	“Chloramine B” 1% solution DV: Chloramine	Chlorhexidine DV: Chlorhexidine bigluconate 0.05%	% %
E. coli A1	+	+	+	+	-	80
E. coli B1	+	+	+	+	+	100
Total						**90**

Table 2 shows that E. coli obtained from soil contaminated with human and animal faeces have the highest sensitivity to disinfectants (90%). 2 strains presented in this group (E. coli A1 and B1) were sensitive to almost all disinfectants (by 80% and 100%, respectively).

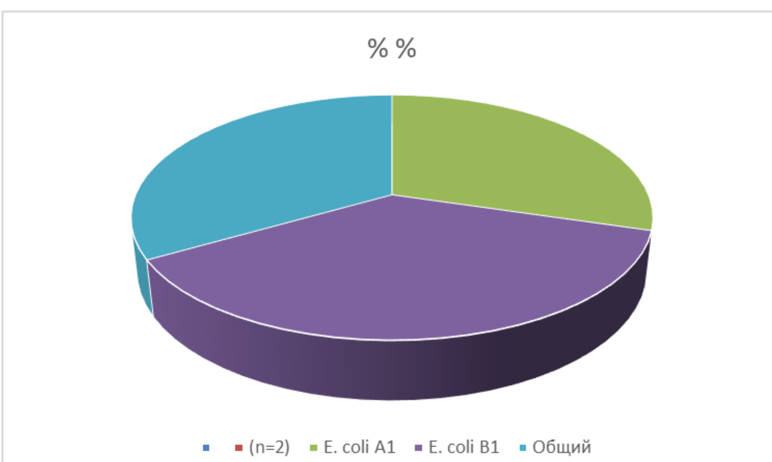

Fig. 3. Susceptibility of Escherichia coli strains obtained from soil contaminated with human and animal faeces.

4 Conclusions

E. coli are the main infectious agents of nosocomial infections, resulting in significant morbidity and mortality in healthcare facilities, as well as additional hospital costs [20]. In a hospital, microbial pathogens can be found on equipment such as endotracheal tubes, catheters, soap dispensers, and stethoscopes [13]. If insufficient disinfection is carried out, contaminated equipment can become the vector of a nosocomial pathogen to a predisposed host. In an attempt to reduce nosocomial infection, preventive mechanisms must be taken to destroy the infectious agent, the means of transmission, and the susceptible patient host. Disinfection and antiseptics using biocides are the main mode of action used to control the growth of nosocomial pathogens [16]. The indiscriminate and misuse of biocides in agriculture, food production, medicine, and personal care has led to the emergence of biocide-resistant microorganisms [5]. Verifying the effectiveness of disinfection is a vital but often difficult task.

It is relevant to use in this aspect in medical institutions precisely those disinfectants that are more affordable, environmentally friendly, but have high bactericidal activity.

The problem of microbial contamination not only of environmental objects, but also of medical equipment, has led to the study of this issue related to the effect of chlorine-containing liquids on bacteria that pollute them, in particular, on E. coli.

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