

Effect of Various Disinfectants Used, on Resistant Bacteria in Our Hospital

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ABSTRACT

BACKGROUND

We wanted to determine the sensitivity status of microorganisms isolated from our patients with regard to the various disinfectants used in our hospital.

METHODS

An in vitro study was performed. The sensitivity of microorganisms against disinfectants was studied by qualitative suspension method. In the study, it was planned to study the sensitivity status of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* with regard to A (2-propanol, ethanol, benzalkonium chloride, glucoprotamine), B (0.55 % orthophthalaldehyde), and C (5 % sodium hypochlorite) on using the following standard strains - *S. aureus* NCTC® 12493, *A. baumannii* ATCC® BAA 1605, *K. pneumoniae* ATCC® 700603, *P. aeruginosa* ATCC® 27853. Bacterial strains were prepared as per turbidity of 0.5 McFarland standard. Microdilution was carried out with disinfectant solutions. Passages were made to the solid medium at the end of 1-, 5-, 10- and 20-minutes contact periods, respectively bacterial growth was evaluated.

RESULTS

A, B were effective at 5th minute against hospital strains and standard bacterial strains. Two hospital strains of *K. pneumoniae* and one hospital strain of *P. aeruginosa* were detected at the first concentrated duration of C. In addition, a hospital strain of *K. pneumoniae* culture positivity was detected at the 5th and 10th minute of contact.

CONCLUSIONS

According to results of the study, disinfection policy must be established in all hospitals so that patients can be protected from hospital infections. Each hospital needs to identify effective antiseptics / disinfectants against microbial flora isolated in their hospital.

KEY WORDS

Disinfection, Disinfectant Effectiveness, Qualitative Suspension Test, Nosocomial Infection

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BACKGROUND

Hospital-acquired infections are an important health problem in developing and developed countries. In 5 - 10 % of the patients admitted to the hospital, a hospital-acquired infection is observed during their treatment.¹ In recent years, hospital-acquired infections caused by multidrug-resistant bacteria increase morbidity and mortality in patients. The transmission of this microorganism is mostly by cross-carriage through the hands of people who are in close contact with the patient, such as healthcare professionals and their relatives.²

The source is hands of healthcare professionals in 20 - 40 % of the transport and spread of microorganisms that show high transmission and multiple drug resistance within the hospital. Decreased compliance with hand hygiene causes changes in hospital flora, increase in hospital infections and emergence of new resistant microorganisms.³ Skin antiseptics used in hospitals are fast-acting and are as effective as hand-washing in maintaining hand hygiene.⁴

The selection and use of appropriate disinfectants is one of the important steps in the prevention of nosocomial infections. For this reason, every hospital should know the sensitivity of microorganisms that causes the most problems to the disinfectants used.⁵ It has been reported in the literature that bacteria develop resistance to phenol, alcohol, chlorine compounds.^{6,7}

The sensitivity of microorganisms to disinfectants varies depending on their structural features. While natural resistance is generally related to the reduction of the disinfectant uptake into the cell; The acquired resistance develops through chromosomal mutations and plasmids.⁸ Tests measuring the effectiveness of disinfectants are divided into two groups according to the test structure: Disinfectant activity measurement tests; determination of minimal inhibitory concentration are suspension tests, capacity tests and application tests. Specific tests are aimed at measuring the effectiveness of surface disinfection, instrument disinfection, hand and skin antiseptics. Suspension tests are the most used, inexpensive, easy to apply and repeatable tests.⁹

In this study, the susceptibility and duration of action on 32 multi-drug resistant bacterial strains and 4 standard strain samples isolated from three disinfectants, which are frequently used in our hospital, were investigated.

METHODS

This in-vitro study was conducted in the Medical Microbiology Laboratory of Health Sciences University Kayseri Training and Research Hospital. Disinfectants used are presented in Table 1, The bacteria used are presented in Table 2. C disinfectant was used without diluting, 1 / 10 diluted (1 ml disinfectant + 9 ml distilled water) and 1 / 100 diluted (1 ml disinfectant + 99 ml distilled water). Final concentrations of neutralizing solution substances were prepared with 3 % saponin, 0.1 % L-cysteine, 0.1 % L-histidine, 3 % Tween 80 with Tryptic Soy Broth (TSB). Sterilized in an autoclave at 121° C for 15 minutes. Culture procedures from blood samples and identification of microorganisms grown in culture were performed in the Microbiology Laboratory of our hospital. Strains detected in blood culture were further identified with

BD Phoenix automated system (Becton Dickinson USA). Strains identified and antibiotic susceptible according to EUCAST criteria were processed.

Disinfectants

A (Incidin Foam®)	2 Propanol, Ethanol, Benzalkonium Chloride, Glucoprotamine
B (Cidex OPA®)	0.55 % Ortho-Phthalaldehyde
C (Power Clean®)	5 % Sodium Hypochlorite

Table 1. Disinfectants Used in the Study

Bacteria	Number of Strain
<i>Acinetobacter baumannii</i>	8
<i>Klebsiella pneumoniae</i>	8
<i>Pseudomonas aeruginosa</i>	8
<i>Staphylococcus aureus</i>	8
<i>A. baumannii</i> ATCC® BAA-1605™	1
<i>K. pneumoniae</i> ATCC® 700603™	1
<i>P. aeruginosa</i> ATCC®27853™	1
<i>S. aureus</i> NCTC® 12493	1

Table 2. Bacteria Used in the Study and the Number of Strains

Sensitivity Study method: The effectiveness of disinfectants on resistant bacteria was done by the qualitative suspension method.^{10,11} 36 bacteria used for the test were planted in TSA and incubated in the oven for 24 hours at 37° C. Bacterial suspensions in 0.5 McFarland turbidity (1.5×10^8 CFU / mL: Colony Forming Unit / millilitre) were prepared with TSB, a 24-hour culture of microorganisms.

1000 microliters (μ L) of disinfectant to be tested for each bacterium were put into the tubes, 10 μ L of the bacterial suspension prepared in 0.5 McFarland turbidity was added to these tubes and kept for 1-5-10-20 minutes. At the end of each period, 100 μ L of bacteria and disinfectant mixture is taken and added to the tubes containing 900 μ L of neutralizing agent, which were prepared separately for each working minute before; 10 μ L of this mixture was taken and inoculated to TSA.

Petri dishes were incubated at 37° C for 48 hours. For control cultivation, solutions without disinfectant were prepared and cultivated in petri dishes. Lack of reproduction at the end of periods was interpreted as disinfectant having a bactericidal effect.

RESULTS

Reproduction status of bacteria after contact times with disinfectants are shown in Table 4. Accordingly, in the disinfectant A, the growth of a hospital strain *K. pneumoniae* was observed during the first minute contact time. The disinfectant A was effective from all hospital strains and standard bacterial strains from the fifth minute.

At the end of the first minute contact to the B disinfectant, the growth of a hospital strain *K. pneumoniae* and a hospital strain *A. baumannii* was monitored. The B disinfectant was effective from the fifth minute against all hospital strains and standard bacterial strains.

When working without diluting to the C disinfectant, the growth of two hospital strains *K. pneumoniae* and one hospital strain *P. aeruginosa* were detected after the first minute of action. Also 5th - 10th reproduction of a hospital strain *K. pneumoniae* with minute contact time was detected. In the 1 / 10 dilution of C disinfectant, two *A. baumannii*, three *K. pneumoniae* and one *P. aeruginosa* growth were observed in the hospital strains during the first minute action time.

Time Bacteria	A				B				C				C 1 / 10				C 1 / 100			
	1 min	5 min	10 min	20 min	1 min	5 min	10 min	20 min	1 min	5 min	10 min	20 min	1 min	5 min	10 min	20 min	1 min	5 min	10 min	20 min
<i>A. baumannii</i> ATCC@BAA-1605™	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>K. pneumoniae</i> ATCC@ 700603™	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
MRSA NCTC@12493	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. aeruginosa</i> ATCC@27853™	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. baumannii</i> (8)	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	6	3	2
<i>K. pneumoniae</i> (8)	1	0	0	0	1	0	0	0	2	1	1	0	3	2	1	1	7	6	6	5
MRSA (8)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5	4
<i>P. aeruginosa</i> (8)	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	5	5	4	4

Table 3. Reproduction of Strains after Bacteria, Contact Time with Disinfectant, and Their Number

A; 2 propanol, Etnanol, Benalcolium Chloride, Glucoprotamine B; 0.55 % Ortho-Phthalaldehyde, C; 5 % Sodium Hypochlorite

In addition, two *K. pneumoniae* strains from the hospital strains and one *K. pneumoniae* growth were observed at the 10th, 20th minute contact time. From the bacteria studied at 1 / 100 dilution of C disinfectant, *K. pneumoniae* standard strain, six hospital strains *A. baumannii*, seven hospital strains *K. pneumoniae*, five hospital strains MRSA (Methicillin-Resistant *Staphylococcus aureus*) and five hospital strains *P. aeruginosa* growth were detected. During the fifth minute contact times, three hospital strains *A. baumannii*, six hospital strains *K. pneumoniae*, five hospital strains MRSA and five hospital strains *P. aeruginosa* growth were detected. During the tenth minute contact times, two hospital strains *A. baumannii*, six hospital strains *K. pneumoniae*, five hospital strains MRSA, four hospital strains *P. aeruginosa* growth were detected. Twenty-minute contact times revealed growth of one hospital strain *A. baumannii*, five hospital strains *K. pneumoniae*, four hospital strains MRSA and four hospital strains *P. aeruginosa*.

DISCUSSIONS

According to surveillance data of our hospital, there were 326 bacteraemia cases in the Anaesthesia and Reanimation Intensive Care Unit in 2016. Reproductive contamination in sixty blood cultures, 52 % of the 266 factors found growth in blood culture, gram negative, 44 % gram-positive bacteria, 4 % yeast fungus reproduced. Gram negative bacteria were *A. baumannii* (47 %), *Klebsiella* spp (23 %), *P. aeruginosa* (9 %), respectively. Gram-positive bacteria are CNS (Central Nervous System) (62 %), *Enterococcus* spp (20 %), *S. aureus* (16 %).

The effectiveness of the disinfectant; Use according to the prospectus or non-prospectus changes, use with different concentrations, different contact times.¹² Disinfection of the environment and materials with the right disinfectant and applications can prevent the emergence of the hospital infection. In order to achieve this result, it is necessary to demonstrate that disinfectant is effective against microorganisms that can be found in the hospital environment with reliable tests, and the application method and application concentrations can be determined accurately.^{13,14}

Bleach contains 5.25 % hypochlorite, i.e. 52,500 ppm free chlorine; Reconstitution of the bleach 1: 100 provides about 525 ppm of free chlorine. CDC (Centers for Disease Control and Prevention) on non-porous surface, < 10 ml splash: 1: 100 dilution (prepared with 5.25 % sodium hypochlorite), > 10 mL, if large splash or large lab-based splash; To reduce the risk of infection during the procedure, it is recommended to apply a 1:10 dilution before cleaning and terminal disinfection with a 1: 100 dilution after this decontamination.¹⁵

Sodium hypochlorite (< 5 %) used in our hospital, without dilution, showed that growths were detected in the patient isolates at the end of a 10-minute encounter, and that it was necessary to contact all bacteria for at least 20 minutes. It was observed that it was not effective in the contact periods followed in dilutions. There is a need for new research for this.

In another study, the direct and 1 / 10 diluted form of sodium hypochlorite (5 %) was found to be effective from the 1st minute, and the growth was detected in both standard bacteria and patient isolates at 1 / 100 dilution after 2 minutes of exposure. It suggested that it should be applied for at least 5 minutes.¹¹

In a similar study, the efficacy of five disinfectants (phenolic compounds, Didecyl-Dimethyl-Ammonium Chloride (DDAC), sodium hypochlorite, Isopropanol + Ammonium Compounds (IACs), hydrogen peroxide) against 187 bacterial strains containing clinical isolates and thirty *P. aeruginosa* strains taken from hospital water samples that were environmental isolates were examined. Hydrogen peroxide has been shown to be effective against both clinical and environmental strains. IAC and DDAC are only effective on gram positive bacteria; Sodium hypochlorite and phenol compounds were not effective on any strain.¹⁶

In another study, 20 *P. aeruginosa*, 20 *Acinetobacter* spp and 20 *K. pneumoniae* strains reported as nosocomial infection agents were studied with sodium hypochlorite. The effects of sodium hypochlorite at 1 / 10, 1 / 100 and 1 / 1000 dilutions were investigated after contact times of 1, 2, 5, 10 and 30 minutes. In sodium hypochlorite 1 / 10 dilution, growth was detected in *Acinetobacter* spp. in only 1 minute. Sodium hypochlorite was found effective in 1 / 100 dilution after 2 minutes of contact with *P. aeruginosa*, 5 minutes of contact with *K. pneumoniae* and 10 minutes of contact time with *Acinetobacter*.

CONCLUSIONS

As a result, it was determined that the disinfectants we used in our study had different effects against standard strains and hospital strains, and some of them did not have any effect. When choosing a disinfectant, selection should be made by considering factors such as toxicity, ease of use, storage conditions, stability, recommended use dilution, application time and price. In the selection of disinfectants in every hospital, disinfectants with questionable effects should be avoided, especially in the surface disinfection of critical equipment and high-risk areas. It will be more beneficial for each hospital to determine the effective disinfectants and

antiseptics against its own microorganisms in preventing hospital infection.

Limitations

Advanced analysis for selected hospital strains is required. Use of quantitative testing would be more suitable for disinfectant effectiveness.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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