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Antimicrobial Susceptibility Pattern of Newly Formulated Disinfectants Against Pathogenic Bacterial Contaminants in Different Veterinary Research Laboratories in Beni-Suef City, Egypt

Mohamed A. El Bably, Asmaa N. Mohammed*, Manar Bahaa El Din Mohamed and Dina A. Mohamed

Department of Hygiene, Zoonoses and Epidemiology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt,

Abstract

IN VETERINARY research facilities, antimicrobial disinfectants are thought to be the primary line of protection against any harmful bacteria on various inanimate surfaces to aid in the prevention of healthcare association infections (HAIs). The study goals were to estimate the prevalence rate of bacterial pathogens in the surrounding environment of veterinary research facilities, assess the antimicrobial pattern of newly formulated disinfectants (Sporocide Glu®, Cox killer®, and Klorsept 25®) and two antiseptics (ethyl alcohol 70% (w/v) and chlorohexidine HCL (125mg/100ml)) against all isolated bacterial pathogens, and establish a control strategy for preventing the spread of bacterial contaminants to researchers and the lab environment. To isolate and identify pathogenic bacteria from the lab surrounding environment, a total of 236 swab samples were taken from the lab environment (n = 149), equipment (n = 57), and lab researchers (n = 30) in the seven research veterinary laboratories. The agar-well diffusion assay was used to evaluate the sensitivity profile of thirty strains of bacterial isolates to various disinfectants and antiseptics under investigation. Results, the most common bacterial isolates in all lab environmental samples, including switches, fans, benches, doors, floors, containers, and basins, were *E. coli* and *S. aureus* (35.5% each). The largest rate of coagulase negative staphylococci (*CNS*) isolates was found on fume hoods, refrigerators, and incubators. The most predominant bacterial strains from researcher shoes were Escherichia coli (*E. coli*) and Staphylococcus aureus (*S. aureus*), which accounted for 50% each, 40% from coveralls, and 30% from hands, respectively. At 0.7 and 1.0% concentrations, SG® disinfectant exhibits 100% biocidal action against *S. aureus*, *CNS*, *Klebsiella* spp., and *Pseudomonas* spp. Oppositely, hydrogen peroxide (H_2O_2) was 100% effective against all bacterial isolates, except for of *S. aureus*, which was 83.3% effective at the highest dose tested (6.0%). In conclusion, the environment and laboratory equipment are potential sources of contamination when there is a large concentration of bacterial contaminants. Sporocide Glu® (1%), Klorsept 25® (0.4 mg/l) disinfectants, and chlorohexidine HCL (125 mg/100 ml) antiseptics proved their bactericidal action (100%) against all bacterial isolates in the surrounding environment of labs.

Keywords: Bacterial contaminants, Antimicrobial profile, newly disinfectants, Research laboratories.

Introduction

The lab environment is subjected to a multitude of contaminants, including microbes. These tiny creatures have carved out a large ecological niche for themselves, allowing organisms to exist in a variety of indoor microhabitats. This provides us with a complicated ecosystem that necessitates a deeper comprehension [1]. Animal research institutions' contamination by microbes is turning into a serious worldwide problem. There is potential for treating certain laboratory-acquired infections (LAI) and hospital-acquired illnesses by characterizing these microbial pollutants. Healthcare workers, especially technicians, are primarily exposed to infections in these labs. Microorganisms on benches, floors, media, and equipment can be caused by a variety of factors, including humidity, temperature, the kind of nutrient media used in the lab, and storage conditions for the media. Consequently, it is crucial to identify, isolate, and determine the microbial

*Corresponding authors: Asmaa N. Mohammed, E-mail: asmadel82@yahoo.com Tel.: 01227525459
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origins when performing typical microbiological manipulations [2].

Infectious pathogens can be transmitting directly through contact, injection, inhalation, or ingestion. These agents include parasites, viruses, fungi, and bacteria (including *S. aureus, E. coli*, *Klebsiella* spp., and *Pseudomonas* spp.). LAI (Laboratory-acquired infection) is a significant concern in biosafety of labs for pathogenic microorganisms. It aims to protect laboratory workers from potentially harmful pathogens and avoid the spread of communicable diseases [3]. The *Pseudomonas* species of bacteria are among those that are most dangerous to human and animal health. Consequently, a precise cleaning process is needed to stop the spread of illnesses linked to *pseudomonas* in both humans and animals [4]. *S. aureus* is a significant Gram-positive bacterial pathogen on a global scale because of its ability to produce toxins that cause gastrointestinal illnesses [5]. On the other hand, *Klebsiella* spp., are important human bacterial pathogens that can result in both opportunistic nosocomial infections and community-acquired illnesses. As a result, they seriously threaten public health [6].

Antiseptics and disinfectants used in veterinary laboratories are crucial for the management of infectious agents, such as zoonotic and antibiotic-resistant pathogens, in addition to being used for biosecurity and biosafety goals. Reduce or stop the growth of bacteria and other pathogens that could cause infectious diseases in humans and animals when cleaning surfaces or items to a level that is considered safe for the health of the general population [7]. Therefore, consideration must be given to the disinfectants' safety, efficacy, and simplicity of washing when selecting which ones to use [8]. Disinfectants work together on different target areas to dehydrate bacterial cells (ethyl alcohol 70%), denaturate bacterial proteins (glutaraldehyde), release emerging oxygen (such as hydrogen peroxide and Klorsept 25®) and damage the bacterial cell membrane (chlorohexidine Hcl). The number of microorganisms in the environment is decreased by this procedure [9].

Methods for disinfectant testing are required for efficacy, safety, and quality control. Furthermore, there are several methods for evaluating disinfectant efficacy; nevertheless, the diffusion strategy is the most commonly used. This process involves creating wells in the contaminated agar and filling them with the right disinfectant. Different disinfectants were tested against bacteria recovered from human samples, equipment, and the environment using the agar well diffusion method [10]. Two crucial goals are accomplished with the application of aseptic procedures and other appropriate microbiological precautions. These include keeping the laboratory clean from organisms handled there and keeping the operation clean from organisms in the surrounding environment. These include employing manipulation techniques that lessen the possibility of producing aerosols and keeping the laboratory tidy and orderly. Furthermore, the number of infections connected to medical care has been successfully decreased by infection prevention strategies [11]. Thus, the main goals of this work are to ascertain the bacterial pathogens' frequent distribution in the veterinary laboratories' surrounding environment, evaluate the susceptibility pattern of the isolated pathogens to newly formulated disinfectants besides antiseptics used in research laboratories, and develop a control strategy for preventing the spread of bacterial contaminants to the researchers and the lab environment.

Material and Methods

Study location and frame time

This study was conducted in seven research veterinary laboratories in the Beni-Suef province of Egypt (coordinates: 29° 04' N-31° 05' E) throughout the period from April 2023 to February 2024. The labs under investigation had expertise in pathology, animal hygiene, fish diseases, poultry diseases, parasitology, virology, and microbiology. The investigated laboratories' biosafety level and sanitary measures were deemed acceptable.

Sampling

Using sterile cotton swabs moist in treptone soya broth, a total of 236 samples were taken from the lab environment (n = 149; includes all switches, fans, benches, doors, floors, containers, and basins), equipment (n = 57; includes biosafety cabinets, incubators, hot air ovens, fume hoods, balances, PCR, microscopes, fridges, and deep freezers), and lab researchers (n = 30; includes hands, coveralls, and shoes) in the seven research veterinary laboratories according to methods described by [12].

Isolation and identification of bacterial pathogens in labs environment

To identify bacterial infections such as *E. coli, S. aureus, pseudomonas* species, and *Klebsiella* species, all swabs were obtained from the lab environment, equipment, and researchers. For both E. *coli* and *Klebsiella* spp. isolation, samples were looped from each tube exhibiting turbidity onto MacConkey Lactose Agar (Oxoid, Basingstoke, UK) plates after being enriched on tryptic soya broth (Oxoid, Basingstoke, UK) at 37°C for 18–24 hours. Brown [13] detailed the process of streaking colonies of lactose-fermenting pink and smooth onto Eosin Methylene Blue (EMB: Oxoid, Basingstoke, UK) agar plates. The putative colonies were selected for additional identification based on their physical shape. In order to isolate *staphylococci* spp., samples were enhanced at 37°C for 18-24 hours on tryptic soy broth (Oxoid, Basingstoke, UK). Thereafter, the Baird-Parker agar (Becton Dickinson and Co.,

Sparks, MD) plates were streaked with loopfuls from each tube exhibiting turbidity, and the plates were then incubated for 48 hours at 37°C. distinctive colonies that emerged [14]. A solid selective medium called cetrimide agar is used to separate and identify *pseudomonas* from various surfaces and materials. Based on cultural, morphological, and biochemical testing, the isolates of the chosen strains were identified [15]. On the other hand, urease testing, Voges-Proskauer and citrate utilization, methyl red, and indole formation were among the biochemical tests (HiMedia Rapid Biochemical Identification Kit) that were employed for bacteriological identification [16,17]. In the meantime, S. aureus was identified using a slide coagulase test. On a glass plate that had been cleaned, one drop of the bacterial solution and one drop of citrated rabbit plasma (Baltimore Biological Laboratories, Cockeysville, MD) were combined. After gently rocking the slide for five to ten seconds, clumping was found [18].

Assessing the susceptibility pattern of pathogenic bacteria to different tested compounds

The sensitivity profile of thirty strains of bacterial isolates to several investigated disinfectants and antiseptics was assessed using the agar well diffusion assay. Disinfectants that are tested include hydrogen peroxide (H_2O_2 6%, Pure-Misr, Egypt), Klorsept 25® (sodium dichloroisocyanurate, Medentech, (Ireland); Sporocide Glu (SG®) [glutaraldehyde 20%, benzalkonium chloride 12%, pin oil 4%, and trepeniolin 2.5%, High Kim for chemical and disinfectants, Egypt], and Cox Killer® (glutaraldehyde, benzalkonium chloride, and sodium orthoborate, High Kim for chemical and disinfectants, Egypt). Tested antiseptics include ethyl alcohol 70% (w/v), Medimix, Egypt, and chlorohexidine HCL (125gm/100ml, the Arab Drug Company (ADCO), Egypt). Following the manufacturer's instructions, all disinfectants were assessed at the suggested concentrations.

Antimicrobial activity assay of tested compounds against all bacterial pathogens In-vitro

All data from the questionnaires was assembled in the susceptibility pattern of four disinfectants at varying concentrations [Klorsept 25® (0.2, 0.3, and 0.4mg/L), SG® (0.5, 0.7, and 1.0%), Cox killer® (0.5, 0.7, and 1.0%), hydrogen peroxide (3.0 and 6.0%) is commonly used in the disinfection of veterinary research laboratories. In addition, two antiseptics [ethyl alcohol 70% (w/v) and chlorohexidine HCL (62.5 mg/100 mL and 125mg/100ml)] that are used for hand washing were assessed. The susceptibility testing was done using an agar-well diffusion assay, as reported by [19, 20] with slight modifications. Distilled water was used to create the test dilutions of all antiseptics and disinfectants. The bacterial suspensions that were seeded onto Muller-Hinton agar (Oxoid, Basingstoke, UK) at 6 mm agar depth was match with a 0.5 MacFarland tube. Prior to reading, wells were filled with the appropriate disinfectants at varying concentrations and incubated upside-down for the entire night at 37°C. The wells were then excavated using a sterile well puncher 6 mm in diameter. The inhibition zones were interpreted in accordance with [20] because the particular disinfectants lack defined cutoff values. Measures of diameter ≤ 10 mm were classified as resistant (R); measures larger than 10 mm were classified as susceptible (S).

Data analysis

All the data collected was assembled for statistical analyses using SPSS, version 26. The distribution of all bacterial isolates from various laboratory samples was examined using the non-parametric Chi-square test. Besides, the susceptibility patterns of different tested disinfectants and sanitizers against all bacterial isolates. Data on the inhibition zone (mm) of testing sanitizers and disinfectants against bacterial isolates from research labs were analyzed using the one-way ANOVA test. Statistical significance was determined by considering a *P*-value of < 0.05.

Results

The different collected samples from all investigated veterinary research laboratories (n=7) as shown in Table 1, clarified that the total examined samples from different labs environment, equipment and researchers was 236. In addition, the total positive (%) of all collected labs samples was 70.7% (167/236). The labs environment had the highest percentage of positive samples (73.1%; 109/149), followed by equipment (66.6%; 38/57) and researchers (66.6%; 20/30) at $\chi 2 = 119.86$, and $P \leq 0.05$.

Distribution of isolated bacteria from different collected samples of the labs environment in Table 2 exhibited that the most predominant bacterial isolates were *S. aureus* and *E. coli* (53/149; 35.5% each), followed by *CNS* (35/149; 23.5%), *Klebsiella* spp. (31/149; 20.8%), and *Pseudomonas* spp. (20/149; 13.4%) in all lab environmental samples. Furthermore, the highest percentages of *E. coli* were isolated from floors, and benches (13/22; 59.0% and 26/60; 43.3%, respectively), followed by basins, and switches (7/20; 35.0% and 3/14; 21.4%, respectively). Meanwhile, *Staph aureus* was isolated from the doors, floors, and benches (7/14; 50%, 10/22; 45.4%, and 17/60; 45.0%, respectively) in the highest percentages followed by the containers (3/10; 30.0%). CNS isolates showed their existence on floors, doors, and benches at a high rate (7/22; 31.8%, 4/14; 28.5%, and 14/60; 23.3%, respectively). Oppositely, the high rate of *Klebsiella* spp. was isolated from doors (7/14; 50%), containers (3/10; 30.0%), and basins (5/20; 25.0%). *Pseudomonas* spp.

was isolated from basins, benches, and floors (4/20; 20.0%, 11/60, 18.3%, and 4/22; 18.1%, respectively).

Distribution of isolated bacteria from different collected samples of equipment in Table 3 clarified that the most predominant bacterial isolates were *E. coli (*16/57; 28%**)** followed by *S. aureus* and *Pseudomonas* spp. *(*11/57; 19, 2% each). Meanwhile, *CNS* was 10/57; 17.5% and *Klebsiella* spp. was 8/57; 14.0% in all equipment samples. Furthermore, the highest percentages of isolated *E. coli* from biosafety cabinets, followed by deep freezers was 3/4;75.0% and 3/5; 60%, respectively, then balances, and microscopes (1/2; 50.0% and 3/7; 42.8%, respectively). Meanwhile, isolated *S. aureus* from biosafety cabinets, microscopes, and fridges was 2/4; 50%, 2/7; 28.5%, and 4/19; 21.0%, respectively in the highest percentages, followed by deep freezers (1/5; 20.0%) and incubators (2/12; 16.6%**).** CNS isolates showed their existence on fume hoods, fridges, and incubators at the highest rate (1/2; 50.0%, 5/19;26.3%, and 3/12;25.0%, respectively). Oppositely, the high rate of *Klebsiella* spp. was isolated from fridges (5/19; 26.0%), deep freezers (1/5; 20.0%) and microscopes (1/7; 14.3%). The highest percentages of *Pseudomonas* spp. were isolated from deep freezers (3/5; 60.0%), followed by fume hoods, PCR, and balances (1/2; 50.0% each).

Distribution of isolated bacteria from collected researchers' samples in Table 4 clarified that the most predominant bacterial isolates were *S. aureus,* followed by *E. coli* (13/30; 43.3% and12/30; 40.0%, respectively). While *CNS, Klebsiella* spp., and *Pseudomonas* spp. were (4/30; 13.3%, 1/30; 3.3%, and 8/30; 26.6%, respectively) in all researcher's samples. In addition, the highest percentages of *E. coli* were removed from shoes, coveralls, followed by hands (5/10; 50.0%, 4/10; 40.0%, and 3/10; 30.0%, respectively). Meanwhile, the highest level of *S. aureus* was isolated from shoes (5/10; 50.0%) followed by coveralls, and hands (4/10; 40.0%). Moreover, *CNS* isolates showed their existence on shoes at a high rate (3/ 10; 30. 0%). Oppositely, the highest level of *Klebsiella* spp. was isolated from coveralls (1/10; 10.0%). *Pseudomonas* spp. was isolated at a high rate from shoes, and coveralls (3/10; 30.0% each), followed by hands (2/10; 20.0%).

The biocidal effect of testing disinfectants (Klorosept 25®, Cox killer®, SG®, and H_2O_2) and antiseptics (ethyl alcohol, and chlorohexidine HCL) against all bacterial isolates from different investigated samples in Table 5 exhibited that both *E. coli, and CNS* isolates were highly sensitive (100%) to Klorosept 25® at both concentrations of 0.3 mg/l and 0.4 mg/l, followed by *Klebsiella spp.,* and *Pseudomonas* spp. (66.6% each). The sensitivity pattern of each bacterial isolate (*CNS, Klebsiella* spp.*,* and *Pseudomonas* spp.) to Cox Killer® disinfectant was not exceeded by 33.3 % at the highest tested concentrations of 0.7%, and 1.0%. On the other hand, the biocidal activity of testing SG® disinfectant was 100% at 0.7 and 1.0% against *S. aureus, CNS, Klebsiella* spp.*,* and *Pseudomonas* spp. while its biocidal effect against *E. coli* was not exceeded *by* 50.0%. Oppositely, the effectiveness of hydrogen peroxide (H_2O_2) against all bacterial isolates was 100%, except *S. aureus* which was 83.3% at the highest tested concentration of 6.0%. On the other hand, the efficacy of antiseptics such as ethyl alcohol 70% against *Pseudomonas* spp. was 100%, followed by CNS (66.6%) and *S. aureus* (50.0%), while the sensitivity of both *E. coli* and *Klebsiella* spp. wasn't exceeded by 33.3 %. Oppositely, chlorohexidine HCL proved its bactericidal effect (100%) against all bacterial isolates at 125mg/100ml at $P \leq 0.05$.

The inhibition zone (mm) of testing disinfectants against different bacterial isolates was significantly noticeable, as shown in Table 6 and Fig. 1. The susceptibility pattern of bacterial pathogens (*E. coli, S. aureus, CNS, Klebsiella* spp.*,* and *Pseudomonas* spp.) to Klorosept 25® disinfectant was clear, whereas the inhibition zone for both *E. coli* and *Pseudomonas* spp. was 47.5±0.33 and 45.0±0.20 mm, respectively, followed by *Klebsiella* spp. (30.0±0.11 mm*)*, and *S. aureus* (27.0±0.05 mm) at a concentration of 0.4mg/l. The susceptibility pattern of bacterial pathogens (*E. coli, S. aureus, CNS, Klebsiella* spp.*,* and *Pseudomonas* spp.) to Cox Killer® disinfectant, the inhibition zone of *Pseudomonas* spp. was 46.2±0.23 mm, followed by *CNS,* and *Klebsiella* spp. (30.0±0.15 and 30.0±0.11 mm, respectively) at a concentration of 1.0 %. The sensitivity of bacterial pathogens to Sporocide Glu® disinfectant was obvious, whereas the zone size for *S. aureus* was 45.0±0.08 mm, followed by *CNS* and *Klebsiella* spp. (37.5±0.04 and 37.5±0.27 mm, respectively). In addition, *E. coli* and *Pseudomonas* spp. were 30.0±0.03mm each at a concentration of 1.0 %. The susceptibility of bacteria to H_2O_2 disinfectant showed the inhibition zone for *E. coli* was 45.1±2.4 mm followed by *Pseudomonas* spp. and *S. aureus* (43.5±2.3 and 40.0±1.4 mm, respectively). As well, *CNS* and *Klebsiella* spp. were 37.5±0.01, and 37.5± 0.16 mm, respectively at a highest concentration of 6.0 %. The bacterial pathogens sensitivity to ethyl alcohol 70% revealed the diameter of zone for both *CNS* and *S. aureus* was 30.0±0.0 and 20.0±0.03 mm, respectively followed by *Pseudomonas* spp. and *Klebsiella* spp. (17.5±0.0, and 15.0±0.21 mm, respectively). Furthermore, *E. coli* was 10.0±0.0 mm. For chlorohexidine HCL disinfectant at a concentration of 125gm/100ml, the inhibition zone for both *E. coli* and *S. aureus* was 35.0±0.01and 30.0±0.03mm, respectively, followed by *CNS, Pseudomonas* spp. (27.5±1.04, and 25.0±0.11 mm, respectively), and *Klebsiella* spp. (22.5±0.04 mm) at $P \leq 0.05$.

Discussion

In light of the one Health concept, training on the dynamic and complex indoor microflora's variation and density are influenced by the sources and related environmental conditions. The permissible thresholds for microbiological pollutants in indoor environments are not standardized. Inhaling germs in an indoor environment can cause microbial infections, allergies, and cancer, among other respiratory disorders [1]. Microbes are found in all areas of the environment and are involved in a variety of settings, including laboratories. Microbial contamination is a significant worldwide obstacle for researchers working with microbial cultures. It might lose valuable strains from the lab. A microbiological lab may practice high microbial contaminants as a result of improper management. It is a widespread health concern that makes it challenging to obtain reliable research results. It harms the caliber of our job when it is mechanically or methodically introduced into our society [21]. The current study exhibited the frequent distribution of bacterial pathogens in labs surrounding environment in veterinary laboratories and it has been found that the most predominant bacterial isolates were *E. coli*, and *S. aureus* (35.5% each), followed by *CNS* (23.5%), *Klebsiella* spp. (20.8%), and *Pseudomonas* spp. (13.4%) in all lab environmental samples include switches, fans, benches, doors, floors, containers and basins. Moreover, the highest percentages of *S. aureus and E. coli* were isolated from floors, and benches. *CNS* isolates showed their existence on floors, doors, and benches at a high rate. Oppositely, the high rate of *Klebsiella* spp. was isolated from doors, containers, and basins. As well, *Pseudomonas* spp. was isolated from basins, benches, and floors. Halatoko et al. [22] clarified that the most contaminated sites in laboratory were basins (66.6%), followed by lab benches (61.9%), refrigerator door handles (47.6%) and the percentage of *Klebsiella* spp. contaminants on surfaces was 44.3%. Ghayoor et al. [23] showed that bacterial contaminants in different areas of microbiological laboratory include tables, floors were exhibited the most common bacterial isolates was *S. epidermis* (36.36%) followed by *B. subtilis* (18.18%). Furthermore, the current results were in accordance with [24] who found that the prevalence rate of bacterial strains isolated from both door locks, and working benches in the clinical lab were (*S. aureus* (26%), *E. coli* (22%), *CNS* (8%), *P. aeroginosa* and coliforms (4% each). Meanwhile, The *Pseudomonas* spp. prevalence was higher in all floor sampled sites at 23.50% than *Shigella* spp. 11.71% [2].

The frequent distribution of bacterial isolates from different lab equipment clarified that the *CNS* isolates showed their existence on fume hoods, fridges, and incubators at the highest rate. *E. coli* was isolated from biosafety cabinets, followed by deep freezers, balances, and microscopes in the highest rate. *S. aureus* was also isolated from biosafety cabinets, microscopes, and fridges in the highest percentages. Conversely, a high percentage of *Klebsiella* spp. was isolated from fridges, deep freezers, and microscopes. *Pseudomonas* spp. were isolated from deep freezers (3/5; 60.0%), followed by fume hoods, PCR, and balances (Table 3). Ayalew et al. [25] found that the most widespread bacterial isolates in lab fomites were *S. aureus, K. pneumoniae,* and *E. coli* (57.6%, 19.2%, and 6.4%, respectively). Meanwhile, Salim [26] stated that the incidence rate of bacterial isolates from biological lab fomites was *S. aureus* (58.57%) and *S. epidermidis* (26.84%), followed by *Klebsiella* spp. (11.98%), and *Protus* spp. (4.29%). The highly varied distribution of bacteria relative to the region suggests that the occurrence of fomites is mostly dependent on personnel to the greatest extent, which could explain these results [27]. Oppositely, MOSE [2] revealed that the incubator had the highest percent of *S. aureus* (50%), followed by *B. subtilis* (12.5%). Biosafety cabinets showed *pseudomonas* spp. (26.60±2.52%) and *S. aureus* (2.80±1.16%).

Handling blood or any other biological sample puts lab workers at risk for exposure or unintentional harm. Workers in laboratories, whether in the public or commercial sectors, are always at risk of contracting an occupational infection due to their constant exposure to known or undiscovered microorganisms [28]. The frequency of pathogenic bacterial isolates from lab researchers' in Table 4 illuminated that *E. coli* and *S. aureus* were the most predominant bacterial isolates from shoes (50% each), coveralls (40% each), followed by hands (30%, and 40%, respectively). Moreover, *CNS* isolates showed their existence on shoes at a high rate. Oppositely, the highest rate of *Klebsiella* spp. was isolated from coveralls (10.0%). *Pseudomonas* spp. was isolated at a high rate from shoes, and coveralls (30.0% each), followed by hands (20.0%). Regarding these findings, Margarido et al. [29] clarified that the most popular bacterial isolates from clothes and coveralls swab samples were *S. aureus and S.* epidermidis (21.5% and 50%, respectively). Gurjeet et al. [30] found that the majority of pathogenic bacteria that were isolated from the hands of workers were *S. aureus,* and *CNS* (40.58%, and 21.74%, respectively), followed by *P. aeruginosa* (8.70%). Additionally, Pegu et al. [31] showed that the most predominant bacterial isolate from participant hands was *S. aureus* (12%). Halatoko et al. [22] revealed that *Staphylococcus* spp. was isolated at the highest rate from staff hands, followed by *Klebsiella* spp., and *E. coli* (75%, 15%, and 5%, respectively).

Cleaning and disinfecting equipment and surroundings helps to disrupt the transmission chain of these agents by preventing the growth of harmful

germs and the buildup of contaminants [32]. Disinfectants are the primary treatment choices against pathogenic bacteria on surfaces in medical facilities because they are broad-spectrum antimicrobials [33]. In clinical labs and healthcare facilities, popular antimicrobials used for disinfection of inanimate surfaces include hydrogen peroxide, quaternary ammonium compounds (QATS), and chlorine-based solutions [34, 35]. The primary determinant of disinfection action is the type of bacteria that the disinfectants target. Because of this, bacterial strains utilized in experiments to evaluate the efficacy of disinfectants ought to be typical of the bacterial community**.** This is accomplished by employing *S. aureus* and *E. coli* as food contamination indicator strains [36].

The biocidal effectiveness of testing disinfectants and antiseptics against all bacterial isolates from various investigated samples in the veterinary laboratories (Tables 5 and 6) exhibited that Klorosept 25® disinfectant has a biocidal activity (100%) against both *E. coli, and CNS* at both concentrations of 0.3 mg/l and 0.4 mg/l. whereas the inhibition zone for both *E. coli* and *CNS*. was 47.5±0.33 and 42.3±0.15 mm, respectively, at a concentration of 0.4mg/l. Whilst, Cox Killer® disinfectant exhibited that its efficiency against *CNS, Klebsiella* spp*.,* and *Pseudomonas* spp. was not exceeded by 33.3 % at the highest tested concentrations of 0.7% and 1.0%. The inhibition zone of *Pseudomonas* spp. was 46.2±0.23 mm, followed by *CNS,* and *Klebsiella* spp. (30.0±0.15 and 30.0±0.11 mm, respectively) at 1% concentration. As well, the biocidal activity of testing SG® disinfectant was 100% against *S. aureus, CNS, Klebsiella* spp*.,* and *Pseudomonas* spp. at 0.7 and 1.0% concentrations whereas the zone size for *S. aureus* was 45.0±0.08 mm, followed by *CNS* and *Klebsiella* spp. (37.5±0.04 and 37.5±0.27 mm, respectively). Oppositely, the effectiveness of hydrogen peroxide (H_2O_2) against all bacterial isolates was 100%, except *S. aureus,* which was 83.3% at the highest tested concentration of 6.0%. The inhibition zone for. *S. aureus* (40.0±1.4 mm) at the same concentration. Mohammed et al. [37] revealed that the Klorsept 25® disinfectant had biocidal activity (100%) against *E. coli, K. pneumonia, S. garoli*, *S. kentucky*, and *Shigella* spp. at 2.0 mg/l and 180 min contact time. In addition, all bacterial isolates were susceptible (100%) to H_2O_2 disinfectant at 5.0 % and 60 min contact time, compared to its efficacy which wasn't exceeded 87.5% at 3% concentration within the same contact time. Montagna et al. [38] pointed out that the only disinfectant that is effective against *P. aeruginosa* strains observed in both clinical and environmental settings is H_2O_2. Furthermore, hydrogen peroxide vapour seems to be extremely close to the perfect disinfectant because of its effectiveness against a number of pathogens, safety, and lack of toxicity issues [39]. The OH radical, which is produced when hydrogen peroxide breaks down in the presence of catalysts such as iron and copper ions, which are frequently present in microorganisms, is responsible for hydrogen peroxide's biocidal action. The microorganism's membrane, DNA, and other biological components are targeted by the radical through an oxidative mechanism [40]. Additionally, Ríos-Castillo et al. [41] discovered that a disinfectant based on hydrogen peroxide demonstrated bactericidal activity against *E. coli, S. aureus*, and *P. aeruginosa* at low concentrations (0.5%). Wanja et al. [42] found that hydrogen peroxide at 3% exhibited broad spectrum antibacterial action against *K. pneumonia* and *E. coli*, with inhibition zones of between 20 and 23 mm in diameter.

Regarding our finding, the efficacy of ethyl alcohol 70% (w/v) as an antiseptic against *Pseudomonas* spp. was 100%, followed by CNS (66.6%) and *S. aureus* (50.0%), while the sensitivity of both *E. coli* and *Klebsiella* spp. wasn't exceeded by 33.3 %. The zone diameter for *Pseudomonas* spp. was 17.5±0.0 mm and for *E. coli* was 10.0±0.0 mm. Conversely, chlorohexidine HCL proved its bactericidal effect (100%) against all bacterial isolates at 125mg/100ml. The inhibition zone for both *E. coli* and *S. aureus* was 35.0±0.01 and 30.0±0.03mm, respectively. The ethanol sterilization action is mainly due to the dehydration of proteins and the enzymes that deactivate and prevent bacterial growth [43]. The efficiency of the antiseptics (ethanol 70%, and chlorohexidine gluconate 6%) on the tested bacteria (*E. coli, P. aeruginosa,* and *S. arueus*) had different sterilization pattern and from the obtained results, ethanol had the highest efficacy of 70% against the studied microorganisms, whereas chlorohexidine gluconate had the lowest efficiency of 6% [44]. Additionally, gram-positive bacteria with ethyl alcohol resistance of 60–95% showed a small decrease in resistance, including *S. aureus* and *S. pyogenes* [45]. The most widely used active component in alcohol-based disinfectants is ethyl alcohol (CH3CH2OH), which has been applied as a surface antiseptic. It works well against several non-enveloped viruses, fungi, yeasts, and vegetative types of bacteria [46, 47]. Vuai et al. [48] showed that alcohol-based hand sanitizers were more successful in preventing *P. aeroginosa*, and *S. aureus* growth, which had an inhibition zone of 12.47 mm, and 12.13 mm, respectively. Meanwhile, Nia et al. [49] found that *S. aureus* was effectively inhibited by chlorhexidine solution, followed by *E. coli,* and showed an inhibition zone of 24.33±0.57mm and 16.00±0.00 mm, respectively.

Conclusion

Controlling and preventing the source of bacterial pathogens and their potential to spread to lab workers, and researchers requires regular monitoring and investigation of bacterial contaminants in the

surrounding environment of the labs. In addition, the usage of disinfectants and antiseptics is essential in eliminating and preventing the transmission of infectious diseases in veterinary labs, among researchers as well as in the community. Furthermore, the frequent distribution of bacterial pathogens in the research laboratories environment revealed that the most predominant bacterial isolates were *E. coli* and *S. aureus*, followed by *CNS*, *Klebsiella* spp., and *Pseudomonas* spp. The most widespread bacterial isolate in lab equipment was *E. coli* followed by *S. aureus* and *Pseudomonas* spp. The biocidal activity of testing SG® disinfectant was 100% against *S. aureus, CNS, Klebsiella* spp*.,* and *Pseudomonas* spp. at 0.7 and 1.0% concentrations. Oppositely, the H_2O_2 was highly effective against all bacterial isolates except *S. aureus,* which was 83.3% at the highest concentration (6.0%). The efficacy of chlorohexidine HCL proved its bactericidal effect (100%) against all bacterial isolates at 125mg/100ml.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

There are no experimental trials on either animals or human organs and/or tissue in the manuscript. Before the study started, lab researchers provided us with their informed consent to be voluntary participants in the data gathering. The hand swabs samples that involved researchers in the labs under investigation were authorized by the IRB (Institutional Review Board; Ref. No.: IORG 0009255) of Beni-Suef University. Furthermore, the author proved the fact that all procedures used in the text were carried out in compliance with all applicable rules. All information was logged and subjected to statistical analysis.

TABLE 1. Collected samples from different investigated veterinary research laboratories during study period

Collected samples	Total examined No.	Total positives samples No. (%)	
		No.	%
Labs environment	149	109	73.1
Equipment	57	38	66.6
Researchers	30	20	66.6
Total	**236**	**167**	**70.7**

P-value: $P \leq 0.05$, $\chi 2 = 119.86$

TABLE 2. Frequent distribution of different bacterial isolates (%) from the lab environment during study period

Samples of Labs environment	Distribution of isolated bacteria from lab environment No. (%)				
	E. coli	***S. aureus***	***CNS***	***Klebsiella* spp.**	***Pseudomonas* spp.**
Benches (*n*=60)	26 (43.3)	27 (45.0)	14 (23.3)	10 (16.6)	11(18.3)
Floors (*n*=22)	13 (59.0)	10 (45.4)	7 (31.8)	5 (22.7)	4 (18.1)
Doors (*n*=14)	2 (14.3)	7 (50.0)	4 (28.5)	7 (50.0)	1 (7.1)
Switches (*n*=14)	3 (21.4)	0 (0.0)	4 (28.5)	0 (0.0)	0 (0.0)
Fans (*n*=9)	0 (0.0)	1 (11.1)	0 (0.0)	1 (11.1)	0 (0.0)
Containers (*n*=10)	2 (20.0)	3 (30.0)	2 (20.0)	3 (30.0)	0 (0.0)
Basins (*n*= 20)	7 (35.0)	5 (25.0)	4 (20.0)	5 (25.0)	4 (20.0)
Total (*n*= 149)	**53 (35.5)**	**53 (35.5)**	**35 (23.5)**	**31 (20.8)**	**20 (13.4)**

The association between frequency of bacterial isolates from labs environment is statistically significant at $\chi 2 = 146.53$, $P \leq 0.05$

TABLE 3. Frequent distribution of different bacterial isolates (%) from the lab equipment during study period

Lab equipment	Distribution of isolated bacteria from lab equipment				
	E. coli	***S. aureus***	***CNS***	***Klebsiella* spp.**	***Pseudomonas* spp.**
Incubators (*n*=12)	1 (8.3)	2 (16.6)	3 (25.0)	1 (8.3)	0 (0.0)
Hot air ovens (*n*=4)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Microscopes (*n*= 7)	3 (42.8)	2 (28.5)	1 (14.3)	1 (14.3)	1 (14.3)
Biosafety cabinets (*n*= 4)	3 (75.0)	2 (50.0)	0 (0.0)	0 (0.0)	1 (25.0)
Fume hoods (*n*=2)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)
PCR (*n*=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
Balances (*n*=2)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
Fridges (*n*=19)	4 (21.0)	4 (21.0)	5 (26.3)	5 (26.0)	3 (15.7)
Deep freezers (*n*=5)	3 (60.0)	1 (20.0)	0 (0.0)	1 (20.0)	3 (60.0)
Total (*n*= 57)	**16 (28.0)**	**11 (19.2)**	**10 (17.5)**	**8 (14.0)**	**11 (19.2)**

The association between frequency of bacterial isolates from labs equipment is statistically significant at $\chi 2 = 128.79$, $P \leq 0.05$

TABLE 4. Frequent distribution of different bacterial isolates (%) from researchers in labs during study period

Collected samples	Distribution of isolated bacteria from researchers in labs (No. %)				
	E. coli	***S. aureus***	**CNS**	***Klebsiella* spp.**	***Pseudomonas* spp.**
Hands (*n*=10)	3 (30.0)	4 (40.0)	1 (10.0)	0 (0.0)	2 (20.0)
Coveralls (*n*=10)	4 (40.0)	4 (40.0)	0 (0.0)	1 (10.0)	3 (30.0)
Shoes (*n*= 10)	5 (50.0)	5 (50.0)	3 (30.0)	0 (0.0)	3 (30.0)
Total (*n*= 30)	**12 (40.0)**	**13 (43.3)**	**4 (13.3)**	**1 (3.3)**	**8 (26.6)**

TABLE 5. Biocidal effect of tested disinfectants and antiseptics against all bacterial isolates

Tested disinfectant (concentrations	Sensitivity pattern of isolated bacteria to all tested disinfectants (*n*=30)										*P-value*
	E. coli		***S. aureus***		***CNS***		***Klebsiella* spp.**		***Pseudomonas* spp.**		
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	
Klorosept 25®											
0.2 mg/l	6 (100.0)	0 (0.0)	1 (16.6)	5 (83.3)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	
0.3 mg/l	6 (100.0)	0 (0.0)	0 (0.0)	6 (100)	6 (100)	0 (0.0)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	0.03
0.4 mg/l	6 (100.0)	0 (0.0)	3 (50.0)	3 (50)	6 (100)	0 (0.0)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	
Cox Killer®											
0.5 %	1 (16.6)	5 (83.3)	0 (0.0)	6 (100)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	
0.7 %	1 (16.6)	5 (83.3)	3 (50.0)	3 (50.0)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	0.05
1.0 %	1 (16.6)	5 (83.3)	3 (50.0)	3 (50.0)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	2 (33.3)	4 (66.6)	
Sporocide Glu® (SG®)											
0.5 %	5 (83.3)	1 (16.6)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100%)	0 (0.0%)	
0.7 %	3 (50.0)	3 (50)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100%)	0 (0.0%)	0.01
1.0 %	3 (50.0)	3 (50)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100%)	0 (0.0%)	
Hydrogen peroxide (H_2O_2)	6 (100.0)	0 (0.0)	5 (83.3)	1 (16.6)	6 (100)	0 (0.0)	4 (66.6)	2 (33.3)	6 (100%)	0 (0.0%)	0.02
3.0 %	6 (100.0)	0 (0.0)	5 (83.3)	1 (16.6)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100%)	0 (0.0%)	
6.0 %											
Ethyl alcohol 70% (w/v)	2 (33.3)	4 (66.6)	3 (50.0)	3 (50.0)	4 (66.6)	2 (33.3)	2 (33.3)	4 (66.6)	6 (100%)	0 (0.0%)	0.05
Chlorohexidine HCL											
62.5 gm/100ml	1 (16.6)	5 (83.3)	1 (16.6)	5 (83.3)	0 (0.0)	6 (100)	2 (33.3)	4 (66.6)	4 (66.6)	2 (33.3)	0.001
125 gm/100ml	6 (100.0)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	6 (100%)	0 (0.0%)	

S: Susceptible (absence of bacterial growth) on agar; R: Resistant (presence of bacterial growth) on agar

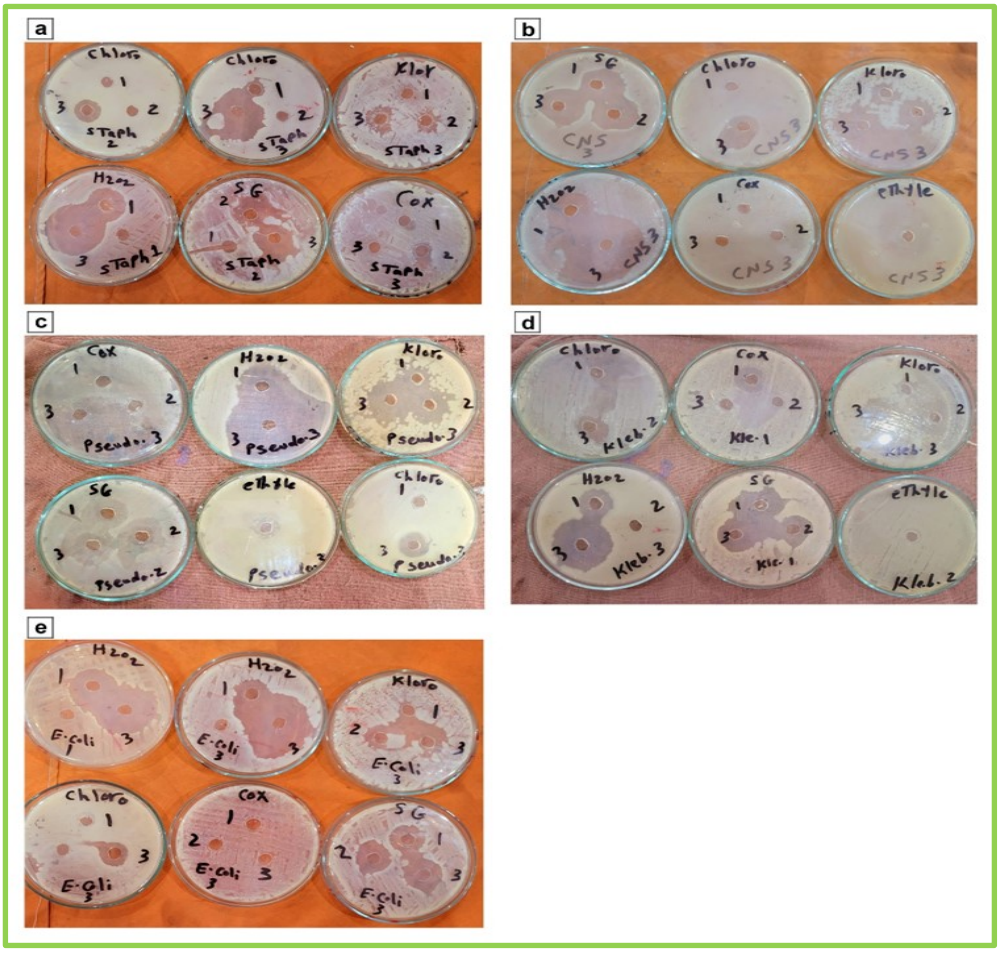

Fig. 1. The inhibition zone of tested disinfectants and antiseptics at different concentrations against all bacteria isolates, *S. aureus* (a), *CNS* (b), *Pseudomonas* spp. (c), *Klebsiella* spp. (d), and *E. coli* (e).

TABLE 6. The inhibition zone (mm in diameter) of all tested disinfectants and antiseptics against different bacterial isolates

Tested disinfectant/ sanitizer (concentrations)	The inhibition zone (mean ± SE) of tested disinfectants				
	E. coli	***S. aureus***	***CNS***	***Klebsiella* spp.**	***Pseudomonas* spp.**
Klorosept 25®					
0.2mg/l	27.5±0.14^{ab}	15.0±0.22	20.5±0.0^{ab}	17.5±1.2^{ab}	30.0±0.01
0.3mg/l	30.0±0.06	25.5±0.30^{b}	35.5±0.02	25.0±0.06	35.0±0.34
0.4mg/l	47.5±0.33^{a}	27.0±0.05	42.3±0.15^{a}	30.0±0.11^{b}	45.0±0.20^{a}
Cox Killer®					
0.5%	10.0±0.02^{c}	0.0±0.0	20.0±1.2^{ab}	20.0±0.42^{b}	30.0± 0.05
0.7%	10.0±0.01	10.0±0.07^{c}	20.0±2.2	20.0±0.07	40.0±0.11
1.0 %	10.0±0.0	10.0±0.0	30.0±0.15^{b}	30.0±0.11	46.2±0.23^{a}
Sporocide Glu® (SG®)					
0.5%	25.0±0.4^{ab}	18.5±1.1	27.5± 1.8^{b}	27.5±0.09	25.0±0.0^{b}
0.7%	30.0±0.05	25.0±0.15^{b}	32.5±0.06	22.5±0.35^{b}	32.5±2.4
1.0 %	30.0±0.03	45.0±0.08^{a}	37.5±0.04^{a}	37.5±0.27^{a}	30.0±0.03
Hydrogen peroxide (H_2O_2)					
3.0 %	37.5±1.5^{b}	21.0± 0.3	27.5± 1.7	22.5± 0.05^{b}	27.5±0.2
6.0 %	45.1±2.4^{a}	40.0±1.4^{a}	37.5±0.01^{a}	37.5± 0.16^{a}	43.5±2.3^{a}
Ethyl alcohol 70% (w/v)	10.0±0.0^{c}	20.0±0.03	30.0±0.0^{b}	15.0±0.21^{ab}	17.5±0.0^{ab}

The association between inhibition zone of isolated bacteria against tested disinfectants with superscript of different letters $^{(a,b,ab\&c)}$ in the same column is statistically significant at $P \leq 0.05$

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نمط الحساسية للمطهرات المحضرة حديثا المضادة للميكروبات ضد الملوثات البكتيرية المسببة للأمراض في المختبرات البحثية البيطرية المختلفة

محمد عبدالرحمن البابلى، أسماء نادى محمد ، منار بهاء الدين محمد و دينا عبدالله محمد

قسم الصحة والأمراض المشتركة والوبائيات - كلية الطب البيطري - جامعة بني سويف - بني سويف - مصر.

المستخلص

في منشآت البحوث البيطرية، يُعد استخدام المطهرات المضادة للميكروبات هي خط الحماية الأساسي ضد أي بكتيريا ضارة على الأسطح غير الحية المختلفة للمساعدة في الوقاية من العدوى المرتبطة بالرعاية الصحية (HAIs) . تهدف الدراسة الى تقدير معدل انتشار مسببات الأمراض البكتيرية في البيئة المحيطة بمرافق البحوث البيطرية، وتقييم النمط المضاد للميكروبات للمطهرات المصنعة حديثًا (Sporoside Glu®، Cox Killer®، Klorsept 25®) واثنين من المعقمات (الكحول الإيثيلي 70% وكلوروهيكسيدين HCL (125 ملجم/100 مل) ضد جميع مسببات الأمراض البكتيرية المعزولة، ووضع استراتيجية تحكم لمنع انتشار الملوثات البكتيرية إلى الباحثين وبيئة المختبر. لعزل البكتيريا المسببة للأمراض وتحديد البكتيريا المسببة للأمراض من البيئة المحيطة بالمختبر، تم أخذ عدد 236 عينة مسحة من بيئة المختبر (149)، والمعدات (57)، والباحثين في المختبر (30) في المختبرات البيطرية البحثية السبعة. استُخدمت مقايسة الانتشار في بئر آجار لتقييم مدى حساسية ثلاثين سلالة من العزلات البكتيرية لمختلف المطهرات و المعقمات قيد الفحص. من النتائج تبين أن المعزلات البكتيرية الأكثر شيوعا في جميع عينات البيئية المخبرية، بما في ذلك المفاتيح والمراوح والأسطح والأبواب والأرضيات والحاويات والأحواض، هي الإشريشيا كولاي و المكورات العنقودية الذهبية35.5) ٪ لكل منهما). بالاضافة الى أن أكبر معدل لمعزلات المكورات العنقودية الذهبية السالبة في أغطية الأبخرة والثلاجات والحاضنات. كانت السلالات البكتيرية الأكثر شيوعًا من مسحات الأحذية للباحثين هي الإشريشيا كولاي والمكورات العنقودية الذهبية ، حيث بلغت النسبة 50٪ لكل منهما، و40٪ من البلاطى، و30٪ من الأيدي، على التوالي. كما أظهر المطهر Sporoside Glu® بتركيزات 0.7 و1.0%، تأثيرًا قاتل بنسبة 100% ضد المكورات العنقودية الذهبية والمكورات العنقودية الذهبية السالبة والكليبسيلا وسلالات السودومونص. وبالنسبة الى فوق أكسيد الهيدروجين (H_2O_2) أثبت أنه أكثر فاعلية بنسبة 100% ضد جميع المعزلات البكتيرية، باستثناء المكورات العنقودية الذهبية ، التى لم تتعدى نسبة 83.3% عند أعلى تركيز تم اختباره (6.0%). خلصت النتائج الى أن تعد البيئة ومعدات المختبر مصادر محتملة للتلوث عندما يكون هناك تركيز كبير من الملوثات البكتيرية. أثبتت مطهرات Sporocide (1%) Glu®و Klorsept 25® 0.4ملغم/لتر) وكذلك الكلوروهكسيدين HCL 125ملجم/100 مل) فعاليتها القاتلة للجراثيم (100%) ضد جميع العزلات البكتيرية في البيئة المحيطة بالمختبرات.

الكلمات الدالة: الملوثات البكتيرية، خصائص مضادات الميكروبات، المطهرات الحديثًه، مختبرات الأبحاث.