Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
QJPS
Al-Qadisiyah Journal of Pure Science

Biocides Susceptibility testing of *Staphylococcus aureus* isolated from the hospital environment

Zahraa Al jboori [1*] and *Aalaa* Fahim Abbas[2]
* sci.env.mas22.6@qu.edu.iq alla.fahim@qu.edu.iq
College of Science, University of Al-Qadisiyah, Iraq

Abstract

Staphylococcus aureus it is one of the most commonly reported pathogens of nosocomial. The extensive use of biocides in hospitals result in development of non-susceptible S. aureus strains. Testing the susceptibility of S. aureus isolated from environmental of the hospital to biocides was the aim in the current study. Using the macrobroth dilution method , 31 bacterial isolates of S. aureus isolated from the environmental of the hospital were exposed to different concentrations of biocides: Benzalkonium chloride 0.05%, Cetrimed, Chlorhexidine digluconate 2% , Ethanol 75%, Hydrogen peroxide 50%, Povidone 10% , Sodium hypochlorite 5% , and Vinegar 6 %. The results indicated that all isolates were sensitive to Cetrimed, Chlorhexidine digluconate 2%, Ethanol 75%, Hydrogen peroxide 50%, and Sodium hypochlorite 5%. Two isolates were resistant to Benzalkonium chloride 0.05% and Vinegar 6 % with percentage (6.5%) and 20 isolates were resistant to Povidone 10% with percentage (64.5%). Mean of MIC and MBC were: Benzalkonium chloride (300 µg/mL, 400 µg/mL), Cetrimed (11115.5 µg/mL, 12634 µg/mL), Chlorhexidine digluconate(2594.05µg/mL, 2930.09 µg/mL), Ethanol (250000 µg/mL, 326612.9 µg/mL), Hydrogen peroxide(57325.2µg/mL, 59005.2 µg/mL), Povidone (75,000 µg/mL,100,000 µg/mL), Sodium hypochlorite(21572.5µg/mL, 28629.03µg/mL), and Vinegar(45000 µg/mL, 60,000 µg/mL).

Keywords: Staphylococcus aureus, Hospital environment, biocide resistance .

Introduction

The main and risk source in spreading S. aureus bacteria is the hospital environment (Kozajda et al., 2019). *S. aureus* is one of the most commonly reported nosocomial pathogens, even after disinfection, it can still be found on hospital common surfaces (Odoya et al., 2015). S. aureus strains can withstand up to seven months in hospital settings without differences found whether the isolate was resistance or sensitive to antibiotics (Gregory et al., 2021). One of the main causes of hospital-acquired illnesses is hand and touch surface contamination by medical staff. Low, medium, and high frequency contact surfaces have been identified in the vicinity of patient areas. Worktables, bedside rail restraints, infusion pumps, suction catheters, glucose meters, multi-parameter ECG monitors, nurses' work computers, and door handles are examples of high-frequency contact surfaces. That many harmful bacteria have been shown to be, such as methicillin resistant *S. aureus* (MRSA), coagulase-negative Staphylococcus, and Staphylococcus aureus, reside on these surfaces (Shi et al., 2015). In healthcare settings, non-antibiotic antimicrobials biocides as disinfectants and antiseptics are widely used, they are used for pre-operative skin decontamination of patients and staff, to control infections and microbial contamination, disinfection of hospital surfaces, general and equipment sterilization (Barakat et al., 2022). Because S. aureus may cluster and develop biofilm on surfaces, which makes them less vulnerable to biocides, the bacterial cells on such surfaces are more persistent and there is a greater chance of cross-contamination (Chieffi et al ., 2023). Extensive infection control programs encouraged the use of biocides which resulted in the development of non-susceptible strains of S. aureus. The basic mechanism of biocide resistance is a decrease in the drug's intracellular concentration, which can be achieved through efflux pumps that can extrude various types of antimicrobial drugs, including both biocides and antibiotics, or by reducing the permeability of the cell wall (Conceicao et al., 2021). the minimum bactericidal concentration (MBC) and Minimum inhibitory concentration (MIC) assay tests are used to ascertain the sensitivity of bacteria and assessto of the efficacy of disinfectants. When growth of bacterial is suppressed at low doses of a disinfectant, its potency, effectiveness, and suitability are increased; this is achieved when the disinfectant's MIC and MBC values are minimal enough. values of MIC are often lower than MBCs because, in most situations, bactericidal rather than bacteriostatic effects require concentrations that higher of an antibacterial agent. When it comes to disinfectant, MBC is typically a significantly larger dosage that kills the germs, whereas MIC is the lowest concentration that prevents the growth of the bacterial tested (Tapouk et al ., 2020). There may be a reduction in susceptibility or even resistance shown by the significant increase in the MIC value that was reported. It is important to take into account the disinfectant's in-use concentration when interpreting the results, as it can be greater than the MIC values that were actually measured (Rozman et al., 2021). In the current study biocides resistance among bacterial isolates of S. aureus isolated from hospital environment was detected..

Material and method :

Processing and Collection of the samples

In the current study, one hundred and eighty eight environmental samples were collected during the study period extended from July 2023 to October 2023 from Al-Qassim General Hospital in Hilla city, and included surfaces and air samples. Environmental samples were taken in accordance with the principles provided in the Centers for Disease Control and Prevention (CDC) environmental cleaning toolkit . Surface samples were collected from frequently touched objects (beds, tables, door knobs, sinks, walls, floor, curtains, cabinets, light switches, medical devices), in addition to patients and employees hands. Surface samples were taken one hour following the daily cleaning and disinfection process. To collect surface samples, by used of the sterile cotton swabs soaked in normal saline. The swabs were taken perpendicularly, with only the tip touched the surface and rotated 360 degrees. The swabs were then streaked onto blood agar plates. After that, the plates were aerobically incubated for twenty four hours at 37 °C to promote growth of the bacterial, and for a further 24 hours if no growth was observed (CDC, 2003). Discrete growing bacterial colonies were sub cultured to achieve pure colonies. The plates underwent a 24-hour aerobic incubation at 37°C. For air samples, the blood agar plates were exposed to air in order to gather air samples using the gravity settlement method. For a duration of thirty minutes, the plates were kept open with a lid by their sides, three to four feet above the floor. The plates were then incubated at 37°C and for 24 hours to promote bacterial growth (CDC, 2003).

Identification and characterization of S. aureus

Standard biochemical tests were used to identify bacterial isolates as Staphylococcus aureus at the species level . Firstly, the growing bacterial isolates on blood agar were subjected to Gram's staining, subcultured on mannitol salt agar, subjected to catalase test, DNAase test and further subjected to slide and tube coagulase test for confirmative identification in accordance with Holt et al. (1994) and MacFaddin (2000).

Preparation of bacterial suspension

On the Brain heart agar plates, a loop full of brain heart broth culture of bacterial isolates were streaked. After incubated them for 24 hours at 37 °C, Each bacterial isolate was selected using a sterile swab, and one or two developing colonies were emulsified in five milliliters of sterile normal saline. The mixture was vortexed, and the turbidity was visually compared to the 0.5 McFarland standard to adjust for roughly 1.5×108 CFU/ml (Tapouk et al., 2020).

Biocide susceptibility testing

The modified macrodilution method, as recommended by the principal of the National Committee for Clinical Laboratory Standards (NCCLS), was employed to determine the bacterial susceptibility of the isolates towards biocides (Tapouk et al., 2020). To prepare different biocide concentrations, a set of sterile test tubes were numbered from one to seven . In aseptic conditions, one ml of Muller Hinton broth was added to each tube. One ml of testing biocide (undiluted) was added into the tube number one. Following mixing, serial two fold dilutions were made by adding one ml of each tube's contents to the subsequent one, the procedure proceeded to tube five . One ml was poured out of tube 5, bringing the total volume of the tubes to 1 ml. This procedure was performed for all biocide and for each bacterial isolate. After preparing serial dilutions, each tube was inoculated with 10 µl (1.5 × 108 CFU/ml) of adjusted fresh bacterial culture, then tubes were incubated for 24 hours at 37 °C. Six and seven tubes were served as negative (biocide + MHB) and positive (1.5 × 108 CFU of bacterial inoculums + MHB) controls, respectively. After incubation, the turbidity of the tubes was examined. A biocide was considered resistant, according to Russell (2003), if it did not exhibit an antibacterial action at the manufacturer's specified concentration. Minimum inhibitory concentration (MIC) is the lowest concentration of a biocide agent which has an inhibitory effect on the cultured bacteria, causing no turbidity in the test tube after incubation for 24 hours and at 37 °C. The lowest concentration of biocide (MIC) that inhibited visible bacterial growth was determined. To determine the minimum bactericidal concentration (MBC), 5 µl of each tube was subcultured on the MHA surface and incubated for 24 hours, at 37°C and colony counts were performed (Tapouk et al., 2020).

Statistical analysis

Using the IBM Statistical Package for Social Sciences (SPSS, United States) Statistics software, version 27, a chi-square test (X2) was used in the statistical analysis to evaluate the independence of the variables. $P < 0.05$ was deemed statistically significant (Daniel, 2009).

Results

Prevalence and distribution of Staphylococcus aureus isolates in hospital samples

The environmental of the hospital is increasingly recognized as a reservoir of multidrug-resistant bacteria. From the environment, Healthcare professionals may carry these germs on their hands, which can then colonize patients' skin and mucous membranes and result in nosocomial pathogens. (Boyce, 2016). Therefore in current study 188 different environmental samples were taken from the environmental of the hospital. The results of primary culture on blood agar media showed significant variation between positive bacterial growth which appeared in 148 (78.72%) samples, and negative bacterial growth in 40 (21.27%) samples that did not show bacterial growth on blood agar. Rates of nosocomial contamination vary from facility to facility and even within a single facility, both qualitatively and quantitatively, and over time depending on the services, patients care, and techniques used (Chaoui et al.,2019) Furthermore, differences in incidence may be due to differences in identification techniques (Zahornacky et al, 2022). Negative bacterial cultures may be resulted from controlling environmental contamination by cleaning and disinfection (Afle et al., 2019), or they may be viable but non culturable (VBNC) bacteria (Nikaeen et al., 2023), or other contaminants like fungi (Al-Douri., 2011).

Table (1) : Prevalence of bacterial contamination in hospital samples

Bacterial growth	Number	Percentage %
Positive	148	78.72
Negative	40	21.28
Total	188	100
X2	124.08	
P value	<0.0001*	

* Highly significant association at P<0.05

Out of 188 environmental samples, coagulase negative staphylococci were significantly predominant (50.53%), followed by Staphylococcus aureus (16.48%) (Table 2), a similar outcome was found in the study conducted by (Paudel et al., 2022). In study of (Espiritu and Villanueva., 2022) 103 (41.9%) CoNS were isolated from26 air samples and 220 surface samples, the medical ward had the highest prevalence of Coagulase negative staphylococcus NS at 64%. In study of (Fernandes et al ., 2020) 42 (84%) isolates were identified as coagulase-negative Staphylococci. Other study (Moore et al ., 2023) found out of 173 surfaces sampled, 40 (21.1%) harbored coagulase-negative Staphylococci. Hospital environment often harbor CoNS that considered as the most frequent cause of healthcare-associated infections (HCAIs), the pathogenesis of which is facilitated by the use the devices of medical. CoNS are a class of bacteria that have gradually changed from being basic pollutants in healthcare and non-healthcare environments to being drug-resistant (Espiritu and Villanueva., 2022) . In the study of (Al-Saadi ., 2022) the percentage of S. aureus was 23.7%, in the study of (Al-kaabi et al ., 2016) the percentage was 19.5% for S. aureus isolated from the hospital environment. In study of (Al-Abdli & Baiu, 2016) S. aureus was found in 100 (25.3%) of the 395 environmental swab samples, and in study of (Mirzaii et al., 2015) from the 268 environmental samples, 17 colonies were selected as S. aureus. However isolation percentage were within the expected range due to Staphylococcus aureus has diverse habitats, which has led to its widespread spread in hospitals (Cheung et al .,2021) . In the current study Staphylococcus aureus isolates were distributed as following: tool and device 14 (18.91%), employees and worker 4(22.22), floor 3(37.5), table 3(21.42), curtains 2(33.33), beds 2(22.22), doors 1(7.14), patient 1(7.14), locker 1(50). While faucet, Sink swabs and air samples were negative for S. aureus presence. Furthermore, the results of statistical analysis revealed that the distribution of Staphylococcus aureus isolates was statistically non-significant. Staphylococcus aureus can survive on inanimate environmental surfaces, and it is highly contagious and has the ability to spread, so there are global health concerns (Khairalla ., et al 2017). In a hospital setting, where surfaces may be exposed to low or residual biocide concentrations, resulting in decreased efficacy. Even when applied at the doses recommended by the manufacturer, concentrations that might persist on surfaces following washing could put bacteria under selective pressure (Vali et al .,2008).

Table (2): Prevalence and distribution of Staphylococcus aureus according to the isolation site

Isolation site	Total number of samples	S. aureus	Coagulase negative Staphylococci	Other bacteria	No growth
Beds	9	2(22.22)	7(77.77)	0(0)	0(0)
Curtains	6	2(33.33)	4(66.66)	0(0)	0(0)
Floor	8	3(37.5)	4(50)	1(12.5)	0(0)
Faucet	7	0(0)	3(42.85)	1(14.28)	3(42.85)
Sink	5	0(0)	3(60)	1(20)	1(20)
Tool and devices	74	14(18.91)	25(33.78)	10(13.51)	25(33.78)
Walls	9	0(0)	5(55.55)	2(22.22)	2(22.22)
Doors	14	1(7.14)	9(64.28)	2(14.28)	2(14.28)
Table	14	3(21.42)	11(78.57)	0(0)	0(0)
Locker	2	1(50)	1(50)	0(0)	0(0)
Patients	14	1(7.14)	11(78.57)	1(7.14)	1(7.14)
Employees and worker hands	18	4(22.22)	6(33.33)	3(16.66)	5(27.77)
Air	8	0(0)	6(75)	1(12.5)	1(12.5)
Total	188	31(16.48)	95(50.53)	22(11.7)	40(21.27)
X2		14.14	25.95	6.03	22.3
P value		0.292*	0.011**	0.913*	0.034**

* No significant association at P<0.05, **Significant association at P<0.05

In Al-Khudairi's study (2008), Staphylococcus aureus were diagnosed with a percentage (37.5%) in the floors samples, and (25%) for doors samples. In study of (Ahmadi et al .,2016), the most swab samples contaminated with Staphylococcus aureus were those taken from the exterior of emergency rooms (8.8%), followed by beds (5%) and carts (5%), it has been suggested that bacteria are the source of environmental diseases which persist throughout time. In study of (Odoya et al ., 2015), 54.05% of hospital surfaces were contaminated with Staphylococcus aureus. Staphylococcus aureus can live on inanimate surfaces, and can be found on common surfaces of hospitals such as floors and doors even after disinfection. Every surface that was swabbed had frequent touch with people, which could have been a source of infectious disease transmission, even with consistent daily disinfectant cleaning(Odoya et al ., 2015). Result of the study of (Mir et al., 2021), the most common microorganisms on the surface of medical equipment were S. aureus (16.66%) and CoNS (16.66%). The transmission of resistant microorganisms on lab-contaminated surfaces among patients and in the environmental hospital is accelerated. Additionally, it has been noted that certain bacterial isolates linked to inanimate surfaces, equipment, and other patient samples do exhibit commonalities. These similarities contribute to support the horizontal transmission of microorganisms (Mir et al., 2021).

Biocide susceptibility

In the present work, the susceptibility of S. aureus isolates to eight biocides (Benzalkonium chloride 0.05%, Cetrimed, Chlorhexidine digluconate 2%, Ethanol 75%, Hydrogen peroxide 50%, Povidone 10%, Sodium hypochlorite 5% , and Vinegar 6 %) was tested using macro broth dilution method. The results indicated that Cetrimed, Chlorhexidine digluconate 2%, Ethanol 75%, Hydrogen peroxide 50%, and Sodium hypochlorite 5% were effective against tested S. aureus isolates. Two isolates were resistant to Benzalkonium chloride 0.05% and Vinegar 6 % with percentage (6.5%) and 20 isolates were resistant to Povidone 10% with percentage (64.5%). Because acetic acid is a component of vinegar's composition, the study used vinegar 6% to examine vinegar's effectiveness against isolates of S. aureus, the outcomes revealed two isolates exhibited resistant with percentage 6.5%.

Table (4): Efficacy of biocides solutions against *Staphylococcus aureus*

Biocide	Resistance	
	No./ total	%
Benzalkonium chloride 0.05%	2/31	6.5
Cetrimed	0	0
Chlorhexidine digluconate 2%	0	0
Ethanol 75%	0	0
Hydrogen peroxide 50%	0	0
Povidone 10%	20/31	64.5
Sodium hypochlorite 5%	0	0
Vinegar 6 %	2/31	6.5

MIC mean for Benzalkonium chloride 0.05%, was (300 µg/mL), and MBC mean (400 µg/mL). The resistance of S. aureus may be attributed to their possession of biofilms and efflux pumps. In study of (AlKhazraji et al ., 2020) MIC values were less than 2 µg/mL. Increasing MIC may be attributed to mutations of six different genes (i.e., qacA/B, qacC (smr), qacH , qacJ and qacG,) that contribute to the development of resistance to QAC in S. aureus (Rozman et al., 2021). MIC mean for Cetrimed was (11115.5 µg/mL), while the MBC mean was (12634 µg/mL). In study of (Kulkarni et al., 2020) When resistant of S. aureus, the MIC of cetrimide ranged from 9.765 to 312.5 mcg/ml.. Study by (Ruiz-Linares et al., 2014) reported a moderate disinfection effect when using 0.2% cetrimide for 1 minute. Transmission electron microscopy studies of the effects of cetrimide on methicillin-resistant Staphylococcus aureus revealed that nuclear regions were obscured by cytoplasmic influx, followed by loss of cytoplasmic granularity, cell wall and membrane disruption, cytoplasmic condensation, loss of cellular components, cell wall destruction, extensive damage to the cytoplasmic membrane, and eventual cell lysis (Kulkarni et al., 2020). MIC mean for Chlorhexidine digluconate 2% was (2594.05 µg/mL), and MBC mean was (2930.09 µg/mL). In study of (Conceicao et al ., 2021) MICs value for Chlorhexidine ranged from 0.125 mg/L to 4 mg/L and MBCs from 0.125 mg/L to 8 mg/L, while 92.0% (185/201) of isolates had MICs between 0.5 mg/L and 1 mg/L. The MIC For chlorhexidine has risen by almost 32–150 times which may be attributed to mutations of smr, qacB,qacA, and norA genes in S. aureus and MRSA (Rozman et al., 2021). Although a large percentage of biocide resistance genes have been found in S. aureus isolates from various African countries, chlorhexidine reduced susceptibility appears to be low. The existence of the qacAB and norA genes was not linked to higher chlorhexidine resistance, despite the fact that staphylococci have frequently been shown to exhibit similar relationships (Conceicao et al ., 2016). The MIC mean was (250000 µg/mL) for Ethanol 75% while the mean MBC was (326612.9 µg/mL). The results in (Guilhermetti et al ., 2015) study showed 70% Ethanol maybe the best hand cleaners for getting rid of methicillin resistant S. aureus strains from hands that are either moderately or substantially polluted. Current study showed high disinfection efficiency for Hydrogen peroxide 50%

where MIC mean (57325.2 µg/mL), while its MBC mean (59005.2 µg/mL), it was lethal to isolates even at the fifth dilution of disinfectant. The study of (Buvelot et al., 2021) defined 20 mM H_2O_2 was the highest sublethal concentration affecting moderate growth of bacterial. This concentration was used for most of the experiments, to confirm that S. aureus growth is indeed affected by H2O2. The study of (Lineback et al ., 2018) found that hydrogen peroxide disinfectants achieved a greater overall significant disinfection effectiveness in eliminating bacterial strains than quaternary ammonium disinfectants. Hydrogen peroxide disinfectant was effective against S. aureus biofilms. Hydrogen peroxide disinfectant have been reported to destroy both matrix of the biofilm and the bacteria cells within, making them efficient antibiofilm agents. MIC mean for Sodium hypochlorite 5% was (21572.5 µg/mL), while MBC mean was (28629.03 µg/mL). Hypochlorite acid has a strong antimicrobial effect, It has the ability to: (i) permeate bacterial cell walls and membranes; (ii) oxidatively denature proteins; and (iii) inactivate bacterial nucleic acids (Jaouhar et al., 2021). The MIC meanfor Povidone 10% was (75000 µg/mL) , and the MBC was (100000 µg/mL). However, 20 isolates showed resistance and their growth not inhibited in stock concentration of Povidone 10%. Povidone 10% is commonly used in Iraqi hospitals, therefore repeated exposure of S. aureus may contribute to povidone resistance development. The study of (AlKhazraji et al ., 2020) reported the MIC and MBC concentration (12500-25000 µg/mL) which was less than in current study. The bacterial resistant to Povidone reached 64.516%. in study of (Guzman et al ., 2024) with antiseptic, this percentage was close to the current study. Tthe indiscriminate use of disinfectants causing mutations in cellular metabolism, lead to changes in the structure of the cell wall of the bacteria, especially when changes that happen to protein channels, it prohibit substances from entering the cell (AlKhazraji et al ., 2020). Many internal and exterior cellular mechanisms that result in reduced susceptibility or even resistance to disinfectants can be caused by bacterial phenotypic and genotypic adaptation, However, resistance of bacteria to disinfectants can also be produced by mutation of chromosomal gene, acquisition of resistant determinants such as integrons, transposons, and plasmids, modifications to the permeability of the cell envelope, and elevated expression of the efflux pump. Additional channels for bacterial adaptation are created by the creation of biofilms and the inactivation or neutralization of disinfectants. (Rozman et al ., 2021). MIC mean for Vinegar 6 % was (45000 µg/mL), while MBC mean was (60000 µg/mL), except for two isolates which were resistant to vinegar and their growth not inhibited in stock concentration. Resistant isolates of S. aureus to vinegar was also noted by (Oliveira et al., 2014). In a hospital setting, where surfaces may be exposed to low or residual biocide concentrations, resulting in decreased efficacy. Even while biocides are bactericidal when applied at the doses recommended by the manufacturer, concentrations that may stay until after cleaning on surfaces could exert a selective pressure on bacteria. Theoretically, sub-lethal biocide doses for a particular cellular target may exist somewhere along this concentration gradient, which would put selection pressure on a variety of cellular targets to undergo mutations (Vali et al .,2008). There's a potential that the MICs will be further enhanced in the hospital setting if disinfectants with subinhibitory doses are used. Diluting cleaning solutions or using alternative strategies such increasing efflux pump activity could cause this. As a result, there could be a greater likelihood of exposing hospital patients to these potent S. aureus isolates (Moghaddam et al., 2022).

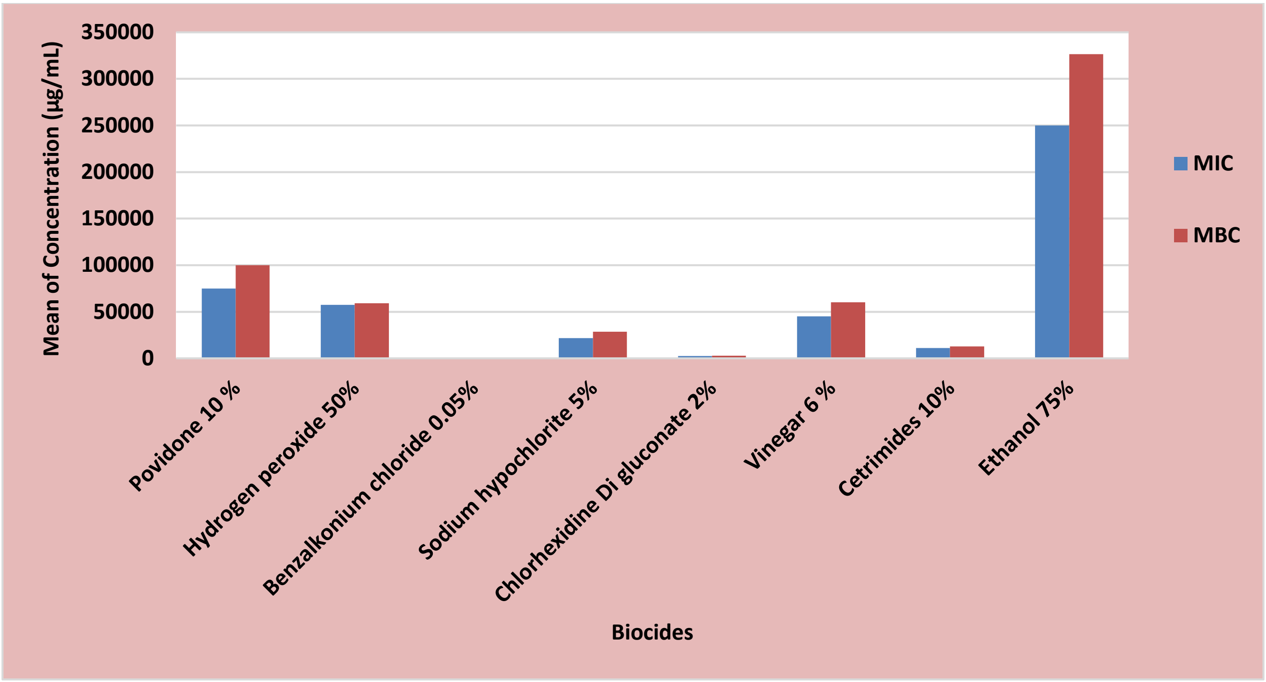

figure (1) shows concentrations of Biocide

Conclusion: Cetrimed, Chlorhexidine digluconate 2%, Hydrogen peroxide 50%, Ethanol 75%, and Sodium hypochlorite 5% were effective against tested S. aureus isolates. Resistance of some isolates of S. aureus to commonly used disinfectants was demonstrated.

References:

Afle, F., Agbankpe, A. J., Johnson, R. C., Houngbégnon, O., Houssou, S. C., & Bankole, H. S. (2019). Healthcare-associated infections: bacteriological characterization of the hospital surfaces in the University Hospital of Abomey-Calavi/so-ava in South Benin (West Africa). BMC Infectious Diseases, 19(1), 1–7.

Ahmadi,K. , Zakeri,H. , Doloo,H.Z. , Kakhki,B.R. , Mohammadshahi,A. , Moghaddam,M.A., and Hashemian,A.M.(2016). Multi-drug resistant Staphylococcus aureus isolated from emergency ward of an Iranian hospital. Tropical Journal of Pharmaceutical Research , 15 (4): 815-819.

Al-Abdli, N.E., Baiu ,S.H,. (2016) . Isolation of MRSA Strains from Hospital Environment in Benghazi City, Libya . American Journal of Infectious Diseases and Microbiology, Vol. 4, No. 2, 41-43 .

Al-Douri, A.Y.M. (2011) Diagnostic and Molecular Genetic Study of the Bacteria Causing Urinary Tract Infections to Patients in the Tikrit City. Ph.D. Thesis, College of Education, Tikrit University, Tikrit.

Al-Kaabi , K.Th ., Al-Kashwan,Th. A,and Al-Fahham,H.R,. (2016). Molecular Study of Methicillin Resistant Staphylococcus aureus Isolated from Different Hospitals in Najaf-Iraq . College of Medicine, Kufa University. JMSCR Vol : 04(08), 11922-11930 .

AlKhazraji ,N.Z., Al Jubouri, A.S., and Al Marjani,M.F.(2020) . Detection of Antiseptic Resistant Genes and Biofilm Formation in Multidrug Resistant Staphylococcus aureus in Baghdad Hospitals . Iraqi Journal of Biotechnology, Vol. 19, 12-27 .

Al-Khudairi,M.K. (2008). Bacteriological and genetic study of methicillin resistant staphylococcus aureus isolated from the hospitals of najaf city. Master thesis college of education ,university of kufa.

Al-Saadi, D.A.A,. (2022). Phenotypic and molecular diagnosis of some antibiotic and disinfectant resistance genes of Staphylococcus aureus bacteria. College of Science - Al-Qadisiyah University.

Barakat,N.A., Rasmy,S.A., Hosny,A.M., & Kashef ,M.T. (2022) . Effect of povidone-iodine and propanol-based mecetronium ethyl sulphate on antimicrobial resistance and virulence in Staphylococcus aureus . Antimicrobial Resistance & Infection Control vol(11) : 139 .

Boyce ,J.M. (2016). Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrob Resist Infect Control ,5:10.

Buvelot,H., Roth,M., Jaquet,V., Lozkhin,A., Renzoni,A., Bonetti,E.V., Gaia,N., Laumay,F., Mollin,M., Stasia,M.M., Schrenzel,J., François,P., Krause,K.H.(2021) . Hydrogen Peroxide Affects Growth of S. aureus Through Downregulation of Genes Involved in Pyrimidine Biosynthesis

Chaoui, L., Mhand, R., Mellouki, F., & Rhallabi, N. (2019). Contamination of the surfaces of a health care environment by multidrug-resistant (MDR) bacteria. International Journal of Microbiology.

Cheung,G.Y., Bae,J.S., and Otto,M. (2021). Pathogenicity and virulence of Staphylococcus aureus .Virulence journal , 12(1): 547–569.

Chieffi,D., Fanelli,F., and Fusco,V. (2023). Antimicrobial and biocide resistance in Staphylococcus aureus: genomic features, decontamination strategies, and the role of S. aureus complex-related species, with a focus on ready-to-eat food and food-contact surfaces. Frontiers in Food Science and Technology, Volume 3.

Conceicao, T., Lencastrea,H., Sousaa,M.A.(2021) . Prevalence of biocide resistance genes and chlorhexidine and mupirocin non-susceptibility in Portuguese hospitals during a 31-year period (1985–2016) . Journal of Global Antimicrobial Resistance , 24 : 169–174.

Conceicao,T., Coelho,c., Lencastre,H.D., Sousa,M.A.(2016). High Prevalence of Biocide Resistance Determinants in Staphylococcus aureus Isolates from Three African Countries. Antimicrobial Agents and Chemotherapy , Volume 60 ; (1):678-681.

Espiritu1, A.C., and Villanueva,S.Y. (2022). Isolation and Identification of Biofilm-Producing, Drug-Resistant Coagulase Negative Staphylococci from a Hospital Environment in Northern Philippines.journal of Pureand Appllied Microbiol, vol 16(1):620-629.

Fernandes, L.F., Souza, G.A.A.D., Ameida, A.C., Cardoso, L., Xavier, M.A.S., Pinheiro, T.P.P., da Cruz, G.H.S., Dourado, H.F.S., Silva, W.S., and Xavier A.R.E.D.(2020) . Identification and characterization of methicillin-resistant Staphylococcus spp. isolated from surfaces near patients in an intensive care unit of a hospital in southeastern Brazil . National Library of Medicine.

Gregory,T.V., Ellis ,K., Valeriani ,R., Khan,F., Wu,X.,Murin,L., Alibayov,B., Vidal,A.G., Tong Zhao,T., and Vidal,J.E.(2021). MoWa: A Disinfectant for Hospital Surfaces Contaminated With Methicillin-Resistant Staphylococcus aureus (MRSA) and Other Nosocomial Pathogens. Front Cell Infect Microbiol

Guilhermetti,M.,Hernandes,S.E.D,Fukushigue.Y, Garcia,L B, cardoso,C.L,.(2015) . Effectiveness of Hand-Cleansing Agents for Removing Methicillin-Resistant Staphylococcus aureus From Contaminated Hands . Cambridge University .

Guzman ,M.D., Cruz ,M.A., Ornelas ,A.L, Cruz ,C.C., Manuel ,E.M., Juan Manuel Lopez ,B. (2024). In situ and in vitro evaluation of two antiseptics for blood bank based on chlorhexidine gluconate/isopropyl alcohol and povidone-iodine. Transfusion and Apheresis Science,Vol 63(1) : 103854.

Jaouhar,S. , Lalami,A.E. , Bouzid,J., Zeouk,I. , and Bekhti,K.(2021). Susceptibility patterns of bacteria isolated from the hospital environment towards disinfectants commonly used for surfaces and medical devices. E3S Web of Conferences, 319: 01081 .

Khairalla ,A.S., Wasf,R. & Ashour,H.M.(2017). carriage frequency, phenotypic, and genotypic characteristics of methicillin-resistant Staphylococcus aureus isolated from dental health care personnel, patients, and environment . scientificreports, 7: 7390 .

Kozajda,A., JeZak,K., and Kapsa,A. (2019). Airborne Staphylococcus aureus in different environments—a review. Environ Sci Pollut Res Int, 26(34): 34741–34753.

Kulkarni ,A., Deo ,A., Nimbarte,S.(2020) . ANTIBIOTIC RESILIENCE PATTERN AND CETRIMIDE INDUCED ULTRA STRUCTURAL CHANGES IN MULTIDRUG RESISTANT S. AUREUS . Journal of Critical Reviews 7(16).

Lineback,C.B. , Nkemngong,C.A., Wu,S.W. , Li,X. , Teska,P.J. and Oliver,H.F.(2018). Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against Staphylococcus aureus and Pseudomonas aeruginosa biofilms than quaternary ammonium compounds . Antimicrobial Resistance and Infection Control , 7:154

Mir,M.A., Ashraf,M.W., Tripathi,V., and Ahmad Mir,B. (2021). Isolation, characterization and prevention of various microbial strains in NIC unit and PIC unit. Scientific Reports 11: 647.

Mirzaii ,M. , Emaneini ,M., Jabalameli ,F., Halimi ,S., Taherikalani,M. (2015). Molecular investigation of Staphylococcus aureus isolated from the patients, personnel, air and environment of an ICU in a hospital in Tehran. Journal of Infection and Public Health, 8, 202—206.

Moghaddam,G.H., Azimian, A., Bayani, G., Dashti, V., Nojoomi, S., Shirazi, N., Solati, A.(2022). High prevalence and expression of antiseptic resistance genes among infectious t037/ST239 methicillin-resistant *Staphylococcus aureus* (MRSA) strains in North Khorasan Province, Iran. Iran Journal Basic Med Sci,25(6):775-780.

Moore ,G., Barry , A., Carter , J., Ready ,J., Wan ,Y., Elsayed , M., Haill , C., Khashu ,M., Williams ,O.M., Brown, C.S., Demirjian ,A., Ready ,D.(2023). Detection, survival, and persistence of Staphylococcus capitis NRCS-A in neonatal units in England . Journal of Hospital Infection ,Vol (140), Pages 8-14 .

Nikaeen, M., Shamsizadeh, Z., & Mirhoseini, S. H. (2018). Direct monitoring of gram-negative agents of nosocomial infections in hospital air by a pcr-based approach. Aerosol and Air Quality Research, 18(10), 2612–2617.

Odoya ,M., Ohenhen,R.E., Adias,T.C.(2015) . An Overview of the Distribution of Staphylococcus aureus in the hospital Environment . IOSR Journal of Environmental Science, Toxicology and Food Technology, Vol 9: (6).

Oliveira ,P.S., , Souzaa,S.G., , Campos ,G.B. , Silvaa ,D.C., Sousaa,D.S. , Araújo ,S.P. , Ferreiraa,L.P. , Santos ,V.M. , Amorima,A.T. , Santos ,A.M. , Timenetsky ,J. , Cruz ,M.P. , Yatsudaa ,R., Marques,L.M. (2014). Isolation, pathogenicity and disinfection of Staphylococcus aureus carried by insects in two public hospitals of Vitória da Conquista, Bahia, Brazil. The Brazilian Journal of INFECTIOUS DISEASES, 18(2):129–136.

Paudel, N., Awasthi, N.P., Lekhak, B., Acharya, A.(2022). Prevalence Of Multi Drug Resistant Bacteria On Environmental And Medical Device Surfaces Of Korea Nepal Friendship Hospital. Europe PMC.

Rozman,U.,Marko Pusnik,M., Kmetec,S., Duh,D., and Sostar ,S. (2021). Reduced Susceptibility and Increased Resistance of Bacteria against Disinfectants: A Systematic Review . Journal of Microorganisms,vol 9(12): 2550

Ruiz-Linares,M., Ferrer-Luque,C.M., Arias-Moliz,T., Castro,P., Aguado,B., and Pilar Baca,P. (2014) . Antimicrobial activity of alexidine, chlorhexidine and cetrimide against Streptococcus mutans biofilm . Ann Clin Microbiol Antimicrob, 13: 41.

Shi,G. Boost,M., Cho,P. (2015). Prevalence of Antiseptic-Resistance Genes in Staphylococci Isolated From Orthokeratology Lens and Spectacle Wearers in Hong Kong. Immunology and Microbiology, Vol 56(5).

Tapouk.F.A, Nabizadeh.R, Mirzaei.N, Jazani.N.H, Yousefi.M and Hasanloei.M.A,. (2020) .Comparative efficacy of hospital disinfectants against nosocomial infection pathogens . Antimicrobial Resistance and Infection Control , 9(115) .

Vali,L., Davies,S.E., Lai,L.G. , Dave,J.,.Amyes,S.G.B.(2008). Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant Staphylococcus aureus isolates .Journal of Antimicrobial Chemotherapy, Vol 61(3): 524–532.

Zahornacky,O., Porubcin,S., Rovnakova,A., and Jarcuska ,P. (2022) . Gram-Negative Rods on Inanimate Surfaces of Selected Hospital Facilities and Their Nosocomial Significance . International Journal of Environmental Research and Public Health .