Evaluation of the Antibacterial Activity of Some Commercial Disinfectants against Methicillin-Resistant *Staphylococcus aureus*

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**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major problem in the hospital as well as in the community setting. The resistance of MRSA to all β-lactam antibiotics and the most commonly prescribed group of antimicrobials for staphylococcal infections poses a significant limitation to the treatment of diseases caused by this multiple drug resistant strain. The study determined the antibacterial activity of some commercial disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from man and animal sources. A total of six disinfectants namely; Isol, Dettol, Roberts, Purit, Savlon, and Z-germicide were used for this study. The disinfectants were tested for antibacterial activity using agar well diffusion method. Maximum Inhibitory Dilution (MID) was also done using agar dilution technique. The extinction time of the different disinfectants used against the eight (8) MRSA isolates were determined by tube dilution method. The results revealed that the disinfectants had plausible inhibition zone diameters (IZD) ranging from 5 – 20 mm. The Maximum Inhibitory Dilution (MID) ranged from 1:125 – 1:500. The range of the Maximum Bactericidal Dilution (MBD) was from 1:62.5 – 1:500 and that of the extinction time was from 30 – 50 minutes. Isol had the highest activity even at lower concentrations when compared to the other disinfectants. There was a significant increase in the mean IZD of the disinfectants against the MRSA isolates. Thus, these disinfectants showed promising disinfecting properties and could be employed in the eradication of infections caused by MRSA pathogen.


**INTRODUCTION**

The history of antimicrobial agents began with the observation of Pasteur and Joubert, who discovered that one type of bacterium could prevent the growth of another (Marquis, 2009). They did not know at that time why one type of bacterium failed to grow was that the other bacterium was producing antibiotic. Antimicrobials include not just antibiotics, but synthetically formed compounds as well (Marquis, 2009).

A wide range of chemicals and natural compounds are used as antimicrobials. Disinfectants are antimicrobial agents that are applied to nonliving objects to destroy microorganisms, but in common usage the term is reserved for chemical substances such as mercury dichloride or phenol (Redmond, 2009). The goal of disinfection is to reduce the risk of endemic and epidemic nosocomial infections in patients (Rutala et al., 1997). The evaluation of the effectiveness of an agent for use as a disinfectant must necessarily include an assessment of its ability to kill a wide variety of infectious organisms and the phenol coefficient tests are often preferred in the evaluation of disinfectants (Okore, 2005).

Different types of microorganisms vary in their responses to antiseptics and disinfectants because of their different cellular structures, composition and physiology. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of those microorganisms of increasing concern in the
realm of healthcare. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to beta-lactam antibiotics such as methicillin, amoxicillin, penicillin, nafcillin, and oxacillin (Klevens *et al*., 2007). MRSA is often referred to in the press as a “superbug” (Klein *et al*., 2007). It is a multi-drug – resistant *S. aureus* or oxacillin-resistant *S. aureus* (ORSA) that has been responsible for many human infections that are difficult to treat (Klein *et al*., 2007) hence, it is a major cause of both nosocomial and community- acquired infections (Klevens *et al*., 2007).

Although bacterial resistance to antibiotics has been extensively studied, only a few reports are available on disinfectant-resistant microorganisms. This study is designed to screen the activities and extinction time of some disinfectants against MRSA isolates.

**MATERIALS AND METHODS**

**Collection of Bacterial Isolates**

Stock cultures of eight methicillin-resistant *Staphylococcus aureus* obtained from man and animal were used in this study. The stock cultures were maintained on nutrient agar slants at 4°C in the Microbiology Diagnostic Unit, Department of Veterinary Pathology and Microbiology, University of Nigeria Nsukka until needed for further studies.

**Disinfectants**

Commercially available disinfectants used in the study include: Isol (Handis and Dromedas Ltd Enugu Nig.), Dettol (Reckett Benckiser Nig. Ltd Ogun Nig.), Roberts (Roberts Pharm. Ltd. Nig.), Purit (Sarolfi Paea Ltd, Apapa, Nig.), Salvon (Phamedica Lab. Ltd, South African) and Z-germicide (Gorgoni Co. Ltd). They were all purchased from Nsukka town, Enugu State, Nigeria.

**Preparation of MacFarland Turbidity Equivalent Standard**

A 1 % v/v solution of Sulphuric acid was prepared by adding 1 ml of concentrated Sulphuric acid to 99ml of water. A 1 % v/v solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride (BaCl₂ - H₂O) in 50 ml of distilled water and adding 0.6 ml of the barium chloride solution to 99.4ml of the sulphuric acid solution (Buchanan and Gibbons, 1984).

**Test for Methicillin-Resistance**

*Staphylococcus* isolates were evaluated for methicillin-resistance using the agar diffusion technique (CLSI, 2002) and antibiotic discs of oxacillin (30µg) (Oxoid Limited Basingstoke Hampshire, England) were used.

A single colony of each test isolate was picked with a wire loop and inoculated into nutrient broth and incubated for 3hours. The turbidity of each broth culture was adjusted to correspond to 0.5 McFarland turbidity standards (corresponding to approximately 10⁶cfu/ml). Each standardized broth culture was used to inoculate the surface of the Nutrient agar plate. The excess broth was drained into disinfectant jar and the surface of each inoculated plate was allowed to dry. Using a disc dispenser, the antibiotic discs were aseptically placed on the surface of the inoculated agar plates, one disc for each plate and the plates were then incubated at 37°C for 24hrs. After incubation, the plates were examined for inhibition zone around the disc. The zone diameters were measured with a meter ruler and recorded. Each test was conducted three times and the mean inhibition zone diameter (IZD) recorded to the nearest whole millimeter (mm). Each test isolate was classified as resistant to oxacillin in accordance with the guide lines given by the CLSI (2002).

**Evaluation of Disinfectants for Antibacterial Activity**

Each of the nutrient agar plates was inoculated with a 10µl of the (0.5 MacFarland turbidity standard) test organism. Sterile Cork borer was used to make wells of 8mm in diameter on the nutrient agar plate after solidification of the medium. 50µl each of the different dilutions (1:3.125, 1:6.25, 1:12.5, 1:25, 1:50) of the various disinfectants was added in the wells with a micropipette. Six wells were made on each plate i.e. two wells for each disinfectant and the plates were incubated at 37°C for 24hours (Pharmaceutical Codex, 1979). The inhibition zone diameter (IZD) produced by the various disinfectants against the test organisms were measured. Each of the experiment was carried out in duplicate and the average values were taken (Pharmaceutical Codex, 1979).

**Determination of Maximum Inhibitory Dilution (MID) of the Disinfectants against the MRSA Isolates**

Agar dilution method was employed to determine the MID of the six commercial disinfectants (Isol®, Dettol®, Roberts®, Purit®, Savlon®, and Z-germicide) against the test MRSA isolates (CLSI, 2002). Nutrient agar was prepared and dispensed in 18ml amount in universal bottles and sterilized by autoclaving at 121°C for 15minutes. For each disinfectant, four different dilutions were made starting with the stock disinfectants prepared with sterile distilled water. Two-fold serial dilutions of each stock solution were made with sterile distilled water contained in test tubes. After the serial dilution, the content of each test tube was added to 18ml of already prepared and sterilized molten agar together with 1ml of 5% sterile yeast solution (representing an organic matter), mixed thoroughly and poured into sterile Petri dish determined as the highest dilution that inhibits visible growth of microorganisms. Plain nutrient agar and 1ml of 5% sterile yeast solution (i.e. without any of the disinfectants) was inoculated as positive control. At the end of incubation period, the absence of growth in the plates was recorded as evidence of no growth (CLSI, 2002).

**Maximum Bactericidal Dilution (MBD) of the Disinfectants**

After completion of the MID procedure, the agar plates showing no growth in the MID tests were used for the determination of the MBD. Blocks were cut out from the plates that showed no growth in the MID test and transferred to a corresponding test tube of fresh nutrient broth, acting as the recovery medium. The newly inoculated broth medium was incubated for 24 hours.
between 32°C and 35°C. At the end of incubation, microbial growth was ascertained by checking the turbidity of the medium. The absence of growth (no cloudiness in the broth) in the recovery medium was recorded as evidence of total cell death of Isol®, Dettol®, Roberts®, Purit®, Savlon®, and Z-germicide against the test MRSA isolates (CLSI, 2002).

**Determination of the Extinction Time of the different Disinfectants**

Using a drop pipette, a standardized suspension of the test bacterium was inoculated into two different dilutions (1:6.25 and 1:3.125) each of the six different commercial disinfectants. Immediately after mixing, two drops were transferred to 42 separate sterile tubes arranged in 6 rows and 7 columns. The tubes were returned to a water-bath at 20°C. The reaction was allowed to continue for 10 minutes time interval for up to 1 hour (60mins.) after which broth was added to quench the reaction in each row of six tubes. Tubes were incubated and examined for growth. The extinction time was the shortest interval required to kill the organism in all six test tubes in a column. A duplicate reaction was performed and the mean death-time was calculated from the reactions.

**RESULTS AND DISCUSSION**

The summary of the sensitivity of MRSA isolates of different disinfectants is shown in table 1 below. The IZD ranged from 5.0 – 20.0 mm. The dilution 1:25 ml/ml of ISOL had more activity with an IZD of 20 mm than the rest disinfectants dilutions. The summary of the Maximum Inhibitory Dilution (MID) of disinfectants against the 8 MRSA strains is shown in figure 1 below. The MID ranged from 1:125 ml/ml – 1:500 ml/ml with ISOL having the highest inhibitory dilutions against the MRSA isolates. The summary of the Maximum Bactericidal Dilution (MBD) of disinfectants against the 8 MRSA strains is shown in figure 2 below. The MBD ranged from 1:62.5 ml/ml – 1:500 ml/ml with ISOL having the highest bactericidal dilutions against the MRSA isolates. Figure 3 shows the extinction time of disinfectants against methicillin-resistant *Staphylococcus aureus*. The time range was 30 – 50 mins with 1:3.125 ml/ml having better extinction time than 1: 6.25ml/ml dilution.

The nature of action of disinfectants in this study was very encouraging as it revealed activities at varying degrees with different concentrations against the bacterial isolates indicating that they can eradicate MRSA from the environment. The goal of disinfection is to reduce the risk of endemic and epidemic nosocomial infections in patients. A great number of disinfectants are used in the healthcare settings which are sporicidal chemicals when used in appropriate concentrations and are recommended for care of patients, items and instruments (Rutala, 1997).

The disinfectant Isol was found to have higher inhibitory zone diameter (IZD), maximum inhibitory dilution (MID) maximum bactericidal dilution (MBD) and better extinction time than Dettol, Roberts, Purit, Savlon, and Z-germicide at different concentrations or dilutions. The reason for this is due to the fact that Isol is a phenol type of antimicrobial agent which acts by coagulating the cytoplasmic constituents leading to irreversible cellular damage. This result is similar to the observation made by Al-Masaudi *et al.* (1991) that there was no decrease in susceptibility of antibiotic resistant strains to phenolics (phenol, cresol, and chlorocresol) or to the preservatives known as parabens. Some studies have shown that Gram-negative bacteria are generally less susceptible to biocides than Gram-positive species like *Staphylococcus*. Such resistance is likely to be intrinsic rather than plasmid – mediated due to outer membrane that acts as a protective barrier (Russell, 1997). According to Reynolds (2002), the variable nature of microbial populations and their ecological habitat alter the efficacy and predictability of the disinfection process. The innate resistance of the microbe and the intrinsic power of the disinfectant must be in balance, keeping in mind the presence of organic matter, turbidity, excessive numbers of organisms, exposure times or dilution, pH and temperature (Reynolds, 2002). Many reports on resistance have often parallel issues including inadequate cleaning, incorrect product use, or ineffective infection control practices, which cannot be underestimated however increased MICs have been confirmed in particular for *Staphylococcus* (Gottardi, 1985).

In conclusion, a great deal remains to be learned about the mode of action of antiseptics and disinfectants. Although significant progress has been made with bacterial investigations, a great understanding of these
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The increased emergence of antibiotic resistant bacteria suggests the need for heavier reliance on disinfection practices to prevent initial infection. The disinfectants used in this study signified or showed marked antibacterial activity against MRSA isolates. There was statistical significant increase (p< 0.05) in the mean IZD of the disinfectants against the MRSA isolates and are thus recommended for reducing or eradicating risks of endemic and epidermic nosocomial infections of methicillin-resistant *S. aureus* in patients, communities, patient-care items and instruments.

**REFERENCES**


