

## Biocides activity against methicillin resistant *staphylococcus*

Maysaa El Sayed Zaki<sup>1\*</sup>, Nahla Hamed Anbar<sup>2</sup>, Ahmed Abd El Aziz<sup>3</sup>, Tarek El Banna<sup>3</sup> and Wedian Sallam<sup>3</sup>

<sup>1</sup>Clinical pathology department, Mansoura faculty of medicine, Egypt; <sup>2</sup>Fellow of biochemistry emergency Hospital, Mansoura university, Egypt; <sup>3</sup>Department of microbiology, Faculty of pharmacy, Tanta university, Egypt

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### Abstract

**Aim:** This study was designed to evaluate the activity of some of the commonly-used biocides against *Staphylococci* species resistant to methicillin at contact time's equivalent to both hand antisepsis and disinfection of surfaces and medical instrumentation for proper antiseptic recommendations in hospitals.

**Material and Methods:** The study included 600 *Staphylococcus* species resistant to Methicillin collected from Mansoura University Hospitals from clinical samples during the period from January 2010 till January 2014. The minimum bactericidal concentrations (MBCs) of several biocides were determined using a dilution-neutralization method

**Results:** Two of the most widely used disinfectants; activated glutaraldehyde solution and Isopropanol 70.5%, chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% were totally effective against all isolates at concentrations recommended by manufacturers. Best results with ethanol were achieved at a concentration of 80%. Povidone-Iodine; was able to eradicate all isolates only at the concentration of 10%.

**Conclusion:** We can conclude from this study that *Staphylococcus* had acquired resistant to commonly used antiseptic solutions, The most effective were the mixtures of both Isopropanol 70.5% chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% and glutaraldehyde.

**Keywords:** Biocides, *Staphylococcus*, Minimal bactericidal concentrations, Hospital isolates, Dilution sterilization methods

### Core tip

We reported in this article the activity of common used biocides in our hospitals, Mansoura University, Egypt toward clinical isolates of Methicillin resistant *Staphylococci* species isolated during the period from January 2010 till January 2014. Two of the most widely used disinfectants; activated glutaraldehyde solution and Isopropanol 70.5%, chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% were totally effective against all isolates at concentrations recommended by manufacturers. Best results with ethanol were achieved at a concentration of 80%. Povidone-Iodine; was able to eradicate all isolates only at the concentration of 10%.

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### Introduction

Biocides are inorganic or synthetic organic molecules used to disinfect, sanitize, or sterilize objects and surfaces, and to preserve materials or processes from microbiological degradation [1]. Because biocides range in antimicrobial activity, other terms maybe more specific, including “-static,” referring to agents which inhibit growth (e.g., bacteriostatic, fungistatic, and sporistatic) and “-cidal,” referring to agents which kill the target organism (e.g., sporicidal, virucidal, and bactericidal) [2].

Biocides are used extensively in hospitals for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections [2].

The mechanisms of the antibacterial action of biocides are still not perfectly understood. Biocides are likely to have multiple target sites within a bacterial cell. Biocides are known to interact with bacterial cell walls or envelopes (e.g. glutaraldehyde), produce changes in cytoplasmic membrane integrity (cationic agents), dissipate the

\*Corresponding author: Maysaa El Sayed Zaki, Professor of clinical pathology, Mansoura faculty of medicine-El Gomhoria street, Egypt, Tel: 0020502258877, Email: may\_s65@hotmail.com

proton-motive force (organic acids and esters), inhibit membrane enzymes (thiol interactors), act as alkylating agents (ethylene oxide), cross-linking agents (aldehydes) and intercalating agents (acridines), or otherwise interact with identifiable chemical groups in the cell [3].

Because the mechanisms of action of biocides are often poorly understood, detailed evaluation of bacterial resistance mechanisms remains disappointing. Nevertheless, it is known that at least some (efflux, impermeability, modification of target sites) of the general mechanisms responsible for antibiotic resistance are also applicable to biocides. The possibility exists of 'cross-resistance' arising between antibiotics and biocides [3].

Research on antibiotics and biocides has traditionally proceeded along separate lines. The reasons for this are probably because antibiotics rely on selective toxicity for their activity, although this does not imply that they are without side effects on human and animal cells. Selective toxicity, on the other hand, is not a prerequisite for the use of biocides, although their actual and potential toxicity should never be ignored [3].

By the very nature of the usage of the two types of antimicrobial agents, tests for evaluating their antibacterial potency differ considerably. With antibiotics, bacterial susceptibility is determined mainly by disc sensitivity and minimum inhibitory concentration (MIC) procedures. MICs can be linked to blood or serum levels and peak drug concentration and mutant prevention concentration in vivo. By contrast, such methodology has limited applications for most types of biocide evaluations. Many biocides diffuse poorly in agar, some biocides interact with agar constituents, and MICs often provide little more than a starting point for the information needed about the lethal effects of 'in-use' concentrations. Therefore, Standard European tests are increasingly being made available to measure such lethal effects for a variety of purposes [4].

This study was designed to evaluate the activity of some of the commonly-used biocides against *Staphylococci* species resistant to methicillin at contact time's equivalent to both hand antisepsis and disinfection of surfaces and medical instrumentation for proper antiseptic recommendations in hospitals.

## Material and Methods

The study included 600 *Staphylococcus* species isolated from clinical samples from Mansoura University children hospital, Egypt from January 2010 till January 2014. The isolates were resistant to methicillin and vancomycin. Biocides susceptibility was applied to these isolates.

### Biocides susceptibility study

The minimum bactericidal concentrations (MBCs) of several biocides were determined using a dilution-neutralization method [5]. Biocides used are found in Table 1.

The principle was to add the bacterial suspension to a test concentration of the disinfectant and after a predetermined exposure time, an aliquot of the mixture was removed, the disinfectant in the aliquot was neutralized immediately by a validated method, and the mixture examined to determine the extent of microbial inactivation.

The biocides chosen for this study were those widely used

Glutaraldehyde
Povidone-iodine
Isopropanol, chlorhexidine digluconate and H <sub>2</sub> O <sub>2</sub> (Alkanol)
Chloroxylenol
Sodium hypochlorite
Ethanol

**Table 1:** Antiseptic solutions used in the study.

in the hospital setting and in the community. The biocides tested were: activated glutaraldehyde solution, povidone-iodine 10%, Isopropanol 70.5%, chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% mixture, chloroxylenol 4.8%, Ethanol and Sodium hypochlorite 5%. Serial dilutions of each disinfectant were prepared below and above the common user concentration (as indicated by the manufacturer).

The Neutralization medium used was that described by the EN1276 [5]. A mixture of 0.3% lecithin (Applichem GmbH, Darmstadt, Germany), 3% polysorbate 80 (Sigma Chemicals, St. Louis, Mo, USA), 0.5% sodium thiosulfate (Sigma Chemicals, St. Louis, Mo, USA), 0.1% L-histidine (Applichem GmbH, Darmstadt, Germany), and 3% saponin (Sigma Chemicals, St. Louis, Mo, USA), in Tryptone-Sodium Chloride diluents was prepared. The diluents were a mixture of 0.1% tryptone and 0.9% sodium chloride in water. The neutralization mixture was then sterilized by autoclaving at 121°C for 15 min and stored in aliquots until use.

McFarland standard 4 was prepared by adding 0.4 ml of BaCl<sub>2</sub> (1.175% w/v) to 9.6 ml of H<sub>2</sub>SO<sub>4</sub> (1% v/v) with constant stirring to maintain a suspension, and verifying the correct density of the turbidity standard (0.67 Absorbance at 600 nm).

Susceptibility testing was performed as follows (6). Isolates were subculture on Tryptone Soya Agar (TSA) twice for 18-24 hours, and suspended in mueller hinton (MH) broth to McFarland Standard 4 (1.2x10<sup>9</sup> cfu/ml). One micro liter of the cell suspension was inoculated twice into 0.1 ml of each the tested biocide concentrations and was exposed for 5 and 60 minutes respectively (equivalent to the exposure times for hand scrubs (5 min) and soaks (60 min)). Immediately after the end of the exposure time, the remaining biocide was inactivated by transferring 1µl of the bacteria-biocide mixture into 0.1 ml of the neutralization medium. Then, 1µl of the mixture was inoculated into an MH broth without biocide. Bacterial growth was observed after incubation at 35°C for 24 h. The minimal bactericidal (MBC) was determined as the lowest concentration of biocide that completely inhibited the growth of the isolates as detected by the unaided eye.

An effective neutralizer must have three criteria. First, the neutralizer must effectively inhibit the action of the biocidal solution. Second, the neutralizer must not itself be unduly toxic to the organisms. Finally, the neutralizer and active agent must not combine to form a toxic compound [7]. In order to test the effectiveness of the neutralizer and the validity of inactivation of antimicrobial activity of disinfectants by the dilution with the neutralizer, a control was used where the test inoculum was added directly to a prepared mixture of the disinfectant and neutralizer. To ensure that the neutralizer is not toxic to the organism itself, another control was used, where the disinfectant was replaced by physiological saline.

## Results

This study was performed on 600 *Staphylococcus* Methicillin resistant isolates. The MBCs of the six biocides for the isolates at 5 and 60 minutes were determined (Figure 1).

Both Isopropanol 70.5%, chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% and glutaraldehyde were able to eradicate all isolates at concentrations below the recommended user concentrations indicated by manufacturers.

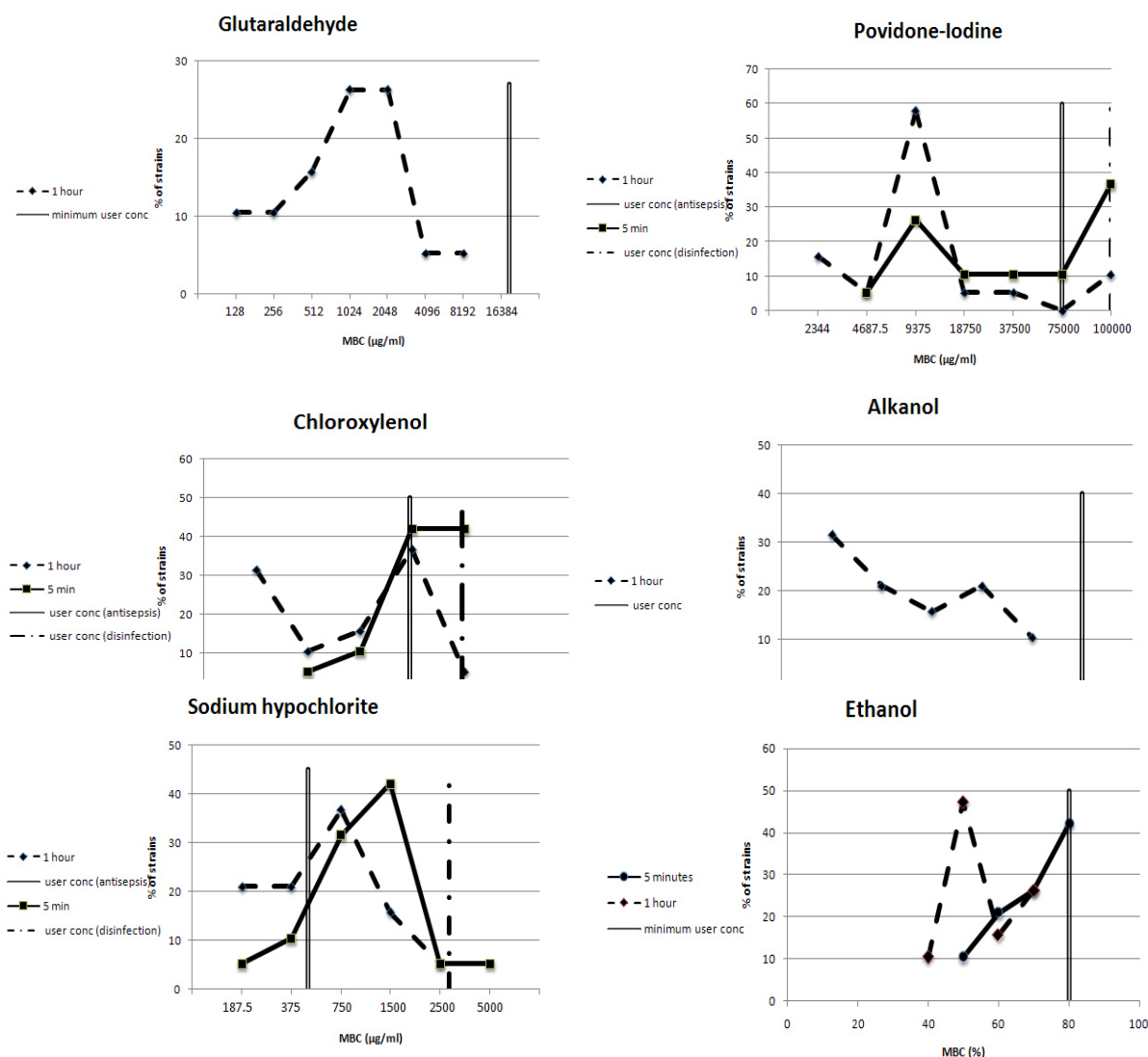
Povidone-Iodine was effective as a bactericidal against all strains at a concentration of 100,000 µg/ml (10% povidone- iodine) at both contact times. However, the minimal user concentration of 75,000 µg/ml (7.5% povidone- iodine) was effective only against 63.8% of the strains at a contact time of 5 minutes.

The MBCs of ethanol at 5 min for the majority of isolates

(42%) were 80%, while for the rest they ranged between 50-70%. MBCs of ethanol at 1 hours contact time was 70% or less for all isolates.

Sodium hypochlorite was effective against 94.6% of the strains at the concentration indicated by the manufacturer (2940 µg/ml) at a contact time of 1 hour. MBC of one strain at a contact time of 1 hour was 5000 µg/ml. Sodium hypochlorite was effective against only 15.8% of the strains at a concentration of 500 µg/ml and contact time of 5 minutes.

Chloroxylenol showed good results as antiseptic against the strains at the concentration recommended by the manufacturer (2285 µg/ml). However, the recommended dilution for environmental disinfection is 1170 µg/ml. One strain was resistant at this concentration and showed an MBC of 2400 µg/ml.



**Figure 1: MBCs of the six biocides at 5 and 60 minutes**

All units are in µg/ml except for ethanol, isopropanol and hydrogen peroxide, which are expressed as %. For Isopropanol, chlorhexidine digluconate and H<sub>2</sub>O<sub>2</sub>, the MBCs a, b, c, d, e and f correspond to the initial undiluted conc. (Isopropanol 70.5%, chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30%), 0.5 the initial conc., 0.25 the initial conc., 0.125 the initial conc., 0.0625 the initial conc. and 0.03125 the initial conc. Respectively.

## Discussion

In this study, the correlation between biocide susceptibility of methicillin-resistant *Staphylococci*, exposure time and user concentrations was investigated in an attempt to determine the best disinfection and antiseptic procedures that can be applied to prevent nosocomial spread of these virulent pathogens.

Results of this study show that both Isopropanol 70.5% chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% and glutaraldehyde were effective disinfectants against multidrug resistant *Staphylococci*.

Chlorhexidine has gained a common use in recent years as skin disinfectants mainly in blood culture collections, preparation of skin in surgical incision and vascular catheter insertion. Our results support its uses [8].

Glutaraldehyde remains a very effective disinfectant despite the concerns about its toxicity. Glutaraldehyde is a powerful disinfectant with a broad spectrum of activity, a rapid microbicidal action, and has the advantage of being non-corrosive to metals, rubber and lenses [9]. Glutaraldehyde has been previously recommended for the cold sterilization and disinfection of several types of medical equipment, including cystoscopes, arthroscopes and laparoscopes and anaesthetic equipment [9]. However, Glutaraldehyde use has been associated with several occupational hazards, including sensitization of skin, eyes and respiratory organs, allergic contact dermatitis, chronic bronchitis and occupational asthma [10]. As a result of its toxicity, glutaraldehyde is no longer used in several countries [8]. It is recommended for health care facilities to follow best practices for safe use of glutaraldehyde, including [11]: i) workers should wear personal protective equipment such as elbow-length gloves or protective sleeves made of glutaraldehyde-impervious material, splashproof goggles or safety glasses, respirators and isolation gowns, lab coats, or aprons whenever there is the potential for skin or eye contact with glutaraldehyde, ii) workers should be educated about the physical and health hazards of glutaraldehyde and the measures they can take to protect themselves, iii) Rooms where glutaraldehyde disinfection/sterilization is performed should be large enough to ensure adequate dilution of vapor and have a good air exchange rate, and iv) glutaraldehyde solutions should be transported only in closed containers with tight-fitting lids and unused glutaraldehyde solutions should be stored in tightly covered containers in a cool, secured, and properly labeled area.

Results also suggest that povidone-iodine might not be totally effective as an antiseptic against multi-drug resistant *Staphylococci* at the concentration of 7.5%. Since povidone-iodine is a widely used antiseptic in hand washing and surgical hand disinfection, skin preparation prior to invasive surgical and non surgical procedures including insertion of intravascular catheters and venepuncture and in antiseptic irrigation [12,13], it is recommended to confine its use to the concentration of 10%.

Both chloroxylenol 4.8% and Sodium hypochlorite 5% were unable to disinfect all strains at the recommended user concentrations. Chloroxylenol 4.8% was effective against all strains at the dilution of 1:20 at both contact times. However,

5.1% and 42.1% of the strains were resistant at the dilution of 1:40 at contact times of 1 hour and 5 minutes respectively. Current recommended dilutions by the manufacturer are 1:20 for skin antiseptic and 1:40 for environmental disinfection. It may be advisable for health-care facilities that use chloroxylenol to use a dilution of 1:20 for both antiseptic and disinfection in order to prevent spread of highly-resistant *Staphylococci*. Similarly, one strain was resistant to 5% sodium hypochlorite at the 1:16 dilution recommended by the manufacturer for disinfection. MBC of this strain was 5000 µg/ml (equivalent to 1:8 dilutions or 12.5% v/v Clorox). It may be advisable for health care facilities in which multi-drug resistant *Staphylococci* are circulating to use lower dilutions.

We can conclude from this study that multidrug resistant *Staphylococcus* had acquired resistant to commonly used antiseptic solutions. The most effective were the mixtures of both Isopropanol 70.5% chlorhexidine digluconate 2% and H<sub>2</sub>O<sub>2</sub> 30% and glutaraldehyde. Revised dilutions and contact durations should be available on large scale studies to improve the efficacy of disinfection processes in health care settings.

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