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Anti-bacterial Activity of N-halamin in Hospital Fabrics: New Synthesis Approach and Examination of Anti-Bacterial Characteristics

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ABSTRACT: An efficient and concise one-pot strategy for the synthesize 1,3-bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione (DMDM) as the anti-bacterial agent has been developed. The reaction took palce in mild and solvent free condition. The synthesized DMDM were characterized by ^{1}H-NMR and Mass analysis. Then DMDM was grafted onto cotton fabrics through new method and examined them for their antibacterial activity against Gram positive and Gram negative bacteria. This fabric provided powerful and rapid antibacterial activity against E.coli as a Gram-negative bacteria and S.aureus as Gram-positive bacteria. These fabrics interestingly eliminate bacteria form hospital textiles. This is very important characteristic in units where the abundance of such bacteria is very high especially in the hospitals.
Key words: DMDM, Antibacterial Activity, Gram positive and negative bacteria, Grafting process.

INTRODUCTION

Vancomycin-resistant enterococci (VRE) and Methicillin-resistant Staphylococcus aureus (MRSA) Infections, caused by antibiotic-resistant gram-positive bacteria are a growing concern, especially in units in which patients are immunosuppressed as a result of trauma or disease. As controlling the spread of these bacteria with antibiotic decreases, bacteria become more and more resistant to antibiotics. The environment can play a important role in the transmission of microorganisms. In 1998, a study showed that outbreak of MRSA was directly attributed to a handheld shower and stretcher [1] in the hospital, and an electronic ear-probe thermometer was ascribed to outbreak of VRE [2]. Admission of patients without VRE infection to a hospital occupied by a VRE-colonized patient was figured out to be an factor for acquisition of VRE by the previously un-colonized individually [3].

Moreover, health workers garments that can easily become contaminated are an important aspect of the environment. Boyce reported that 65% of nurses who had contacted with MRSA-patients, their nursing uniforms or gowns contaminated with MRSA [4]. The ability of that microbe to stay alive on environmental surface is one important factor for transmission of a microorganism from a person to the environment or another person. A few studies have assayed the survival of gram-positive and negative bacteria on different surfaces such as, aluminum foil [5], glass [6], polyvinyl chloride [7], countertops, and stethoscopes [8].

So, given the above, the need for compounds can eliminate these bacteria from hospital fabrics is very necessary nowadays. One of the most important substance that can play this role is N-halamine compounds. N-halamine structures have gained wide attention and appreciation as biocidal agents for a variety of surfaces. N-halamine structures i.e., N-X, where X = Cl or Br have been widely studied and found suitable to be used close to human skin. Chemically, these structures work similar to chlorine bleach in killing the biological agent by oxidation via release of free halogen. The reaction involves release of free halogen, generally chlorine. The N-Cl bond provides the oxidative site which upon reaction with the agents releases chlorine. The released chlorine reduces the agent into less or nonharmful forms, thus helping in reducing the overall toxic threat. N-halamines are stable under the ambient conditions with very low dissociation constants (K < 10-4). Also, since the working reaction is reversible, the surface can be chlorinated to restore the oxidative ability. N-halamines may be inorganic or organic depending on the groups attached to the N atom. If one of the groups is an organic group, the halamine is considered to be an organic N-halamine. Such structures can comprise amines, amides and imides. The types of groups attached to the N-atom determine the efficiency of chlorine release and the relative stability of the compound. For example, in case of the amine structure, the N-X bond is better stabilized due to the electron donating alkyl groups as compared to the imide structure which has two electron withdrawing carbonyl groups. This means that an amine-halamine releases the halogen atom rather slowly as compared to an imide-halamine, thus resulting in an extended activity lifetime. The presence of an α-hydrogen in an amine or amide structure can lead to dehydrohalogenation of the N halamine thus resulting in a loss of biocidal activity. This reaction is promoted by ultraviolet light or heat. To avoid this reaction, a system with no α-hydrogens such as the

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heterocyclic N-halamines is preferred. Recently 5,5-dimethyl hydantoin (DMH) was used as the halamine precursor. For grafting through a free radical mechanism, an allyl monomer of DMH was prepared [9]. The allyl monomer, 3-allyl-5,5- dimehtyl hydantoin (ADMH was graft polymerized onto a variety of surfaces including cellulose, polypropylene, polyester and polyamide. The grafted polymer demonstrated biocidal activity against Escherichia coli (E. coli) and Staphylococcus aureus (S.aureus). The grafted polymer was also durable to laundering and exhibited biocidal efficiency after as many as 20 washes. The biocidal effect of the polymer was easily regenerated by hypochlorite bleach [10-12].

So, thanks to these attractive properties, N-halamine structures have been incorporated into different materials to provide potent, durable, and rechargeable biocidal functions against a broad spectrum of microorganisms. The N-halamine grafted fabrics provided potent antimicrobial activity against both gram-negative and gram-positive bacteria.

MATERIAL AND METHODS

Chemicals and reagents

IR Spectra were recorded on a Shimadzu FT-IR-4300 spectrophotometer; in cm-1. 1H and 13C-NMR Spectra were recorded on Bruker DRX -300 spectrometer (1H NMR 250 MHz); δ in ppm, J in Hz. EI-MS (70 eV) spectra were determined with a HP 5973 GC-MS instrument; in m/z. Melting points were determined with a Electrothermal 9200 apparatus. All reagents and materials were purchased from E-Merck Ltd.

Synthesis of new N-Halamine using formaldehyde and 5,5 dimethyl hydration

20 cc of the commercial 37% formaldehyde aqueous solution (0.2mol) were charged into a bottomed flask with an agitator at 50 °C. The agitator was started and there was admixed about 2 grams of sodium hydroxide (0.05 mol.). Then while continuing the agitation there was admixed 12.8 grams of 5,5-dimethylhydantoin (0.1 mol.). The agitation was continued while holding the reaction mixture temperature at between 39° to 49°C for 4 hours. At the end of that time, the excess formaldehyde solution was removed by rotary evaporation. The residue then was extracted by 50 cc ethyl acetate and 5cc water. The organic layer was vaporized and collected. Finally the oily product was crystalized by n-hexane and ethyl acetate to give white desired product.

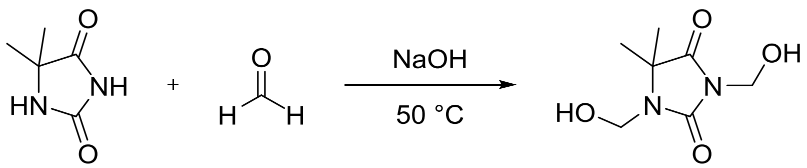

Figure 1. Synthesis of 1,3-bis(hydroxymethyl)-5,5 dimethylimidazolidine-2,4-dione

Products characterization data of N-halamine compound

1,3-bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione (DMDM): white solid (13.7 g, 73% yield); ^{1}HNMR (250 MHz, CDCl3) δ 6.23 (t, j= 7.0 Hz, 1H), 5.85 (t, j= 6.7 Hz, 1H), 4.85 (t, j= 7.75 Hz, 4H), 1.44 (s, 6H; MS(EI) m/z (relative intensity) 187.

Grafting DMDM onto cotton fabric

12 grams of DMDM was added to 0.3 gram of Triton X-100 as a wetting agent in 300 milliliters of deionized. The pH must be adjusted to 3.4 with 1 milliliter HCl (0.1 N). 70 grams of pure cotton fabric (#400 Testfabrics, Inc., Middlesex, N.J.) , 70 grams of cotton/polyester (35/65) and blend fabric (#7409, Testfabrics, Inc., Middlesex, N.J.) were immersed in the bath for five minutes and then padded with a more than 80% pick-up rate through a padder. These fabrics were dipped and padded again, and then dried at 80° C for 5 minutes. Then, the fabrics were cured at 160° C for 5 minutes. Finally, the fabrics were washed with 45 grams of American Association of Textile Chemists and Colorists (AATCC) at a low level of water at 60° C for 30 minutes. The resulted fabrics were dried. The yield was 21 grams (1.36% add-on) of the cotton fabric and 71 grams (0.73% add-on) of the cotton/polyester blend fabric.

Antibacterial Functions

The antibacterial assessments were conducted according to AATCC Test method 147-2004. Staphylococcus aureus (Gram-positive bacteria) and E. coli (Gram-negative bacteria) were used as antibacterial function of the DMDM-coated textiles. Sterile bacteriostasis agar was distributed in petriplates. The bacteria were cultured for 24 hours, 1 loop full of culture was loaded by 2 mm inoculation loop and spread to the surface of the agar plate by making 7.5 cm long paralled streaks 1 cm far of center of the petriplate. The test sample was pressed cross overly, through the five inoculums of streaks to warrant intimate contact with the agar surface. The palates were incubated at 37° C for 18-24 hours. After incubation, a streak of interrupted growth underneath and along the side of the test material indicates antibacterial effectiveness of the fabrics.

RESULTS

We began our study by evaluating various conditions for the hydroxylation of 5,5-dimethyl hydantoin in the presence of 37% formaldehyde solution (Table 1). DMDM was obtained with excellent yield in presence of formaldehyde (0.2 mol.), and NaOH (0.05 mol) (Table 1, entry 1). The reaction was tested in the presence and absence of and found that best results aquire In the absence of solvent led to 73% yield (entry 1,2). It was found that the best temperature for the reaction was 50 °C (entry 5-7). Furthermore, the amount of base plays the key role for the formation of DMDM (entry 7-8).

Table 1. Selected Results of Screening the Optimal Conditions

Entry	Solvent	Base(mol)	Temp.	Yield %
1	-	NaOH (0.05)	50	73
2	H2O	NaOH(0.05)	50	46
3	-	KOH(0.05)	50	61
4	-	Sodium bicarbonate(0.05)	50	45
5	-	NaOH	100	68
6	-	NaOH	R.T	35
7	-	NaOH(0.025)	50	51
8	-	NaOH(0.1)	50	56

The DMDM-grafted fabrics demonstrated potent biocidal activity against gram-positive and negative bacteria. This fabrics showed the antibacterial characteristic against E.coli after 24 hours (Figure 2, a & b). As shown in the Figure 2, all E.coli bacteria eliminate from the DMDM-gfated fabric whereas the sample fabric included simple fabric without DMDM-grafted (Figure 2, c) show no antibacterial activity. This effect was maximized and all bacteria were eliminated from the textile in the second iteration (Figure 2, b). DMDM-grafted textile show same properties against S.aureus (figure 3). White thin strips in the petri plate show the growth of bacteria.

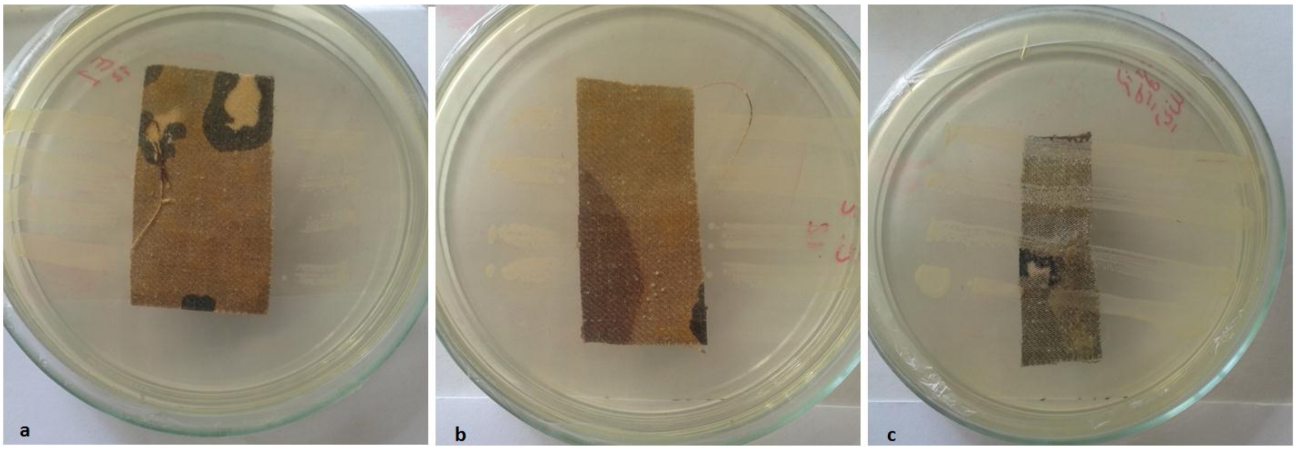

Figure 2. Results after 24 hours; a) first iteration against E.coli; b) second iteration against E.coli; c) the control sample without DMDM-gfated.

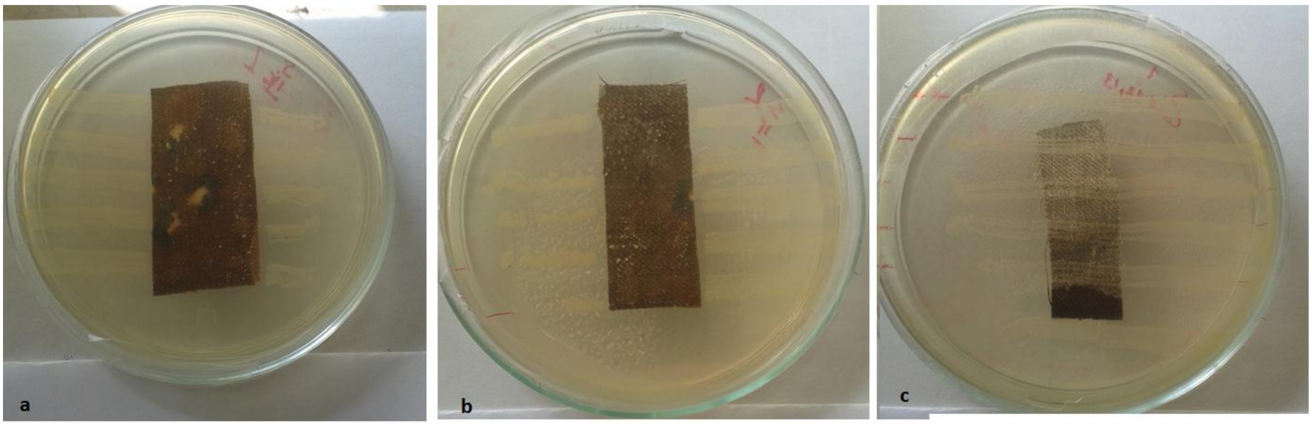

Figure 3. Results after 24 hours. a) first iteration against S.aureus; b) second iteration against S.aureus; c) the control sample without DMDM-gfated.

DISCUSSION AND CONCLUSION

In 2002, Sun and Co-workers reported durable and re-generable antimicrobial textile materials prepared by a continuous grafting process [13]. In this study, a cyclic-amine monomer, 3-allyl-5,5-dimethylhydantoin (ADMH), was grafted onto various textile materials in a continuous finishing process to prepare antibacterial textiles. Research group of Worley described Antimicrobial treatment of nylon [14]. They discussed preparation of an antimicrobial nylon material and its properties. Biocidal cyclic N-chloramine moieties were covalently bonded to Nylon 66. In another study, Sun et al. disclosed refreshable N-halamine polymeric biocides. This group Grafted hydantoin-containing monomers onto high performance fibers by a continuous process [15].

In conclusion, We have demonstrated the novel synthesis approach for 1,3-bis(hydroxymethyl)-5,5-dimethylimidazolidine-2,4-dione (DMDM). Notably, this work provides mild and solvent free conditions to yield the DMDM. This product was grafted onto cotton fabrics upon a AATCC Test method 147-2004. This fabric provided powerful and rapid antibacterial activity against E.coli as a Gram-negative bacteria and S.aureus as a Gram-positive bacteria. This feature can be used in hospital fibers where growth of these bacteria is abundant. These fabrics interestingly eliminate bacteria form hospital textiles.

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