

# A new strategy to control the proliferation of microorganisms in solid hospital waste and the diffusion of nosocomial infections

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## SUMMARY

A possible tool to reduce nosocomial infections is to identify unknown sources of contamination and then to provide a measure for controlling the related infections. In this study, solid hospital waste was considered a potential source of contamination, and a strategy to reduce the potential risk of pathogen contamination was tested. This paper describes a novel technique for waste management in healthcare settings with a view to facilitating infection prevention and control. We explored the innovative use of sodium dichloroisocyanurate (NaDCC) by investigating the microbicidal activity of chlorine, which derives from the hydrolysis of NaDCC mediated by humidity, and by testing its effect on the inhibition of microorganism growth.

NaDCC was inserted in a solid hospital waste bin containing also Lauria-Bertani agar plates, with different dilutions of a known titre of three different microorganisms, namely *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus brasiliensis*. The plates were incubat-

ed in the container with or without the antimicrobial agent (control, CNT) at room temperature for 5 days. The number of colony-forming units (CFUs) present on each plate was then counted. Microorganisms capable of proliferating in the CNT waste bin were not able to grow in the presence of NaDCC. Furthermore, the molecular chlorine which developed and was released in the waste bin under the experimental conditions (T=20°C, t=5 days) was quantified using iodometric titration. NaDCC hydrolysis, mediated by humidity, has a strong and long-lasting microbicide effect. The proliferation of tested bacteria and fungi is totally inhibited. These results demonstrate the effectiveness of NaDCC in controlling and/or inhibiting microbial proliferation and support its possible use in the treatment of hospital waste to control the spread of nosocomial contamination.

*Keywords:* healthcare waste, nosocomial infection, NaDCC, prevention, waste.

## INTRODUCTION

Because of the increasing concern for healthcare associated infections (HAI) by the public and health care systems, preventing the diffusion of infection within the healthcare environment is

assuming a primary importance. HAIs are among the major causes of death and increased morbidity among hospitalized patients, with a minimum of 175000 deaths every year in industrialized countries [1]. Breaking the chain of transmission includes many strategies such as hand hygiene and usage of barrier protection, however, an important but often overlooked aspect is environmental decontamination, such as the identification of unknown potential source of contamination [2, 3]. Although the role of the healthcare

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environment in the spread of some infections is far from universally agreed upon, circumstantial evidence suggests that contaminated hospital environmental surfaces can be a risk factor for infection caused by some pathogens. In addition, there has been increased recognition that environmental measures should form a crucial component of the overall strategy for preventing healthcare-associated infections [4-6]. Cleaning has never been regarded as an evidence-based science and consequently receives little attention from the scientific community [7]. Nevertheless to reduce the diffusion of microbial infection, improved cleaning strategies have been adopted in hospital settings where the uptake of infections is much higher and more frequent [8].

However, there is still not enough attention towards a different possible font of contamination such as the solid waste hospital garbage containing materials possibly infected.

Circumstantial evidence suggests that contaminated hospital waste can represent a risk for public health and for the environment outside from the hospital, such as a pollution of water source [9, 10], thus requiring expensive procedures to be adopted to treat and to dispose the contaminated hospital waste. Those advocating an important role for the solid hospital waste as a reservoir of nosocomial pathogens have argued that effective waste decontamination treatment is important in helping to avoid the cycle of infection transmission inside and outside the hospital.

On one hand, specific attention is paid to the fate of healthcare waste after they leave the hospital facility, in fact there are a number of scientific publications aimed at testing new strategies for their treatment other than incineration. On the other hand, there does not seem to be any publications aimed at proposing a strategy of containment of microbial growth in hospital waste in the main time when the waste remains in the hospital wards [11-15]. Moreover, in some circumstances, infectious or hazardous waste could not be adequately separated from ordinary domestic type waste, thus enhancing the amount of waste classified as infectious and needing special treatment.

In this scenario, we were intrigued to search for a simple and cheap strategy to arrest or inhibit the microorganism proliferation in the healthcare waste decreasing the potential risks of hazardous

waste management and general occasional contact. We focused our attention to promote a new strategy to treat the hospital waste with the aim of minimizing and, where possible, eliminating pathogen microorganism growth during the storage of waste in the healthcare facilities.

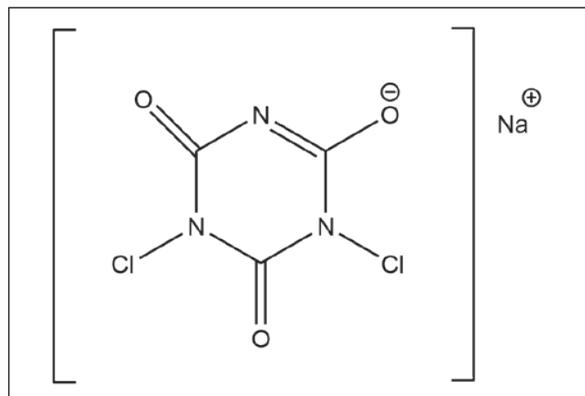
Therefore, we tested innovatively whether the chlorine emitted by the slow hydrolysis of NaDCC, in the gas phase, was able to inhibit the proliferation of pathogen Gram-negative bacteria *Escherichia coli* and pathogen Gram-positive *Staphylococcus aureus* as well as the fungi *Aspergillus brasiliensis*, which is a known sporogenic species [16]. The tested microorganisms, in particular *Escherichia coli* and *Staphylococcus aureus* are known to be present in various clinical solid waste, general waste and clinical sharp waste. Therefore, they were considered ideal candidates for our study [12].

NaDCC is already largely employed in hospital disinfection procedures as antimicrobial agent for its efficacy against both bacteria and fungi; however, it has been adopted as disinfectant only as aqueous solution, at direct contact with microorganisms whereas our approach is principally based on the efficacy of NaDCC gas phase [17]. NaDCC has been recently approved by the United States Environmental Protection Agency (USEPA) and the World of Health Organization (WHO) for the routine treatment of drinking water since many studies, performed to investigate the toxicity and irritancy of this product, have given a positive feedback. These molecules are not metabolized by the organism and do not bioaccumulate, for this reason they are considered safe to handle. The studies on development toxicity have also established that NaDCC is not fetotoxic, teratogenic mutagenic or carcinogenic [18].

## ■ MATERIALS AND METHODS

### *Reagents*

The reagents sodium dichloroisocyanurate (NaDCC, Figure 1) 96% CAS 2893-78-9 and sodium chloride were provided by Sigma Aldrich (St. Louis, MO, USA). The reagents used to prepare the LB growth media for microorganisms (Bacto Tryptone, Bacto Yeast Extract, and Bacto Agar) were provided by BD Bioscience (Franklin Lakes, NJ, USA).



**Figure 1** - NaDCC is the sodium salt of dichloroisocyanuric acid.

#### *Microorganisms and media*

The bacterial strains used for these experiments, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538), were deep frozen for storage (at  $-80^{\circ}\text{C}$ ) and grown on LB agar plates at  $37^{\circ}\text{C}$  overnight to  $10^6$  colony-forming units (CFU)/mL. On the other side, the strain *Aspergillus brasiliensis* (ATCC 16404) was deep frozen for storage (at  $-80^{\circ}\text{C}$ ) and grown on LB agar plates at  $30^{\circ}\text{C}$  overnight to  $10^5$  colony-forming units (CFU)/mL.

#### *Antimicrobial activity of NaDCC vapour phase*

We inserted 2 g of NaDCC in a plastic garbage can (capacity: 60 L) to evaluate its antimicrobial action. In particular, we tested the capability of molecular chlorine ( $\text{Cl}_2$ ), released by hydrolysis mediated by air humidity, to kill the microbial cells. In the garbage can were, also, inserted LB agar plates on which different dilutions of a known tittle of the tested microorganisms (about  $10^6$  CFU/mL *Escherichia coli*,  $10^5$  CFU/mL *Staphylococcus aureus*,  $10^5$  CFU/mL *Aspergillus brasiliensis*) were spread. For each experimental group, we also followed control experiments based on plates with a known number of colonies (dilution  $10^{-4}$  and  $10^{-3}$ ) of bacteria or fungal cells incubated in the garbage can (capacity: 60 L) without the antimicrobial agent. Each plate was left for 5 days at room temperature, at a relative humidity of about 45%, in the garbage bin with NaDCC (experimental groups) or without the antimicrobial agent (control groups). At the end of this period, the number of CFU formed was counted.

#### *Quantification of active $\text{Cl}_2$*

Molecular chlorine ( $\text{Cl}_2$ ) levels were measured using a iodometric titration with a method described in a previous paper [19]. 10 mL of the gas phase was injected in a reactive solution prepared by dissolving 1.0 g of KI (99.9%, Sigma-Aldrich, St. Louis, MO, USA) in 10 mL of distilled water and 5 mL of  $\text{H}_2\text{SO}_4$ /distilled water 1:1 v/v. The formed  $\text{I}_2$  was determined using a 0.1 N sodium thiosulphate solution (Sigma-Aldrich, St. Louis, MO, USA) using soluble starch as an indicator.  $\text{Cl}_2$  mass was determined from the volume of sodium thiosulphate and then its concentration in the can was calculated, referring to a volume of 60 L.

The analysis was conducted over the 5 days of testing phase to estimate the amount of chlorine emitted by the sodium dichloroisocyanurate hydrolysis.  $\text{Cl}_2$  levels were then correlated to the antimicrobial activity in the gas phase of NaDCC.

## ■ RESULTS AND DISCUSSION

As results from the literature, the nosocomial infections are increasing in strategic importance since they are considered chiefly responsible for the chain of events that determine the death of hospital patients [1]. For these reasons we considered this simple, not expensive, and not dangerous strategy as an important innovative approach to control an unknown or hidden source of nosocomial infection.

Widely used disinfectants in nosocomial and domestic settings are based on chlorine solutions [5, 6, 8, 20-24]. One of chlorine-releasing agent, such as sodium dichloroisocyanurate, has been largely used as sanitizers in treatment of drinking water and sewage, disinfection of water for swimming pools and industrial cooling towers, cleaning of sanitary and dental products, disinfection of environmental and hospital surfaces, irrigation system for flowers, medical equipment and laundry [18, 20, 21, 25-27]. Moreover, several papers have reported the higher efficacy of NaDCC with respect to NaOCl against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria as well as fungi and viruses which are often relevant to hospital settings. In many cases, the experimental results have recommended NaDCC instead of NaOCl for its biocidal effectiveness. In fact, it showed an increased resistance to

inactivation by organic material, slow decomposition and release of HOCl, capacity to maintain an appropriate level of available chlorine without affecting the pH of the water, lower corrosiveness to metal, plastic and rubber [20-22, 25].

Table 1 shows the experimental results obtained testing the NaDCC gas phase as microbicide agent. The number of colony-forming units (CFU)/mL for both bacteria and fungi is reported. The molecular chlorine emitted by NaDCC hydrolysis allowed a complete inactivation of *E. coli*, *A. brasiliensis* and *S. aureus*. In fact, these microorganisms freely grew in waste bin without NaDCC, whereas they were completely inhibited in that one containing NaDCC. The experimental results seem to demonstrate that this treatment could be efficient against both Gram-positive and Gram-negative pathogen microorganisms, but also against fungi and spores. At the same time, we monitored the chlorine released from solid tablets of NaDCC in the restricted environment of waste bin (volume = 60L) at room temperature (20°C) by iodometric titration. The results show that the chlorine concentration remained constant (2.0 g/m<sup>3</sup>) for five days.

We have chosen to monitor for 5 days since in the case of dangerous biohazard waste, temporary storage of infectious waste may have a maximum duration of five days from the moment the container is closed (D.P.R. 254/2003 Italian Legislation, which regulates the management of waste at the hospital settings).

According to the D.P.R. 254/2003 the sanitary waste must be managed in order to reduce its dangerousness, to favor its decontamination. Competent authorities and health structures must take initiatives direct to encourage the prevention and proper management of health waste as a priority, especially to minimize the contact

of uninfected materials with potential infectious sources and reduce the production of hazardous waste infectious. The sanitary waste at infectious risk must be sterilized in authorized facilities. However, these latter are always located outside the wards and before the waste reaches them the proliferation of microorganisms can be very high. The director or health manager and the head of sterilization facilities are responsible of the activation of the plants and the effectiveness of the sterilization process in all its phases.

Numerous studies have been conducted to identify the best solid hospital waste management strategy in order to minimize risks to public health and environmental contamination [11-14].

However, the literature on the spread of infectious diseases related to the handling of clinical waste is extremely limited and the strategies adopted to contain the proliferation of microorganisms present in hospital waste are even more scarce [12, 13, 28-30].

Although our objective was not to examine the adverse health effects associated with the presence of contaminated waste in the hospital facilities, the present study shows the possibility to reduce and/or inhibit the proliferation of microorganisms. These latter, in many cases, are pathogens and, in some cases, they are multi-resistant strains [12, 31, 32]. The pathogen concentration reduction in solid hospital waste could be considered as a useful tool to prevent their dispersion in the environment, the contact with workers and also with patients having compromised immunological systems. This procedure would also allow avoiding the dispersion of aerosol and/or simplifying all treatment steps that are foreseen in case of accidental unwinding of the container during transport.

From the results here reported we can infer that NaDCC has the potential to be applied for the treatment of solid hospital wastes by controlling microbial proliferation and consequent potential diffusion of nosocomial infection. This, in turn, will favour hygiene of environment, decrease of health and environmental risks, decrease of economical investment for hazardous waste management.

The presented strategy of hospital waste treatment, avoiding the spreading of microorganisms and bioaerosol in the environment, could be an attractive strategy in the handling of hospital

**Table 1** - Antimicrobial effect mediated by chlorine released by NaDCC during the 5 days. In this table, the number of colony-forming units (CFU) is reported for each tested microorganism and compared between the two experimental groups (CNT+ vs. NaDCC).

Microorganisms	Experimental group (CFU/mL)	
	CNT +	NaDCC
<i>Escherichia coli</i>	33.5 ± 0.4 × 10 <sup>6</sup>	0
<i>Aspergillus brasiliensis</i>	1.7 ± 0.8 × 10 <sup>5</sup>	0
<i>Staphylococcus aureus</i>	1.3 ± 0.6 × 10 <sup>5</sup>	0

and biomedical waste containers that are a potential source of pathogens, as well as providing a better protection for physicians and technicians operating in the aforementioned settings [33]. It is inexpensive and could also reduce the costs of waste disposal treatments, lowering in some way the impact that this hazardous waste could have on the environment and somehow orienting the treatment to decrease of environmental risk and an increase of health security so chased by many recent scientific publication and research project [34].

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### Conflict of interest

The authors declare no conflict of interest.

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