Comparative Immunology, Microbiology and Infectious Diseases 47 (2016) 60–71

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Efficacy of selected biocides in the decontamination of common nosocomial bacterial pathogens in biofilm and planktonic forms

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ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form 13 June 2016
Accepted 16 June 2016

Keywords:
Planktonic bacteria
Biofilm
Biocides
Nosocomial infection

ABSTRACT

The efficacy and use of biocides to eliminate pathogens in the health care environment are based on their testing against planktonic bacteria. In the environment, bacteria exist in biofilms, as they do on medical devices, and as planktonic or viable non-culturable forms as well. This work aimed to evaluate the efficacy of four biocides against the biofilm and planktonic phases of nine common nosocomial bacteria. The bactericidal activity of the biocides against bacteria in the planktonic form was assessed using a broth microdilution technique. The killing activity of the biocides against biofilms was evaluated using cells grown on polyethylene tubes under a dynamic flow-cell system that was designed for biofilm growth. All biocides completely killed the planktonic bacteria at all concentrations; however, they did not eradicate the biofilms of the same pathogens. Our study highlights the need for an alternative strategy, one that utilizes chemicals that have been tested to disrupt or prevent biofilm growth, in order to enhance current disinfection practice.

1. Introduction

Biofilms are complex bioactive structures composed of one or more microbial species protected by a matrix of extracellular polysaccharides. Biofilm formation starts with the initial attachment of planktonic bacteria to a solid surface. The adherent cells multiply leading to microcolony formation and ultimately to the accumulation of multilayered clusters of cells within bacterial polysaccharides and other types of matrices [1]. Eventually, the biofilm becomes thicker and, when disrupted, leads to the dissemination of bacterial cells [1–3]. Bacteria in biofilms are more resistant to antimicrobial agents than are the same organisms grown planktonically [4–6]. Such resistance is demonstrated not only towards antimicrobials but also towards preservatives, disinfectants, and antiseptics [4,5].

In the medical setting, biofilm-associated infections constitute a steadily increasing problem that arises from the surface of different indwelling devices such as intravenous catheters, alloplastic materials, hydrocephalus shunts, and artificial hearts [1]. Implant-associated infections lead to considerable morbidity, repeated surgeries, prolonged antibiotic therapy, and mortality [7]. The mortality rate due to cardiovascular catheter-related biofilm infections, for example, is estimated to be 12–25%, with additional healthcare cost on the order of $33,000 to $35,000 per event [8].

Although surface disinfection is routinely carried out in hospitals to prevent bio-contamination of surfaces, biofilm embedded bacteria usually show resistance to such biocidal treatment [9,10]. Many commercial biocides have been evaluated for their efficacy against planktonic bacteria [11]. However, only minimal data are available on the biocidal activity of such compounds against common nosocomial pathogens in the biofilm mode of growth.

The aim of this study was to evaluate the antimicrobial activity of four biocides, glutaraldehyde, sodium hypochlorite, peracetic acid, and hydrogen peroxide, against the biofilm and planktonic phases of growth of nine nosocomial bacterial pathogens. The tested pathogens include *Acinetobacter baumannii*, *Burkholderia cepacia*, *Enterococcus faecalis*, *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Escherichia coli*.

2. Materials and methods

Unless otherwise indicated, all chemicals were of analytical grade and were purchased from Sigma-Aldrich, Saint Louis, Missouri, USA.

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http://dx.doi.org/10.1016/j.cimid.2016.06.002
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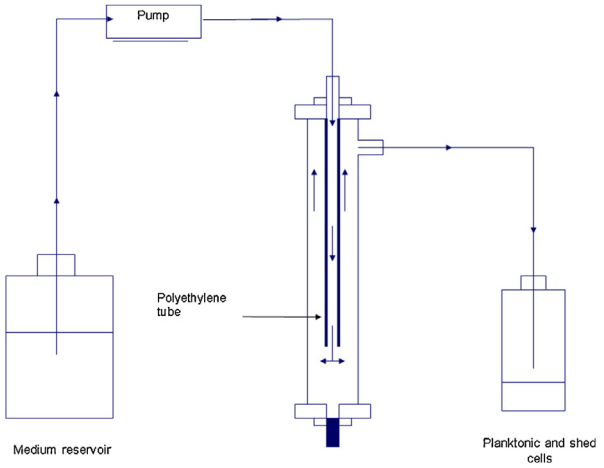

Fig. 1. An *in vitro* biofilm device.
The device consists of a 20 cm stainless steel chamber used to study biofilms. The chamber is plugged with a stainless steel disc in which a polyethylene tube is inserted from inside. Bacterial suspensions in tryptic soy broth (TSB) are used to fill the chambers and initiate biofilm formation on the tube.

2.1. Disinfectants

Glutaraldehyde (GLA), hydrogen peroxide (HPO), peracetic acid (PAA), and sodium hypochlorite (SHC) were purchased from Fisher-Scientific, USA.

2.2. Microorganisms

Eight clinical isolates, including *Acinetobacter baumannii*, *Burkholderia cepacia*, *Enterococcus faecalis*, *Enterococcus faecium*, methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*, and one reference strain of *Escherichia coli (*ATCC 25922), were used in this study (Table 1).

2.3. Evaluation of the killing activity of the biocides against the planktonic phase of bacteria

The minimum bactericidal concentrations of the biocides required to kill all bacteria (MBC_{100}) were determined by using a modified, quantitative method as described by Kawamura-Sato et al. [12]. Briefly, twofold serial dilutions of the disinfectants in distilled water were placed in 96-well microplates. Ten microliters of bacterial suspension in normal saline was added to each well

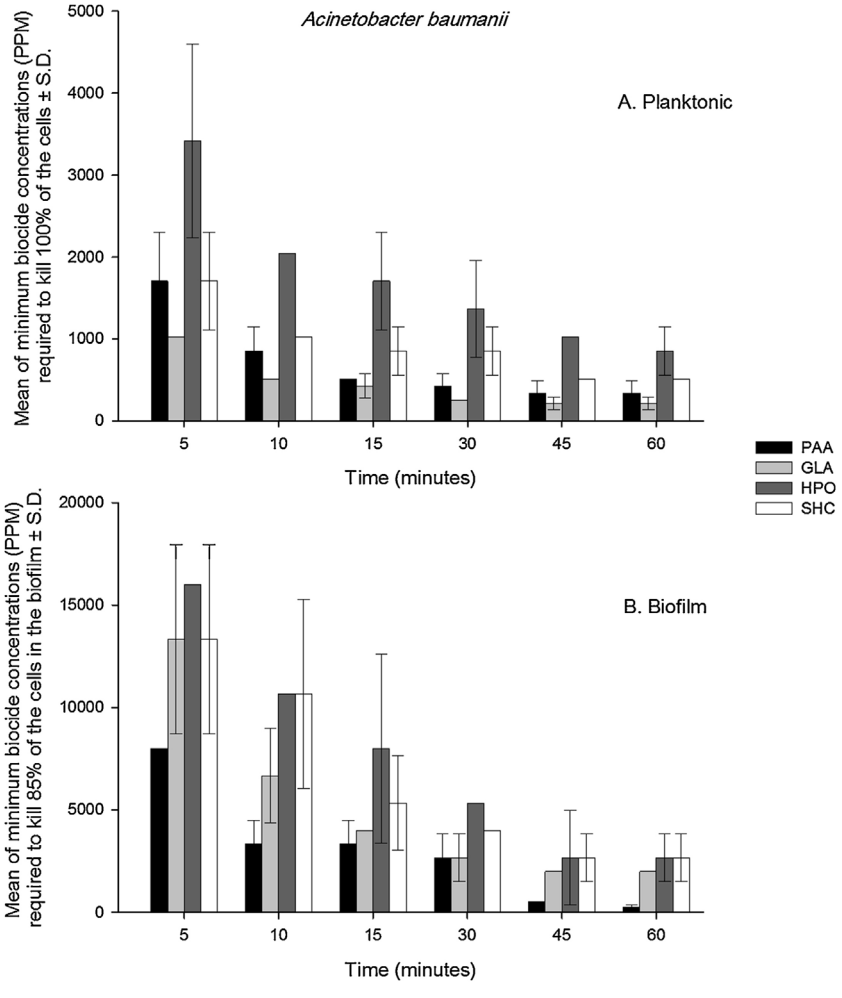

Fig. 2. Antimicrobial activity of the biocides against *Acinetobacter baumannii* in planktonic (A) and biofilm (B) phases at different exposure times.
The minimum concentrations of the biocides required to kill 85 and 100% of the bacteria in the biofilm and planktonic phases, respectively, were determined after different exposure times (5–60 min) using a microdilution-deactivation method.