Int J Clin Exp Med 2014;7(12):5867-5871
www.ijcem.com /ISSN:1940-5901/IJCEM0002986

Original Article

Evaluation of the efficacy of akacid plus® fogging in eradicating causative microorganism in nosocomial infections

Nevzat Unal[1], Keramettin Yanik[2], Adil Karadag[2], Hakan Odabaşı[2], Saban Esen[3], Murat Günaydin[4]

[1]Department of Microbiology, Samsun Maternity and Children Hospital, Samsun; [2]Department of Medical Microbiology, Ondokuz Mayıs University Faculty of Medicine, Samsun; [3]Department of Infectious Diseases and Clinical Microbiology, Ondokuz Mayıs University Faculty of Medicine, Samsun; [4]Department of Medical Microbiology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul

Received October 6, 2014; Accepted December 8, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Objective: The novel polymeric guanidine Akacid Plus® is a member of the cationic family of disinfectants. The aim of the present study was to evaluate the activity of Akacid Plus® against bacteria which cause nosocomial infections and remain viable after contaminating the environment and determine the effects of organic materials to the activity. Methods: Closed room and control room were created for experimental disinfection. Bacterial suspensions of 0.5 McFarland were prepared from methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and vancomycine-resistant *Enterococcus faecium* (VRE) strains. A 0.1 mL of each suspension was applied on the chipboard (25 cm^2) and tile (25 cm^2) test surfaces without albumin and with 2% albumin to simulate organic dirt, and the test surfaces were placed in the test and control rooms after drying. Before testing, cotton swab premoistened with serum physiologic was used to obtain samples from various surfaces in the environment and the samples were transferred onto 5% sheep blood agar for incubation at 37°C. Akacid Plus® solution at a concentration of 0.5% was nebulized with an aerosol applicator (Prowi-06, Germany) for 45 minutes. After a 2-hour waiting period, 1 mL neutralizing broth (Dey-Engley Neutralizing Broth, Fluka) was transferred on the test surfaces, and samples were collected with a swab from the test surfaces and various surfaces in the testing room and inoculated on 5% sheep blood agar for incubation at 37°C for 24 hours. At the end of the incubation period, number of colonies were evaluated on the control and test plates. Results: Although coagulase-negative staphylococci, *Bacillus* spp., and fungi were grown in cultured samples obtained from the environment of experimental laboratory, no growth was observed in the test plates after room disinfection with Akacid Plus®. After room disinfection, MRSA and *A. baumannii* were not detectable in the cultured media prepared from the test surfaces with or without albumin. The bacterial count for vancomyine-resistant *E. faecium was reduced from* 10^7 to 5×10^2 on surfaces without albumin and from 10^7 to 2.5×10^3 on surfaces with albumin. All test plates prepared from the surfaces in the control room showed abundant growth of the microorganism. Conclusion: The nebulization of Akacid plus® solution at a concentration of 0.5% proved to be an efficient means of disinfection for the removal of pathogenic microorganisms that cause hospital outbreaks and use of isolation measures.

Keywords: Akacid plus®, MRSA, *A. baumanni*, VRE

Introduction

It is known that certain pathogenic microorganisms remain viable in the hospital setting for a long period and cause disease outbreaks. The removal of these microorganisms from critical environments is important to break the chain of transmission [1]. Nosocomial infections can be caused by endogenous sources but may also be caused by exogenous microorganisms in the environment [2]. Most disinfectant substances show reduced efficiency due to insufficient contact with the surface. Disinfectant vaporizing and fogging has been used for long years to eliminate microorganisms from the environment. These methods allow penetration of disinfectant into areas which are inaccessible with routine application [3]. The selection of appro-

Table 1. Microorganisms isolated from the environmental samples and number of colonies

Location	Number of colonies-Microorganism	
	Before Application	After Application
Tap	1 CNS	No growth
Counter	4 CNS, 10 fungi, 1 *Bacillus* spp.	No growth
Door handle	1 CNS	No growth
Microwave buttons	No growth	No growth
Drain	1 *Bacillus* spp., 7 CNS, 3 Fungi	No growth

Note: CNS, coagulase-negative staphylococci.

priate disinfectant is only possible through demonstration of the efficiency on microorganisms found in the hospital setting using reliable testing methods and accurate determination of the mode of application and concentration [1, 4]. The efficiency of disinfectant is reduced by the organic and inorganic elements found in the environment [1, 5]. Akacid Plus® is a novel polymeric guanidine compound having high antimicrobial activity and low toxicity among other members of the cationic disinfectants [6]. The aim of the present study was to experimentally investigate the efficiency of Akacid Plus® fogging on various bacterial strains that cause hospital infections and the effect of organic materials in the environment on the efficiency of disinfection.

Materials and methods

Microorganisms and preparation of the testing environment

Closed application and control areas were created for experimental disinfection in the laboratory setting. Before application, samples were collected from five different surfaces for culture analysis. Bacterial suspensions of 0.5 McFarland were prepared from methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and vancomycine-resistant *Enterococcus faecium* (VRE) strains that cause hospital infections. A 0.1 mL of each suspension was applied and evenly distributed on the chipboard (25 cm^2) and tile (25 cm^2) test surfaces without albumin and with 2% albumin and left for drying [5, 7, 8]. Test surfaces were placed in the application and control rooms. Before application, samples were collected from test surfaces and various surfaces in the room using a cotton swab premoistened with serum physiologic and inoculated on 5% sheep blood agar for incubation at 37°C. Culture plates were evaluated after incubation.

Preparation of active substance and neutralizing substance

Akacid Plus® solution at a concentration of 0.5% was nebulized with an aerosol applicator (Prowi-06, Germany) for 45 minutes as per the recommendations of the manufacturer. After a 2-hour waiting period, 1 mL neutralizing broth (Dey-Engley Neutralizing Broth, Fluka) was transferred on the test surfaces, and samples were collected with a swab from the test surfaces and various surfaces in the testing room and inoculated on 5% sheep blood agar for incubation at 37°C for 24 hours. At the end of incubation period, colony counts were evaluated in the control and test plates and expressed as ×100 CFU/cm^2.

Results

Although coagulase-negative staphylococci, *Bacillus* spp., and fungi were grown in cultured samples obtained from the environment before application, no growth was observed in the test plates after room disinfection with Akacid Plus® **Table 1**.

The bacterial count for VRE in samples obtained from experimentally-contaminated tile surfaces was reduced from 10^7 to 5×10^2 on surfaces without albumin and from 10^7 to 2.5×10^3 on surfaces with albumin. All test plates prepared from the surfaces in the control room showed abundant growth of the microorganism. The number of microorganisms isolated from the test surfaces in the control and test rooms are presented in **Table 2**.

The bacterial count for VRE in samples obtained from experimentally-contaminated chipboard surfaces was reduced from 10^7 to 5×10^2 on surfaces without albumin and from 10^7 to 2.5×10^3 on surfaces with albumin. All test plates prepared from the surfaces in the control room showed abundant growth of the microorganism. The number of microorganisms isolated from the test surfaces in the control and test rooms are presented in **Table 3**. There was no growth of *A. baumannii* on plates prepared from both test surfaces after application.

Table 2. Number of microorganisms before and after application on the tile test surfaces (CFU/cm^2)

Microorganism/Surface	With albumin		Without albumin	
	Control	Test room (application)	Control	Test room (application)
	Tile (25 cm^2)	Tile (25 cm^2)	Tile (25 cm^2)	Tile (25 cm^2)
A. baumannii	10^7	0	10^7	0
MRSA	10^7	0	10^7	0
VRE	10^7	5×10^2	10^7	2.5×10^3

Note: MRSA, methicillin-resistant *Staphylococcus aureus;* VRE, vancomycin-resistant *Enterococcus faecium.*

Table 3. Number of microorganisms before and after application on the chipboard test surfaces (CFU/cm^2)

Microorganism/Surface	With albumin		Without albumin	
	Control	Test room (application)	Control	Test room (application)
	Chipboard (25 cm^2)	Chipboard (25 cm^2)	Chipboard (25 cm^2)	Chipboard (25 cm^2)
A. baumannii	10^7	0	10^7	0
MRSA	10^7	0	10^7	0
VRE	10^7	5×10^2	10^7	2.5×10^3

Note: MRSA, methicillin-resistant *S. aureus;* VRE, vancomycin-resistant *E. faecium.*

Discussion

The increased rate of resistance among the microorganisms against antibiotics also causes a increase in the number of nosocomial infections. For prevention purposes, these microorganisms must be eliminated from the critical areas in the hospitals. The methods used to eliminate resistant and sensitive microorganisms in the environment are important to break the chain of transmission to humans [1]. There are many disinfectant substances available in the market. All have specific advantages and disadvantages. The spectrum of effect, area of use, time required for the anticipated efficiency to occur and development of resistance to these substances must be taken into account during selection of these chemicals [8-10]. Toxic effects of these chemicals also limit their use. There are ongoing studies attempting to discover an ideal chemical. Different effective methods must be developed [1, 7]. Akacid Plus® investigated in the present study is a novel substance with no reports of toxic effects to humans, animals and to the environment. The present study showed that Akacid plus® fogging can completely eliminate *Acinetobacter baumannii* and MRSA from tile and chipboard surfaces with or without organic dirt that were shown to remain viable for a long period in the hospital setting. Furthermore, it significantly reduced bacterial cell count of VRE. The studies showed that organic substance load such as protein remnants negatively affect disinfection process [1, 5, 7]. Therefore, we used albumin to simulate protein substances that decrease the efficiency of disinfection. In the present study *A. baumannii* and MRSA showed no growth even on surfaces contaminated with albumin to increase organic material load and significant decrease was observed in the cell count of VRE. In manual disinfection, chemical do not effectively penetrate into areas harboring the microorganisms and the efficiency remains at around 50%. There are many recent studies attempting disinfection in the hospital environment using hydrogen peroxide fogging [11-13]. The present study is the first to evaluate the efficiency of Akacid plus® as an alternative option simulating clean and dirty environments. In a study conducted by Kratzer et al. in Austria, microorganisms in the test room were eliminated after 4 hours of application and there was no change in the number of microorganisms in the control room. They found that Akacid plus® was efficient in eliminating the *Staphylococci* and *Enterococci* strains used in their study. In order to better delineate the efficiency of Akacid Plus® fogging, we collected samples for culture analysis from areas that may harbor microorganisms into which the disinfectant may have little penetration. Gram positive bacteria, spore forming bacilli and fungi were grown in these cultures and no

growth was observed after application [14-16]. This suggests that Akacid Plus® also eliminates other microorganisms found in the environment.

There has been many studies that recommended various disinfectant substances for ideal disinfection. However, there is a limited number of studies that evaluated penetration of fogging into areas which are inaccessible by most disinfectants in routine practice and the efficiency of appropriate disinfectants against microorganisms responsible for hospital infections [3]. Therefore, the present study used fogging technique to penetrate into areas inaccessible by routine application.

In one study that evaluated the efficiency of Akacid Plus®, Kratzer et al. reported Akacid Plus® at a concentration of 0.5% as an appropriate disinfectant to eliminate pathogenic microorganisms from the environment [14]. In the study by Buxbaum et al. from Australia, Akacid Plus® showed lower MIC values for *S. aureus, A. baumannii* and VRE compared to other two disinfectants. This study used a total of 369 clinic isolates in addition to gram negative and gram positive bacteria and reported that this substance could be efficient and useful in preventing bacterial infections. This study also reported this substance as a safe chemical in regards to side effects. They, however, used micro-dilution method [16].

Nosocomical infections associated with severe and lethal consequences in the hospitalized patients and high economic burden are one of the most important problems in the intensive care units of the hospitals [1, 3, 17]. The substances to be used for ambient disinfection in the hospital must show activity against microorganisms that are associated with hospital infections [16]. There is a diversity of causative microorganisms. These infections may change from one patient to another and from one clinical to another. The chemicals to be used in these setting must show activity against *S. aureus*, one of the most common gram positive bacteria, VRE strains that increasingly become problematic, and *A. boumanii*, which has caused significant problems in the intensive care units for years [18]. Therefore, we investigated bacteria which are commonly involved in outbreaks in the intensive care units and which commonly cause shut down of the units involved. The results of the present study are also important due to paucity of studies on VRE and *A. baumanii* and lack of studies that evaluated the effects of fogging technique on these agents.

In conclusion, along with antiseptic and disinfectant solutions with many side effects and different spectrum of effects, Akacid plus® fogging at a concentration of 0.5% provides efficient disinfection for the elimination of pathogenic microorganisms from the surfaces that are found in the hospital environment and cause transmission between the patients.

Address correspondence to: Dr. Keramettin Yanik, Department of Medical Microbiology, Ondokuz Mayıs Üniversitesi Tıp Fakültesi, Kurupelit, Samsun 55139, Turkey. Tel: +90 507 232 92 15; Fax: +90 362 457 60 91; E-mail: keramettinyanik@omu.edu.tr

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