Application of a Next Generation Hand Held Antimicrobial Cold Plasma Technology

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ABSTRACT

Due to the emergence of antibiotic and multidrug-resistant pathogens, there is a growing need for the development of effective antimicrobial therapeutics. Our team has developed a novel device for creating non-thermal cold atmospheric plasma (CAP). Although there are many cold plasma technologies, this device is novel due to its small size, reduced cost, portability with no requirement for any noble gas, and ease of application. The aim of this study is to evaluate the efficacy of this novel cold plasma technology against antibiotic resistant pathogens. Antimicrobial testing results demonstrated efficacy against several multidrug-resistant bacterial pathogens. This novel pocketsize cold plasma technology has significant potential as a countermeasure against multidrug-resistant and hard-to-treat infectious agents in both clinical and field settings. Our non-drug based therapeutic technology is a promising countermeasure for treating infections caused by drug resistant pathogens.

Keywords: cold plasma, antimicrobial, handheld

1 INTRODUCTION

Antibiotic and multidrug-resistant infections are increasing at an alarming rate while placing a burden on our healthcare system. Although the United States' economic loss to antibiotic resistance is difficult to estimate, the Center for Disease Control (CDC) has estimated the loss to be billions of dollars [1].

Bacterial pathogens are learning and adapting to the mechanisms used by antibiotics, quickly gaining resistance, and spreading resistant genes via vertical and horizontal gene transfer. These resistant microbes are a big threat in open wounds. Wound infections are a growing medical concern despite advancements in sterilization of medical equipment and antibiotic prophylaxis. This is especially true when attempting to prevent infection against multidrug-resistant pathogens.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important example from the large pool of multidrug-resistant bacteria thriving in clinical and field settings. Studies have shown that common pathogens found on skin and mucosal surfaces are gram-positive cocci, specifically staphylococci and enterococci. The most common pathogen found in wound infections is *S. aureus* accounting for 20% of these infections. *Enterococci* species (12%), *E. coli* (8%), *P. aeruginosa* (8%), *Enterobacter* species (7%), and *Klebsiella pneumoniae* (3%) consecutively rank after *S. aureus* in regards to their frequency of occurrence [2]. Some strains of MRSA can be extremely durable due to their large range of resistance against antibiotic classes such as aminoglycosides, quinolones, third generation cephalosporins and low to fourth generation cephalosporins. Although MRSA strains show resistance to multiple antibiotics, they are usually not resistant to the broad spectrum of vancomycin [3]. However, there are strains of Enterococci that are vancomycin resistant and these organisms can be fatal especially during long-lasting hospital outbreaks [4]. While vancomycin-resistant Enterococci (VRE) are widely known as culprits to urinary tract infections (UTI), intra-abdominal infections, and cholecystitis, recent studies have shown their growing presence in wound and soft tissue infections at trauma care facilities [5]. Pathogens such as MRSA and VRE are becoming more prominent as antibiotic resistance increases, thus researchers must find and develop novel and effective technologies to combat these hard-to-treat pathogens.

This study presents evaluation of a novel therapeutic device as an effective substitute for antibiotic therapy. This portable handheld device has the capability to generate cold plasma utilizing atmospheric elements such as oxygen and nitrogen as a source of gas. The device's array pad is plugged into the portable power supply and uses dielectric barrier discharge to emit cold atmospheric plasma from the pad. Reactive atmospheric species such as ozone and nitrogen reactive species are produced and contain toxic properties against bacteria. The device was tested for its antimicrobial properties against five gram-positive and four gram-negative multidrug-resistant bacterial clinical isolates.

2 MATERIALS AND METHODS

Bacterial Strains and Preparation

A total of nine multidrug-resistant bacterial clinical isolates were used in this experiment. Methicillin-resistant *Staphylococcus aureus* (MRSA) 6313, vancomycin-intermediate *Staphylococcus aureus* (VISA) NRS1, vancomycin-resistant *Staphylococcus aureus* (VRSA) VRS1, vancomycin-resistant *Enterococcus faecalis* 6401 are among the gram-positive strains that were used in our testing. The gram-negative strains include *Acinetobacter baumannii* 6272, *Enterobacter aerogenes* 6484, *Escherichia coli* 6036, *Klebsiella pneumoniae* 6069, and *Pseudomonas aeruginosa* 6162. All strains were streaked onto trypticase soy agar

(TSA) plates and incubated overnight at 37 °C. Bacterial growth was examined and single colonies were selected and inoculated into 4 mL of trypticase soy broth (TSB) and placed into a shaker incubator at 37 °C and 200 RPM overnight. Optical density readings (600 nm) of the inoculated clinical isolates were conducted to estimate initial concentrations (CFU/mL). Subsequently, the clinical isolates were serially diluted with concentrations ranging from 10^8 to 10^2 CFU/mL for cold plasma application studies.

Cold Atmospheric Plasma (CAP) Treatment

The device power supply can generate CAP through four different frequency settings. We conducted the antimicrobial efficacy studies with the highest frequency setting of 1550 Hz and the lowest frequency setting of 241 Hz. In addition, bacterial concentrations of 10^5 CFU/mL and 10^6 CFU/mL were used in all experiments. Thus, four experimental conditions and two positive controls were studied per clinical isolate (Table 1). Both concentrations were tested at both frequencies therefore CAP generated at 1550 Hz and 241 Hz was applied onto TSA plates containing 10^5 CFU/mL and 10^6 CFU/mL of the desired clinical isolate. A TSA plate was used as a negative control containing 100 µL of TSB. The plates were then air-dried for 10 minutes.

Table 1: Plate outline for CAP treatments per clinical isolate.

	1550 Hz	241 Hz	Untreated
10^5 CFU/mL	Experimental Plate A	Experimental Plate B	Positive Control Plate 1
10^6 CFU/mL	Experimental Plate C	Experimental Plate D	Positive Control Plate 2

The array was plugged into the device driver. The array was inserted into a Teflon cover. The cover acts as a protective barrier to prevent the metals on the array from contacting the TSA plates. The array was pressed onto the TSA plate with a weighted object overtop to keep it in place. The device driver was then set to the desired frequency to generate cold plasma for 10 minutes. After 10 minutes, the perimeter of the array was traced on the underside of the plate to record the location of the CAP treatment area. All plates were placed in an incubator at 37 °C overnight. After incubation, the colonies within the treatment area were counted.

3 RESULTS

Colony Growth Within the CAP Perimeter

High levels of antimicrobial activity were observed for both gram-negative and gram-positive (Fig. 1) strains treated with CAP. Absence and in some cases low numbers of colonies present were counted within the CAP application perimeter. At both frequencies, *K. pneumoniae* had the smallest number of colonies within the CAP application perimeter compared to all other gram-negative strains tested. Among the gram-positive strains, VRE had the lowest colony growth at both frequencies. The two frequency settings that were used to generate CAP (1550 Hz and 241 Hz) were almost equal in their antimicrobial efficacy. Some strains were slightly more susceptible to specific frequencies but the difference was not a significant (less than one-log) reduction.

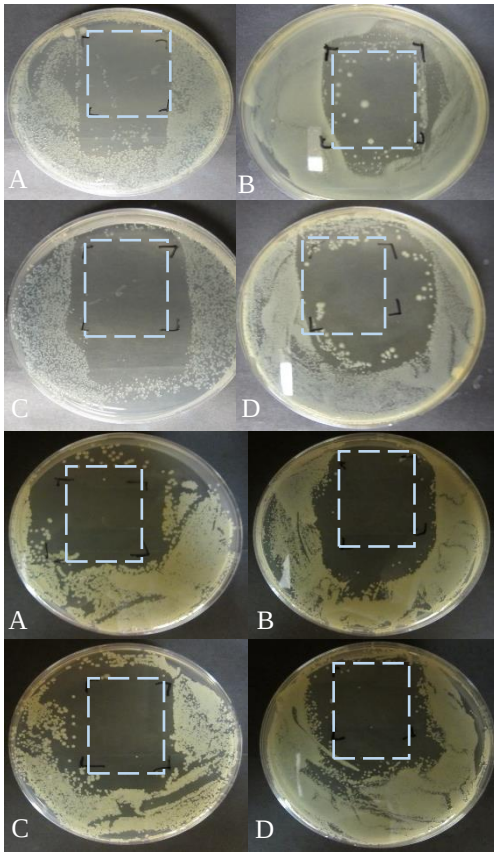

Figure 1: *P. aeruginosa* (top) and VRE (bottom) cold atmospheric plasma treatment areas.

4 CONCLUSION

The CAP technology is an effective countermeasure against multidrug resistant and hard-to-treat infectious agents. Our hand held cold plasma device is ideal for field application due to its size, portability, precision, robustness, reduced cost and ease of application with no requirement for any noble gas.

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