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Determination of the Microbial Population Prevalent in the Prisma Health Ambulance Service System and the Assessment of Disinfection Practices for their Effective Eradication

Sophia Vernieri

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Departmental Honors Thesis

Sophia Vernieri

Advisor: Dr. Jeremy Tzeng

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ABSTRACT

Healthcare-associated infections (HAIs) are significant causes of morbidity and mortality across the United States. These infections may occur due to microbial contamination of ambulatory surfaces, such as door handles and stretchers, and cross-transmission of microorganisms by emergency medical service (EMS) personnel. In this study, we aim to identify the microbial population prevalent in Prisma Health's Ambulance Service System and work to develop efficient and cost-effective disinfection practices that would minimize the transmission of HAIs. To accomplish these objectives, effective protocols were developed to collect surface samples and extract DNA for 16S sequence analysis. For initial process optimization, DNA extraction was conducted on Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria using the MP Biomedicals FASTDNA™ Spin Kit for Soil. In addition, we tested the effectiveness of different disinfectants (Cavicide, Decon7, and Microban-24) using an overnight culture of *S. epidermidis* spread over a glass tile. Our results indicated that the optimal bead-beating duration was 3 minutes at 5 meters per second. We also found that Microban-24 and Decon7 were more effective than Cavicide at 1 minute of contact time against *S. epidermidis*. These results indicated the importance of identifying the microorganisms present in ambulances to optimize disinfection protocol for eradicating those microbial populations in the least amount of time possible.

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INTRODUCTION

Healthcare-associated infections (HAIs) are significant causative agents of morbidity and mortality across the United States. They contribute to an estimated 98,987 deaths across US hospitals [1]. The emergency medical services (EMS) system is an integral component of healthcare, as it provides treatment and transport of patients to hospitals. More than 20 million Americans receive pre-hospital care from the EMS system each year [2]. During ambulatory transport, patients and EMS personnel are at greater risk of exposure to HAIs.

These infections occur due to microbial contamination of ambulatory surfaces, including door handles and stretchers, and cross-transmission of microorganisms by the hands of EMS personnel [3,4]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent microorganism found in the microbiome of ambulances. The presence of this microorganism is significant because MRSA infections cause more than 19,000 deaths a year in the US [5]. Other microorganisms commonly found within ambulances include vancomycin-resistant Enterococcus and *Klebsiella pneumoniae* [6]. The virulence factors of *K. pneumoniae* that contribute to its pathogenicity in humans include the presence of cell wall receptors, capsular polysaccharides, and endotoxins [7].

HAIs of these pathogens are often fatal; therefore, effective disinfection practices are required for their eradication from ambulatory surfaces between services. However, it has been shown that current ambulatory disinfection practices are suboptimal because only 56.9% of EMS personnel wear gloves, 27.8% of EMS personnel wash their hands, and 31.6% of reusable equipment is washed [8]. Effective and standardized disinfection protocols are required to reduce the bioburden of ambulatory surfaces.

RESEARCH OBJECTIVE

The objective of this study is to identify the microbial population prevalent in Prisma Health's Ambulance Service System and to review and develop efficient and cost-effective disinfection practices that minimize the transmission of HAIs. These aims support the three principal goals of healthcare infection control and prevention program identified by the Association for Professionals in Infection Control and Epidemiology and the Society for Healthcare Epidemiology of America, which are 1) to protect patients, 2) to protect healthcare workers, visitors, and others in the healthcare environment, and 3) to achieve these two goals in an efficient, timely, and cost-effective manner. To accomplish these goals, we must first identify the specific microbiome associated with the Prisma Health EMS system and the effectiveness of current disinfection practices against those pathogens. With such knowledge, we can develop efficient and cost-effective disinfection guidelines and practices that minimize the transmission of HAIs and reduce the ambulance turnaround time interval.

It is essential to reduce the ambulance turnaround time interval because the Prisma Health EMS system maintains 30 staffed ambulances, with 20 in the Greenville area and 10 in the Oconee area. On average, the Greenville County trucks respond to 10,000 annual 911 calls, and the Oconee trucks respond to 15,000 annual 911 calls. Therefore, we must understand the prevalent pathogens associated with Prisma Health ambulances to equip the EMS personnel with the knowledge of effective disinfection practices. This protocol will be tailored to high-risk surface areas, and they will ensure minimum contact time and drying time of the disinfectants utilized for overall efficient, timely, and cost-effective control of HAIs.

MATERIALS AND METHODS

DNA Extraction Optimization

DNA extraction was conducted on Gram-negative (*Escherichia coli*) and Gram-positive (*S. aureus*) bacteria using the MP Biomedicals FASTDNA™ Spin Kit for Soil. Optimization was performed by testing different bead-beating settings and durations to get optimal DNA from both Gram-negative and Gram-positive organisms since the environmental samples we collect later will be unidentified.

Disinfectant Testing

Staphylococcus epidermidis, a Gram-positive bacterium, was used as the test organism due to the presence of staphylococcus infections in healthcare settings. An overnight culture of *S. epidermidis* (100 µL) was spread over a glass tile and allowed to dry, forming a simulated biofilm. Paper towels were sterilized in the autoclave, soaked with 500 µL of each disinfectant, and wiped over the entire surface of each tile four times. Once the contact time of 30 seconds or 1 minute was reached, the tile was transferred to a vessel with 20 mL of letheen broth. The tile was sonicated using semi-wave for 30 seconds, then placed in a shaking incubator at 37°C for 5 minutes to recover. Dilutions were made, and 100 µL of each dilution was spread onto plates. After 48 hours of incubation at 37°C, data was analyzed.

RESULTS

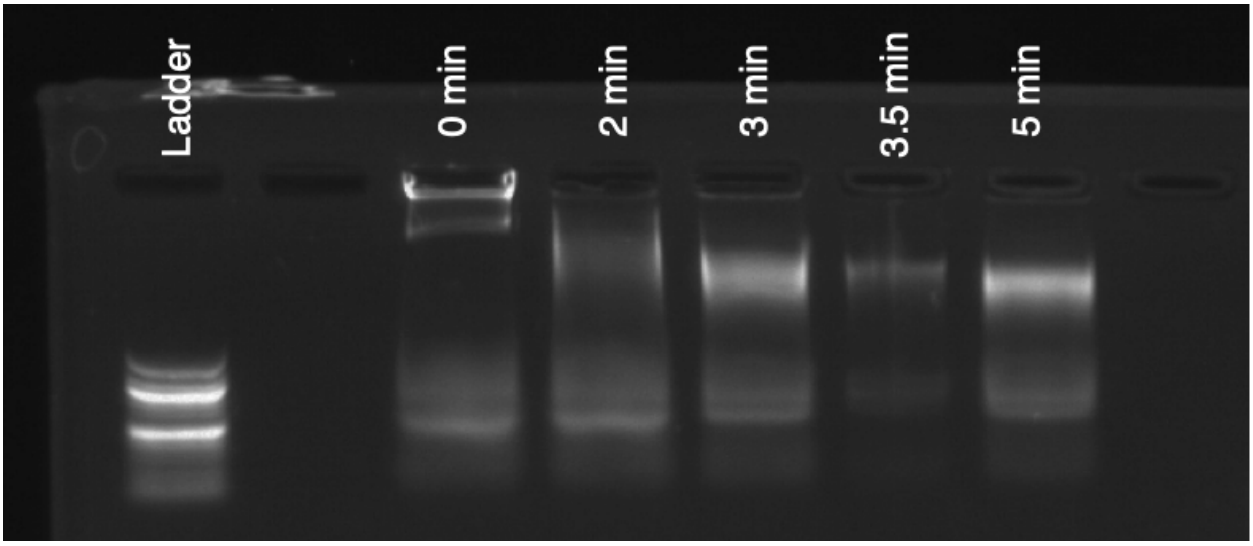

Figure 1: Optimization of power and duration for DNA-extraction.

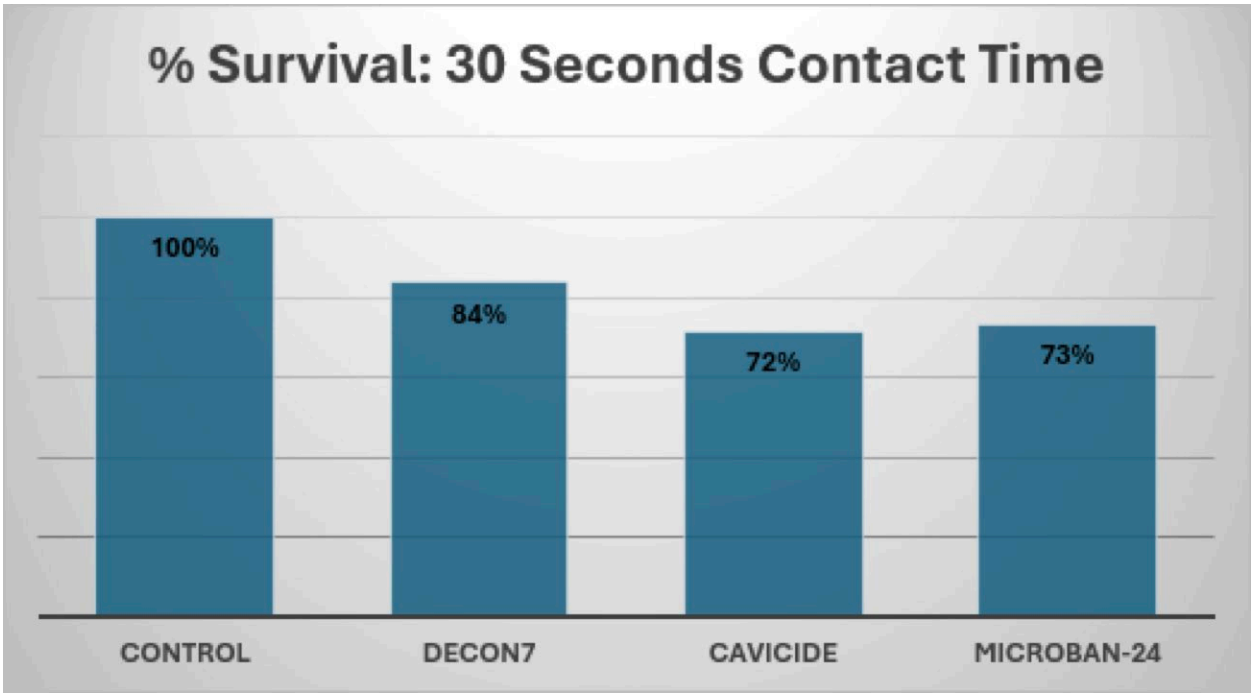

Figure 2: Viable cell count after testing disinfectants (Decon7, Cavicide, Microban-24) with a 30-second contact time.

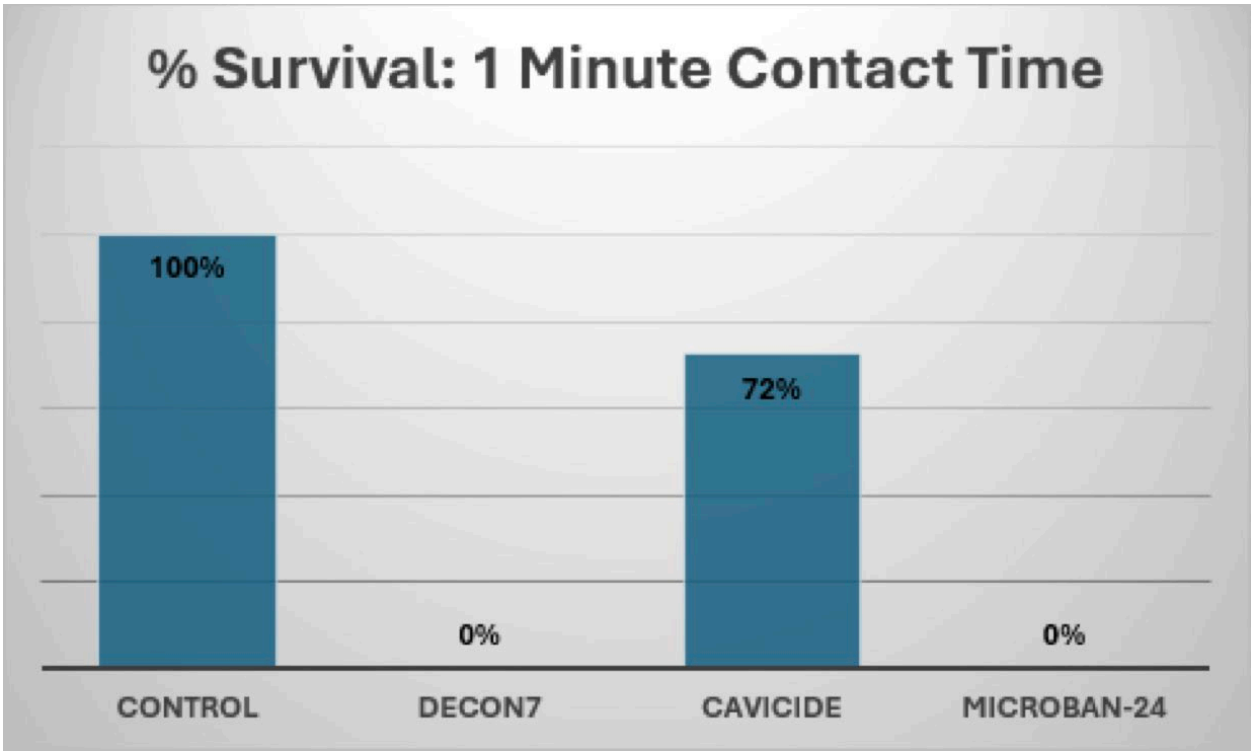

Figure 3: Viable cell count after testing disinfectants (Decon7, Cavicide, Microban-24) with a 1-minute contact time.

DISCUSSION

The optimization of DNA extraction protocol from environmental samples for both Gram-positive and Gram-negative microorganisms revealed that the optimal bead-beating duration is 3 minutes because it releases the most DNA with minimal shearing of genomic DNA (Figure 1). For disinfection efficacy, we found that 30 seconds of contact time was relatively ineffective against *S. epidermidis* (Figure 2). However, Microban-24 and Decon7 reduced the bacterial load of *S. epidermidis* by at least 5 logs at 1 minute of contact time (Figure 3). Therefore, Prisma Health should switch from using Cavicide to either Microban-24 or Decon7 to disinfect their ambulances. These results were significant because they highlighted the importance of optimizing disinfectant protocol across Prisma Health ambulances to reduce the amount of time taken to eradicate microbial populations effectively.

In a similar study, the bactericidal efficacy of a commercial quaternary ammonium compound (QAC)-based surface coating was tested on three different surfaces (polyvinyl chloride, glass, and stainless steel) against three pathogens (*E. coli*, *Acinetobacter baumannii*, and *Listeria monocytogenes*). It was found that the antimicrobial coating was effective against all pathogens, with a reduction of >5.0-log CFU/cm^2 in less than 1 minute for the three surfaces [9]. These results are consistent with our findings because the active ingredients in Microban-24 are QACs, which were found to be effective against both Gram-positive and Gram-negative bacteria.

Future research could include the long-term effects of using QAC-based disinfectants in a healthcare setting. Potentially, the widespread use of these disinfectants could result in the development of antimicrobial resistance. As evidenced in previously published literature, strains of *Pseudomonas* developed resistance against the tested QACs. Specifically, about 30% of the collected strains grew in 200 μg·ml^{-1} benzalkonium chloride [10]. Thus, further testing is required to determine the long-term efficacy of QACs against prevalent microbial populations in healthcare settings.

CONCLUSIONS

These findings underscore the significance of systematic testing of disinfectants against specific microbial strains, as different disinfectants may vary in their effectiveness depending on the target organisms. Through locating prevalent pathogens, more effective disinfection strategies can be implemented in healthcare settings where HAIs pose significant risks to human health. In addition, contact times are an important consideration, especially in Prisma Health's Ambulance Service System, which only has 30 staffed ambulances. Therefore, optimal disinfection protocol would include a combination of the most effective disinfectant against the identified pathogens with the least amount of contact time.

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