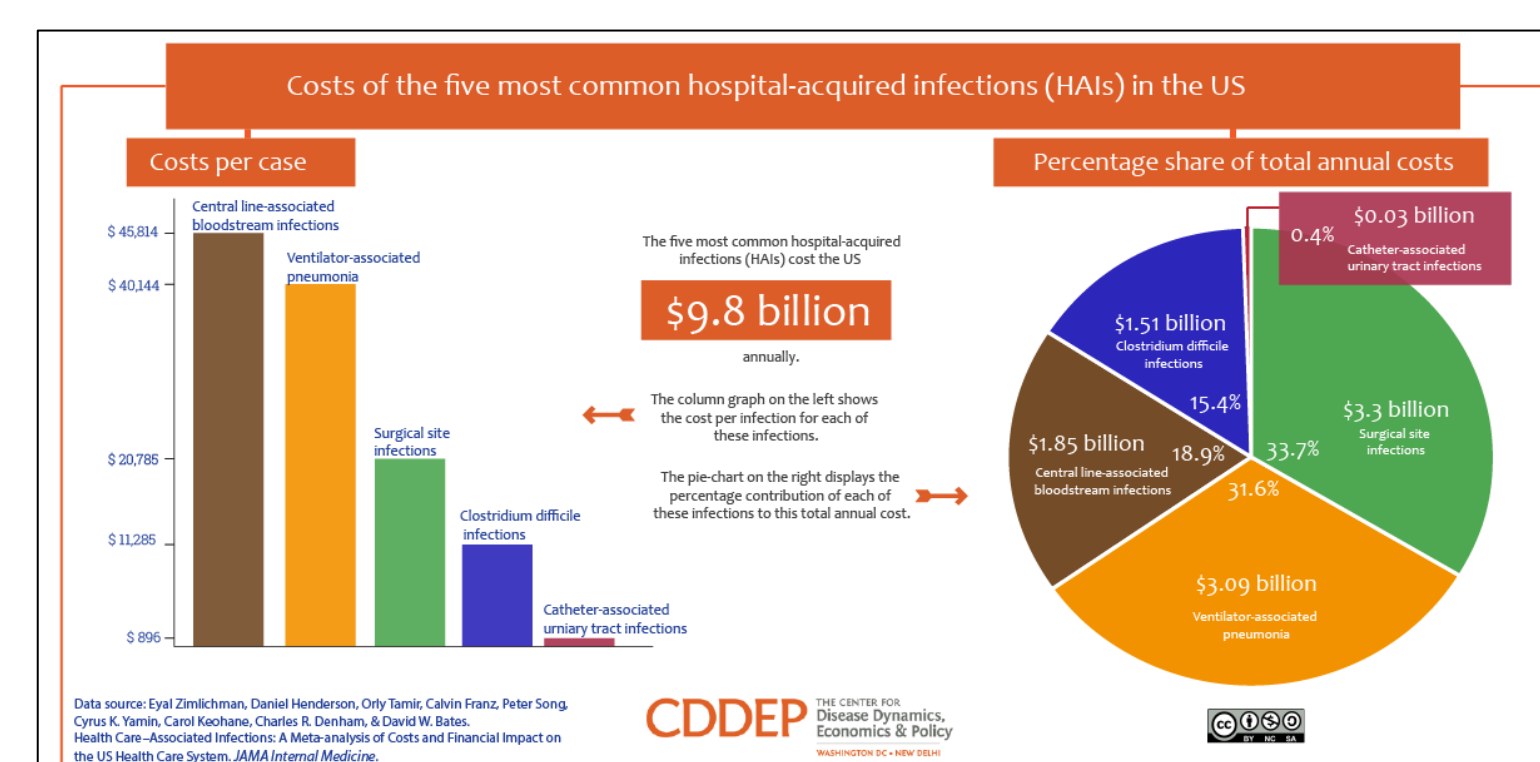


ABSTRACT

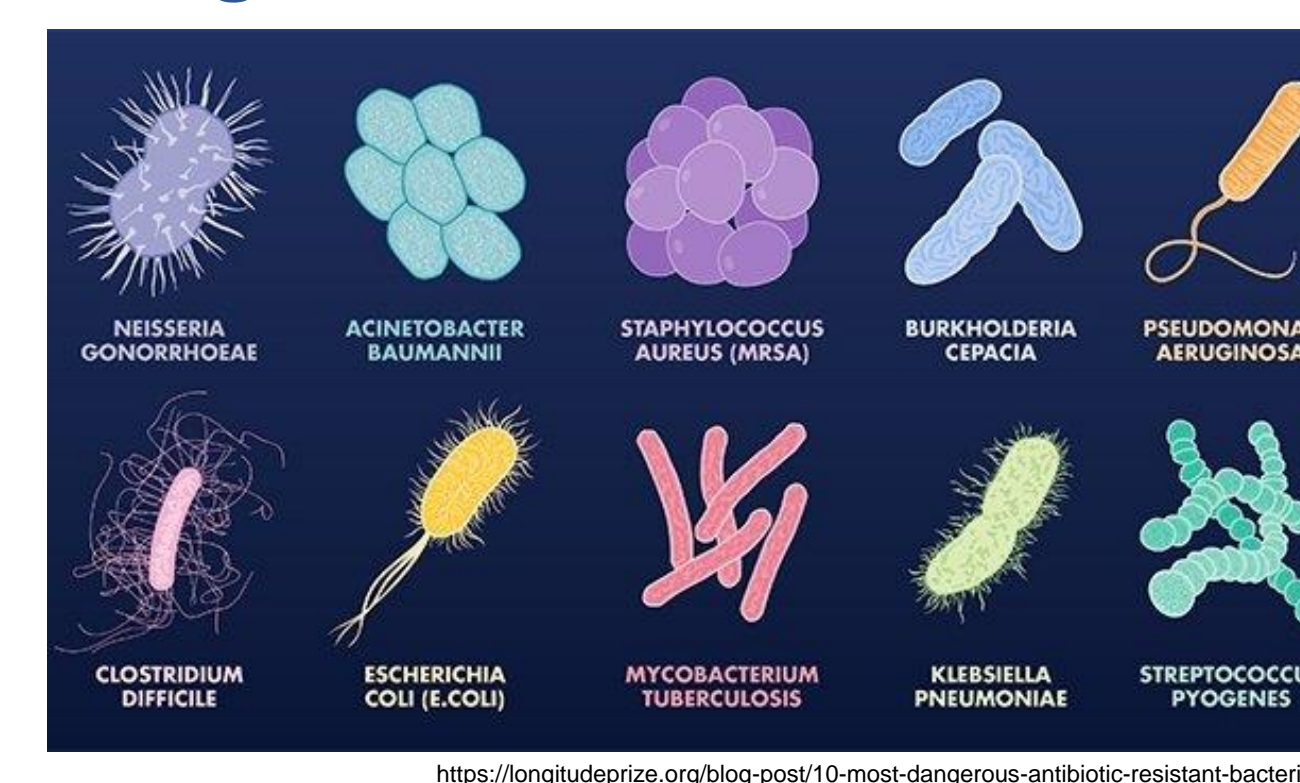
Medical records of the Department of Defense TRICARE beneficiaries from 2005 to 2010 revealed 62,326 bacteremia cases and 181,317 wound infections from which *Staphylococcus aureus* was isolated in 12% and 62% of these conditions, respectively. Up to 88% of combat wounds in U.S. armed conflicts involve extremity injuries, with 15% of these patients developing osteomyelitis caused by methicillin resistant *S. aureus* (MRSA). *S. aureus* often causes biofilm-associated infections such as diabetic foot ulcers and osteomyelitis which are refractory to current antibiotic treatments. Furthermore, the efficacy of vancomycin, the primary drug for treatment of MRSA infections, is decreasing due to the accumulation of mutations. Traditional drug development efforts are diminished by the rapid emergence of resistance to new antibiotics. Rapid emergence of resistance demands a critical need to develop alternative anti-MRSA therapeutics. Chitosan is a natural biocompatible polymer derived from deacetylation of chitin. It is currently approved by FDA as a hemostatic agent to control bleeding in battlefield injuries. Chitosan also has a broad spectrum antimicrobial effect, presumably mediated by interaction of the cationic groups on chitosan with negatively charged bacterial surface components which inhibit trafficking of nutrients and other molecules, resulting in bacterial death. However, most chitosan antimicrobial tests have been performed to determine the minimal inhibitory concentration of chitosan with in vitro experiments. In this study, we determined the anti-biofilm effect of chitosan solution against MRSA. We generated a genetically modified *S. aureus* ATCC 6538 strain to harbor the green fluorescence gene (*S. aureus*-GFP) in the chromosome for accurate quantification and qualification of biofilm. To coat the surface of tissue culture plate with chitosan, 0.15, 0.3%, 0.5%, 1% chitosan solutions were added to individual wells and incubated at 4 °C for overnight. After removing the chitosan solutions and washing with phosphate buffered saline (PBS), bacterial growth media (brain heart infusion broth) was added to the tissue culture plates and inoculated with *S. aureus*-GFP. The plates were then incubated at 37 °C for 24 hours to form biofilms. After washing with PBS, biofilm formation was quantitatively assessed by a fluorescent plate reader. Chitosan solutions successfully inhibit biofilm formation in a concentration dependent manner. No detectable biofilm was observed at 1% chitosan solution and significantly reduced but detectable biofilm was observed at 0.5% chitosan solution. We, then, determined the long-term antimicrobial effect of chitosan after surface coating. The surfaces of tissue culture plates were coated with 0.15%, 0.3%, 0.5%, 1% chitosan, and negative control solutions. The plates were then stored at room temperature for 1, 3, 7 and 10 days. At each time point, the surfaces were inoculated with a bacterial suspension in water containing 4x10⁶ colony forming unit (CFU) *S. aureus*/ml. After incubating at 37 °C for 24 hours, the number of viable bacteria was determined by the standard plate count method. No viable *S. aureus* was detected from the surface coated with 0.3% chitosan. Significantly reduced, but detectable, viable *S. aureus* was recovered from the surfaces coated with 0.15%, 0.5%, 1% chitosan solutions. Since chitosan is a biopolymer that interacts to form a specific structure, our results suggest that a specific biopolymer structure is formed at 0.3% chitosan solution with a stronger antimicrobial effect. We are currently characterizing physicochemical properties of the biopolymer structure formed at different concentrations of chitosan solution to optimize the antimicrobial and antibiofilm effects of chitosan solutions. As chitosan is a cheap, abundant, non-toxic, and biodegradable material, it can be easily applicable to battle-field first-aid treatment for preventing infections and long-term sterilization of military equipment and food supplies.

INTRODUCTION

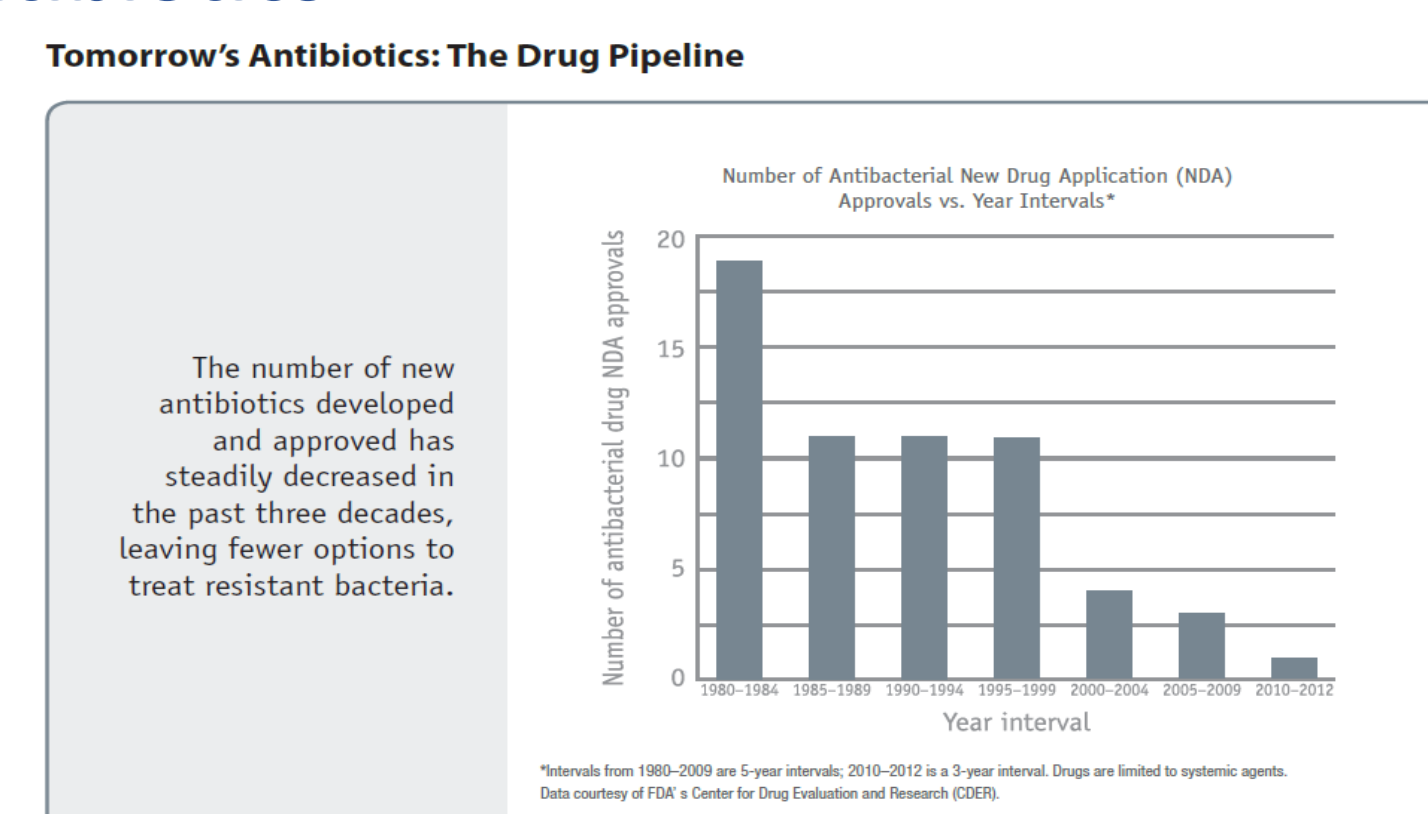
1. Nosocomial infections



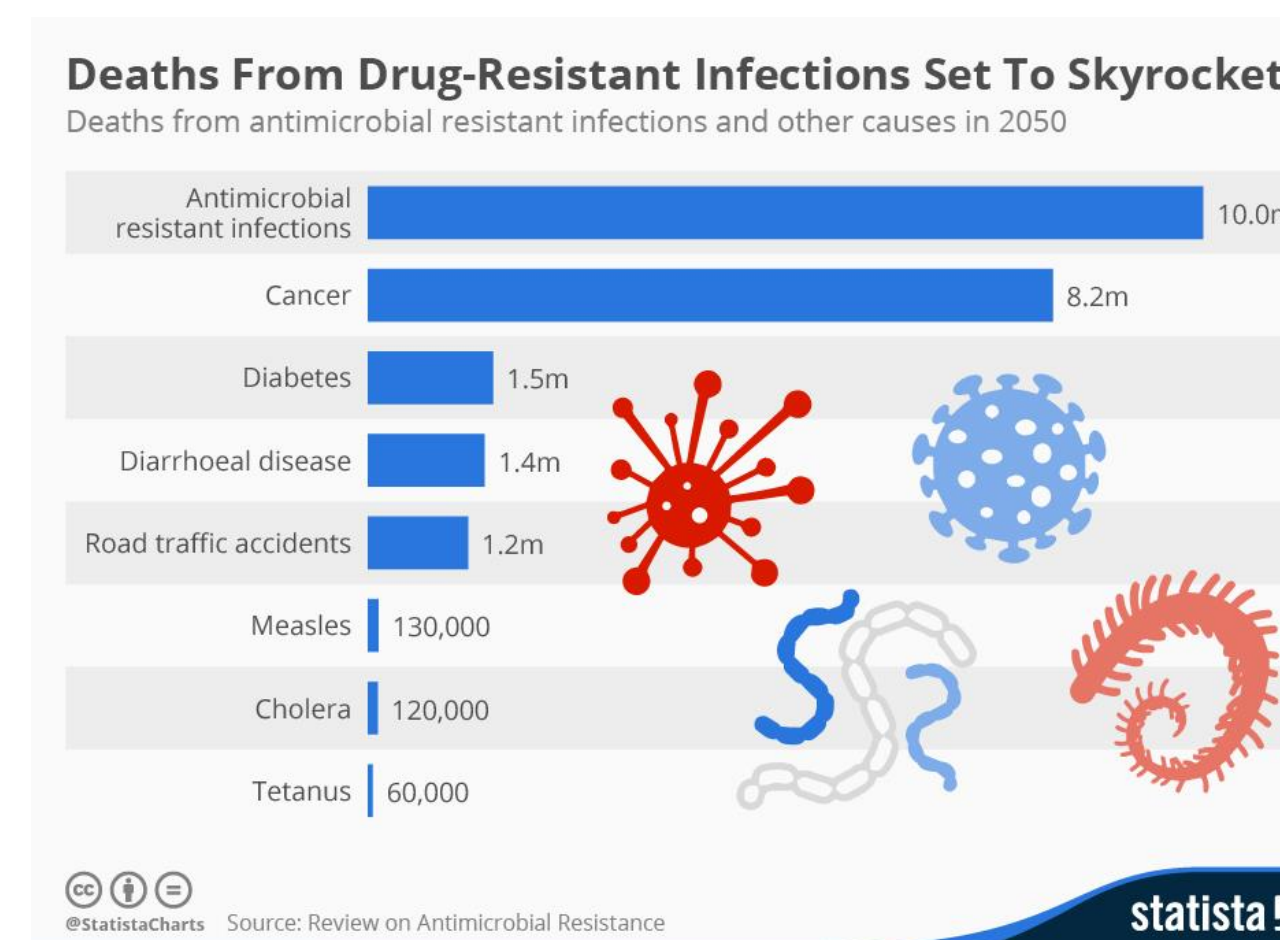
2. Multidrug resistant pathogens



3. Drought of new antibiotics



4. Global crisis of multidrug resistant pathogens

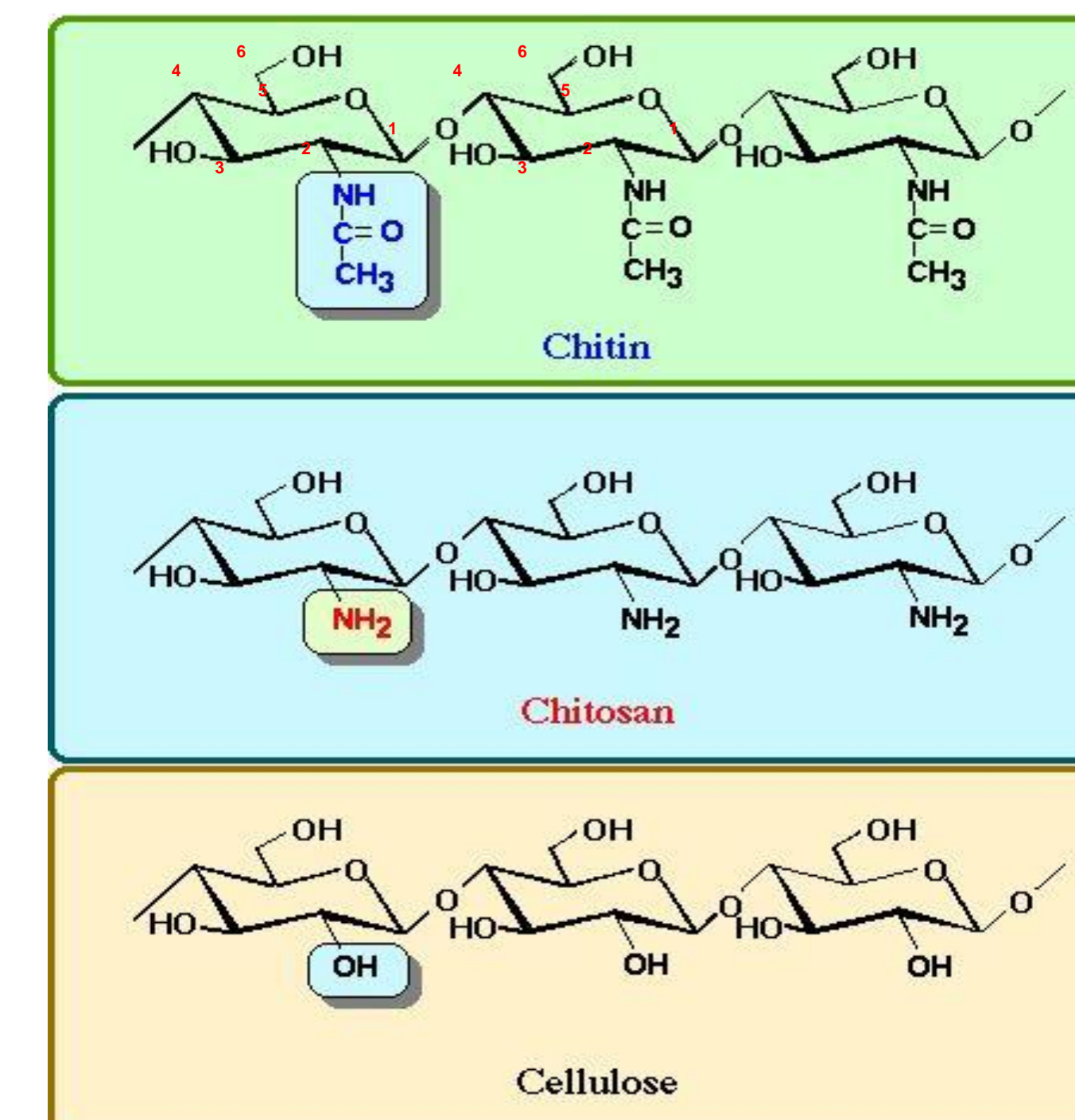


4. What is chitin?

- Chitin is a natural polysaccharide
- Structure similar to cellulose with hydroxyl group replaced by acetamido group
- N*-acetyl-glucosamine units in β -(1 \rightarrow 4) linkage
- Chitosan is the *N*-deacetylated derivative of chitin
- N*-glucosamine units in β -(1 \rightarrow 4) linkage
- N*-deacetylation of chitin into chitosan is achieved by treating with 50% NaOH

5. Why chitin or chitosan?

- Biocompatible
- Biodegradable
- Non-toxic
- Remarkable affinity to proteins
- Ability to be functionalized
- Renewable
- Abundant



6. Applications of chitin and chitosan

Application area	Specific use
Biomedical	Burns and wounds dressings Anticoagulant and antithrombogenic Hemostatic agent Antimicrobial effect
Agricultural	Fertilizer Plant seed coating Antifungal effect
Food/Health supplements	Food additives Food processing Filtration Decreasing cholesterol
Biotechnology	Drug delivery, gene delivery Enzyme immobilization

Chitosan showed antimicrobial effect, presumably mediated by interaction of the cationic groups on chitosan with negatively charged bacterial surface components which inhibit trafficking of nutrients. Thus, chitosan has a potential to be applied as a nosocomial infection control agents.

Objective of study

- Determine a long-term antimicrobial and anti-biofilm effect of chitosan against most common nosocomial pathogens

METHODS

1. Chemically modified chitosan

2. Bacterial strains

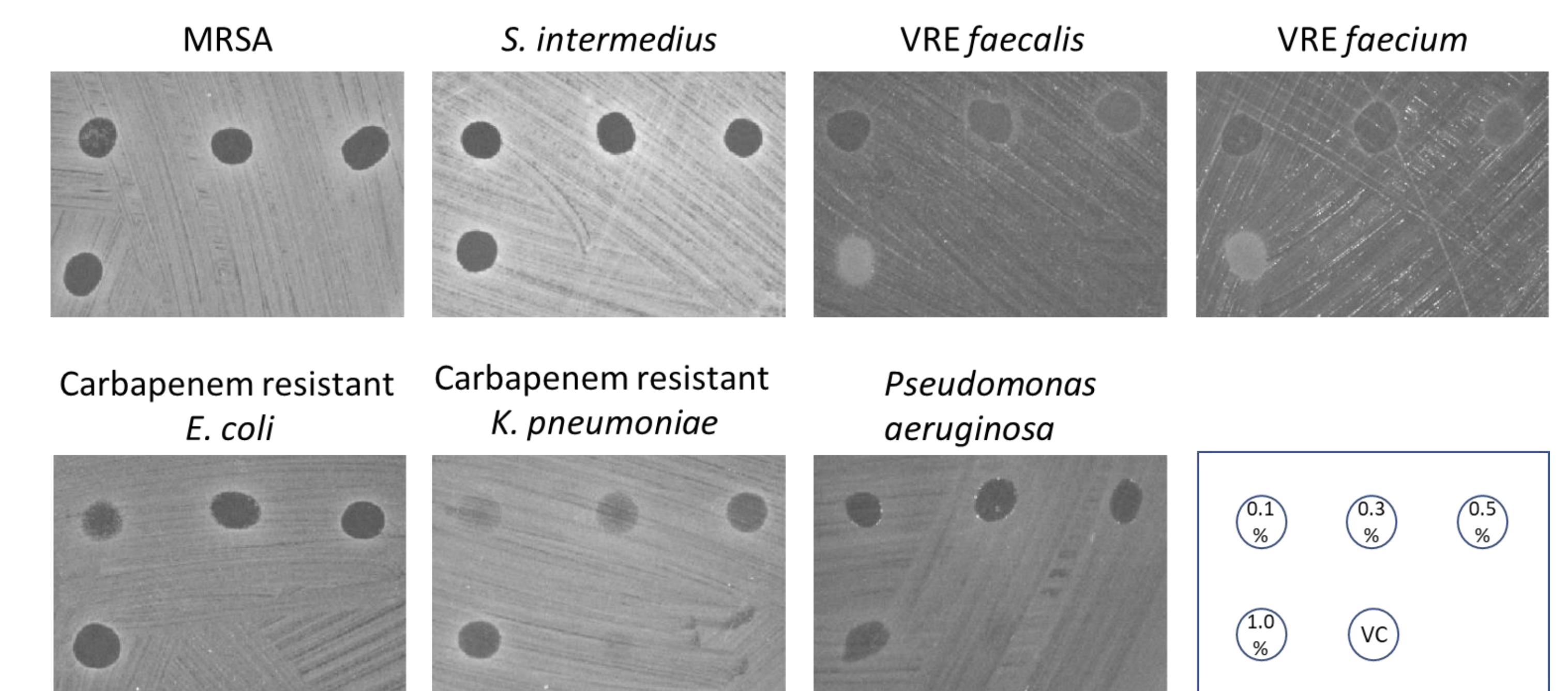
- Methicillin resistant *Staphylococcus aureus*
- Vancomycin resistant *Enterococcus faecalis*
- Vancomycin resistant *Enterococcus faecium*
- Carbapenemase producing *Escherichia coli*
- Carbapenemase producing *Klebsiella pneumoniae*
- Extended Spectrum Beta Lactam resistant *Pseudomonas aeruginosa*

3. Antimicrobial effect tests

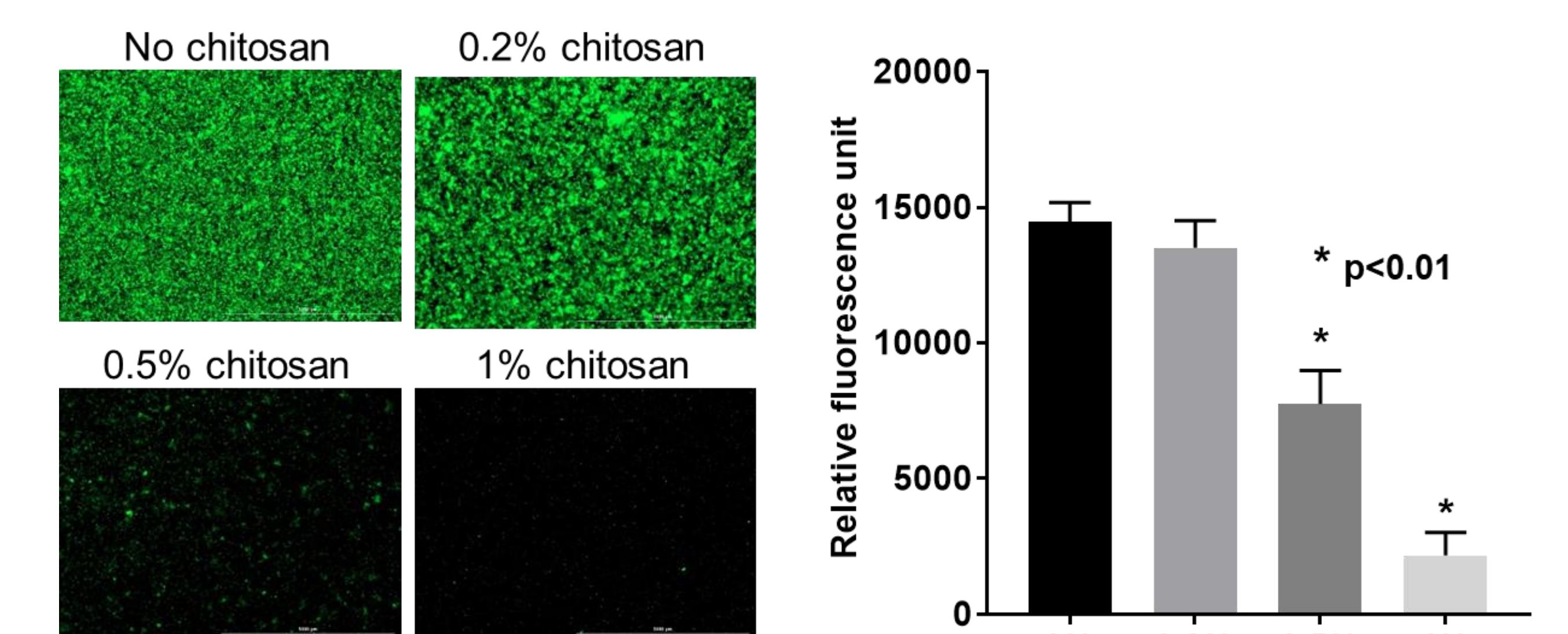
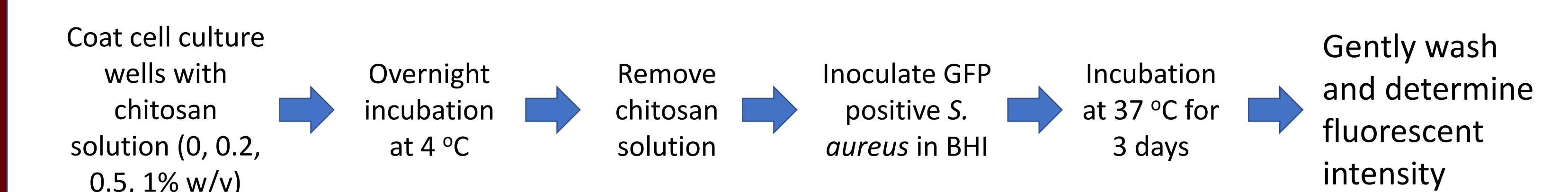
- Minimal inhibitory concentration
- Anti-biofilm effect
- Long-term antimicrobial effect

RESULTS

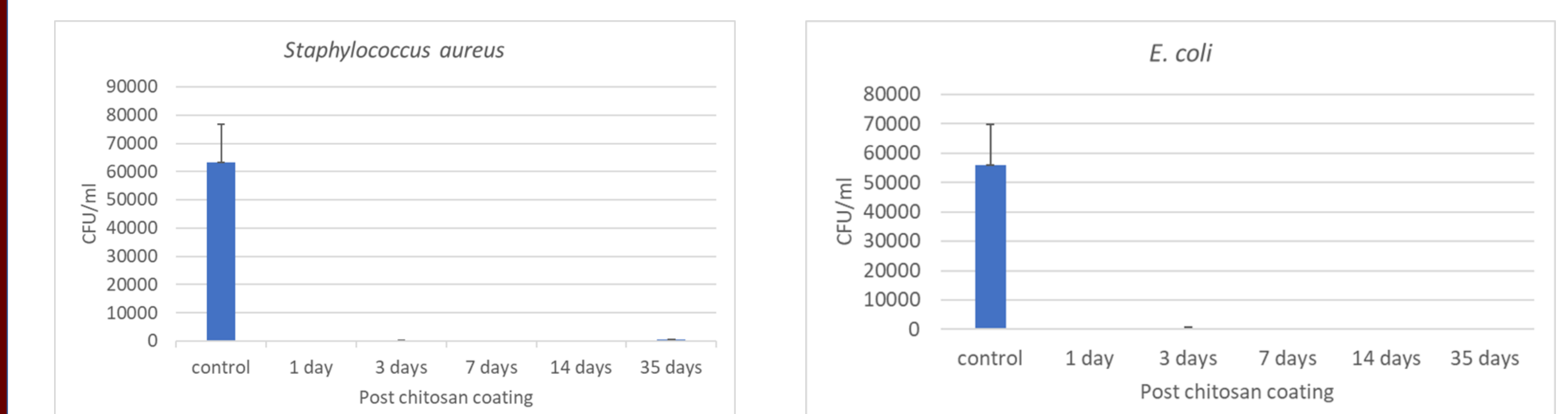
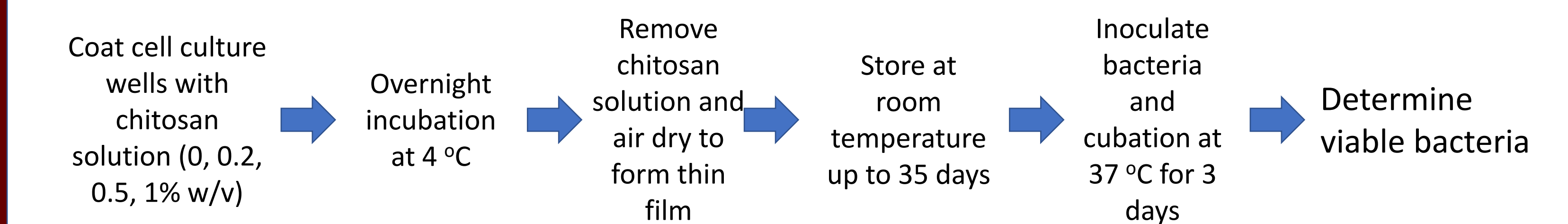
1. Antimicrobial effect of chitosan solution



2. Antibiofilm effect of chitosan solution



3. A long term antimicrobial effect of chitosan film



Conclusion

Chitosan is an ideal antimicrobial polymer

- Easily and inexpensively synthesized
- Biodegradable, not toxic
- Antimicrobial effect against most medically important antibiotic resistant nosocomial pathogens
- Not soluble in water for water associated anti-biofilm application
- Stable in long-term usage and storage at the temperature
- Easily applicable to battle-field first-aid treatment for preventing infections
- Long-term sterilization of military equipment and medical devices

Acknowledgement

Method for applying a persistent antimicrobial film of chitosan effect is protected by US patent (US9149036B1). This work is partially supported by STERIS contract and by National Institute of Health, Center for Biomedical Research Excellence in Pathogen-Host Interactions (1P20GM103646-01A1).