Introduction
1. Introduction

1.1 Antimicrobial resistance and its global threat

There is a dramatic increase in multidrug-resistant bacterial pathogens across the globe over the years. The World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) are considering infections caused by antibiotic resistant bacteria as a major public health problem.\(^1,2\) WHO defines antibiotic resistance as resistance of a microorganism to an antibiotic that was originally effective for treatment of infections caused by it. The emergence of resistant microorganisms is mediated either by mutation or by the acquisition of mobile genetic elements carrying resistance genes. The exposure to antibiotics provides the necessary selective pressure for the rise and spread of resistant pathogens. Therefore, the driving force behind the increasing rates of resistance can ultimately be found in the overuse of antibacterial agents, whether in patients or livestock.\(^3\)

The continuing emergence of nosocomial pathogens such as multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, vancomycin resistant enterococci and *Staphylococcus aureus* (*S. aureus*) with intermediate susceptibility to vancomycin and other glycopeptide antibiotics threaten the lives of hospitalized individuals. Even the common community acquired infections, such as *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Salmonella*, *Campylobacter* species, *Neisseria gonorrhoeae* and Human Immunodeficiency virus are developing resistance to standard therapies.\(^4-6\) Infections caused by antibiotic resistant microorganisms fail to respond to the standard medical treatments, resulting in prolonged illness, higher health care expenditures and a great risk of death. For example, people infected with methicillin resistant *S. aureus* (MRSA) are estimated to be 64% more likely to die than people with a methicillin sensitive *S. aureus* (MSSA).\(^3\)
The annual economic burden associated with the treatment of antibiotic resistant infections have been estimated to be between $21,000 and $34,000 million in the United States alone, and around €1500 million in Europe. In the Indian context, Chandy et al., 2014 estimated the economic burden and health consequences due to antibiotic resistance in hospital inpatients in a tertiary care hospital at Vellore. Results showed the median difference between ‘resistant’ and ‘susceptible’ groups in overall costs, antibiotic costs and pharmacy costs as INR 41,993 (P =0.001); 8,315 (P <0.001) and 21,492 (P <0.001) respectively. Across all parameters, the ‘resistant’ group showed poorer outcomes such as increase in length of stay by three days, intensive care admissions, complications and mortality were 23% (P <0.001), 19% (P =0.006) and 10% (P =0.011) respectively. Studies, looking only at part of the impact of antimicrobial resistance, show that a continued rise in resistance by 2050 would lead to an estimated 10 million people dying every year and a reduction of 2 to 3.5% in Gross Domestic Product (GDP). It would cost the world up to $100 trillion. Antimicrobial resistance is no longer a medical issue instead, it has become a global threat that will require the action of many different stakeholders (i.e. policy makers, public health authorities, regulatory agencies, pharmaceutical companies and the scientific community at large) to tackle antibiotic resistance and come up with a coordinated set of strategies to fight antimicrobial resistance in a multifaceted approach. If resistance rates continue to rise at the present rate, there is a growing concern that therapeutic choices will be limited.
After the development of penicillin, there was a ‘golden age’ of antibiotic discovery during the 1950s and 1960s. However, after 1985 there has been a sharp falloff in new class of antibiotic discovery. Unfortunately during this same time-period MRSA, multidrug-resistant as well as extensively drug resistant Mycobacterium tuberculosis, multiple cases of hospital-acquired infections with Clostridium difficile, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii have emerged across the globe.\textsuperscript{10, 11} Since 1985, only two new classes of antibiotics have been approved for clinical use which includes an oxazolidinone (linezolid) in 2000 and a cyclic lipopeptide (daptomycin) in 2003. Until ten years ago, all major drug companies were taking part in antibacterial research programmes. Today, these programmes have been drastically pruned and many have been cut altogether as companies pursue more rewarding areas, such as chronic illnesses and mood disorders. The antimicrobial drug pipeline is running dry. According to the Tufts Center for the Study of Drug Development in Boston, estimated costs to bring a new drug to market is about $800 million on average over 10 to 15 years. This cost, combined with a likely low return on investment, makes pharmaceutical companies reluctant to invest funds in development of new antibiotics.\textsuperscript{12, 13}

1.2 Need for the study

Among gram positive bacteria, \textit{S. aureus} remains one of the most significant pathogens causing disease in animals and human. MRSA is the most important drug resistant pathogen emerged in the early post antibiotic era. The prevalence of MRSA in hospitals varies from 25 to 60\%.\textsuperscript{1, 3} Currently vancomycin is the drug of choice for MRSA infections. However, the efficacy of vancomycin was found to be low in the treatment of MRSA infections in compromised patients. The rate of mortality from invasive MRSA
Infections is reported to be 10 to 30% even after treatment with vancomycin. On the other hand, infections caused by *S. aureus* with intermediate resistance to glycopeptide or heterogeneously expressed intermediate level glycopeptide resistance are found to be associated with therapeutic failure. Linezolid was approved by the Food and Drug Administration (FDA), USA in April 2000 to treat both community and hospital acquired pneumonia and skin and soft-tissue infections (SSTIs) caused by MRSA. Tsiodras et al., 2001 reported the first clinical staphylococcal isolate resistant to linezolid, which was recovered from a patient receiving oral linezolid for treatment of peritoneal dialysis associated peritonitis. Subsequently, additional reports of clinical *S. aureus* isolates resistant to linezolid emerged.

The emergence of multidrug-resistant *S. aureus* has spurred the need for development of new antimicrobial agents. Scientists are showing a renewed interest in the enzymatic action of lysostaphin to treat staphylococcal infections. Lysostaphin produced by *Staphylococcus simulans* biovar *Staphylolyticus* is a glycylglycine endopeptidase zinc-containing metalloenzyme which causes the rapid lysis of the bacteria by hydrolyzing a pentaglycine cross bridge of the staphylococcal cell wall. Previous studies from 1960s to 1970s have reported lysostaphin as an effective antistaphylococcal agent. The studies on lysostaphin were discontinued due to lack of homogeneous preparations of lysostaphin and the availability of effective antibiotics. With the rapidly increasing resistance to available antibiotics and the availability of recombinant lysostaphin, have rekindled the interest in using lysostaphin as a therapeutic agent for staphylococcal infections. The present study was undertaken to evaluate the *in vitro* and *in vivo* bactericidal activity of recombinant against MRSA isolates.