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Introduction
INTRODUCTION

The word “antimicrobial” is derived from the Greek words anti (against), mikros (little) and bios (life) and refers to any substance of natural, semi synthetic or synthetic origin that kills or inhibits the growth of microorganisms at relatively low concentrations, but causes little or no damage to the host. The antibiotic era began with therapeutic applications of sulfonamides in 1930s, followed by a “golden” period (1935-1970) with a flurry of discoveries of effective antibiotics. With the discovery of antimicrobials, scientists prophesied the defeat of infectious diseases that had plagued humankind throughout the history. However, 1980s saw a decline in the discovery of new agents for clinical use. This period with a reduced rate of introduction of new agents has been accompanied by an alarming increase in drug-resistant microorganisms (Norrby et al., 2005). The development of resistant strains has prompted continuous efforts to exert control over antibiotic usage. Thus antimicrobial resistance has threatened the effective prevention and treatment of an ever increasing range of infections caused by pathogenic bacteria (Song, 2008)

The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. Antimicrobial drug resistance may occur due to a pre-existing factor in the microorganisms or it may be due to some acquired factors by genetic changes or nongenetic mechanisms. The most common mechanisms of genetic transfer are conjugation, transformation and transduction. Biological mechanisms include antibiotic transformations, active efflux, receptor modifications and alteration of target metabolic pathways (Kumar and Schweizer, 2005; Wright, 2005).

The highly concerned resistant bacteria causing community and hospital acquired infections include Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin Resistant Enterococcus (VRE), Vancomycin Resistant Staphylococcus aureus (VRSA), Extended Spectrum β-Lactamase (ESBL) producing Escherichia coli and Klebsiella spp., Haemophilus influenzae, Multi-Drug Resistant Mycobacterium tuberculosis (MDR-TB), Pseudomonas aeruginosa, Acinetobacter baumannii and
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*Enterobacter* spp. (Alanis, 2005; Billström et al., 2008; Lam et al., 2013; Pande and Bhailume, 2014). Thus, antimicrobial resistance has cast a shadow over the medical miracles we take for granted, undermining every clinical and public health program designed to contain infectious diseases worldwide (Davies and Davies, 2010).

Gram-negative bacteria accounts for more than 30 percent of common hospital acquired infections and are the leading causes of nosocomial pneumonia and urinary tract infections (Peleg and Hooper, 2010). The development of antimicrobial resistance among Gram-negative pathogens has been progressive and relentless. Pathogens of particular concern include ESBL-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, and multidrug-resistant *P. aeruginosa*. Classic antimicrobial agents used to treat Gram-negative pathogens include carbapenems, fluoroquinolones, β-lactam inhibitors *viz.* sulbactam, clavulanate, and tazobactam, aminoglycosides, and cephalosporins. Carbapenems and fluoroquinolones are considered potential agents in treating infections caused by ESBL-producing organisms. Recently, WHO’s first global report on antibiotic resistance released on April 30, 2014 reveals that *K. pneumoniae* and *E. coli* strains resistant to carbapenem and fluoroquinolones, respectively has spread to all regions of the world.

Among the infections caused by Gram-positive bacteria, one third nosocomial infections occur due to multiple drug resistant strains, and MRSA and VRE are of particular concern (Rice, 2006; Cookson et al., 1997; Brusselaers, 2011). Many potent antibiotics such as vancomycin, teicoplanin, synercid (quinipristin and dalfopristin), tigecycline and linezolid were developed against them. During 1970’s, vancomycin (glycopeptide) was considered the last line of treatment for MRSA and enterococci. Nevertheless, some MRSA strains and enterococci developed resistance to vancomycin (Murray, 2000). Synercid^AE^ (quinipristin and dalfopristin) and linezolid (Zyvox^AE^), active against most Gram-positive bacteria, were approved in 1999 and 2000, respectively by Food and Drug Administration (FDA) for the treatment of streptococci, VRE and MRSA. Unfortunately, increasing reports of resistance and treatment failures have been reported with synercid and linezolid. In 2003, daptomycin a novel cyclic lipopeptide was approved by FDA for the treatment of skin and soft tissue infections caused by MRSA and VRE for intravenous administration (Carpenter
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and Chambers, 2004). Daptomycin is the newest FDA approved lipopeptide, however, rare incidences of clinical resistance have also been reported against it (D'Costa et al., 2012). Since resistance to each new antibiotic ultimately emerges, within few years after it is marketed thus, there is always a need to find new antimicrobial agents to overcome antibiotic resistant strains of pathogenic microorganisms.

Recent advances in technology have sparked resurgence in the discovery of natural product antibiotics from bacterial sources. In particular, efforts have refocused on finding new antibiotics from streptomycetes and other actinomycetes, cyanobacteria and uncultured bacteria. Members of the genus *Streptomyces* have long been recognized as a rich source of useful biologically active compounds (Watve et al., 2001; Goodfellow and Fiedler, 2010; Kim et al., 2012). The genus is the largest in domain *Bacteria*, encompassing nearly 600 species with validly published names (Euzéby 2014). They are the origin of about 70% of marketed antibiotics and continue to be a major source of new antimicrobial compounds. However, to discover new therapeutic important compounds we need to screen phylogenetically novel streptomycetes, that are speculated to produce new bioactive compounds, and this will also avoid the costly rediscovery of already known compounds from them (Antony-Babu and Goodfellow, 2008). In the light of this, present study was carried out to discover a new *Streptomyces* isolate producing novel and potent antimicrobial compound(s) and work was carried out on the following lines:

- Isolation and screening of actinobacteria for antimicrobials, and selection of potent *Streptomyces* isolate.
- Determination of taxonomic position of selected isolate using polyphasic approach.
- Optimization of antimicrobial production using classical and statistical approaches, and recovery of compound(s).
- Purification of antimicrobial compound(s) by using a combination of silica gel, size exclusion and reversed phase chromatography techniques.
- Characterization of active compound(s) using Tricine-SDS-PAGE, MALDI-TOF-MS, MS/MS sequencing and GC-MS.
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- Safety evaluation of purified compound(s) by mutagenicity testing, DNA nicking assay and *in vitro* cytotoxicity assay.
- Improvement of the strain by protoplasm regeneration for enhanced production of antimicrobial compound(s).