INTRODUCTION

ANTIBIOTIC RESISTANCE

Antibiotic resistance is a drug resistance by which many bacteria are able to survive exposure to one or more antibiotics. Accordingly, pathogenic bacteria which have become resistant to several antibiotics cause infections which cannot be treated with the usual, formerly efficacious antibiotic drugs and their usual, formerly efficacious, dosages and concentrations. Resistance can be acquired or intrinsic. Many clinically relevant pathogens have developed resistance to large group of antibiotics and are called as multidrug resistant (MDR) pathogens. In recent years, the term superbug has become popular.

A Global challenge

Antibiotic resistance is a major public health issue worldwide. Although the natural selection of bacteria makes some resistance inevitable, the problem is largely driven by misuse and overuse of antibiotics. An increasing number of bacteria are becoming resistant to many drug developed to treat the infections they cause. Factors contributing to the resistance are:

- The rising number of healthcare associated infections
- Over-prescription of broad spectrum antibiotics
- Increasing cross-continental travel and global trade.

As the gap widens between the rising number of MDRO infections and the development of new antibiotics to treat the resistant bacteria have become one of healthcare’s biggest threats. There are few antibiotics in the development pipeline to meet the challenge of multi-drug resistance, and the most prudent use of existing antibiotics is crucial to preserve their efficacy.
Healthcare-associated infections (HAI) remain a major cause of mortality, morbidity and excess healthcare cost. In United States, methicillin resistant *Staphylococcus aureus* (MRSA) infections alone kill nearly 19,000 people a year and account for over 60% of the total number of hospital onset *S. aureus* infections.

The global problem of increasing trend in antibacterial resistance is particularly pressing in the developing countries, where the methicillin-resistant *Staphylococcus aureus* (MRSA) is often the severe casual agent in hospital-acquired infections. There is now an increase in difficulties to treat such patients because of emergence of resistance to all current antibiotic classes. Antibiotic resistance particularly in pathogenic organisms has become a serious and growing phenomenon in medicine and has emerged as one of the public health concerns of the 21st century. Clinically relevant organisms have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. Typically, the first-line antibiotics is selected on the basis of several advantages including availability, cost and safety. Comparatively, the second-line antibiotics are broad spectrum, are more expensive or may be unavailable. In the case of some MDR pathogens, resistance to second and even third-line antibiotics is sequentially acquired; a case illustrated by *Staphylococcus aureus* in some nosocomial settings.

**Staphylococcus aureus**

*Staphylococcus aureus* is a gram-positive cocci, non motile, non sporeforming, catalase positive, oxidase negative, facultatively anaerobic bacterium that exists as a skin commensal in a significant proportion of the population. Despite its ubiquitous nature, it is a recognised potential pathogen. *Staphylococcus aureus* is one of the most common causes of nosocomial or community based infections, leading to serious illnesses with high rate of morbidity and mortality. *S. aureus* can cause a range of infectious disease from mild conditions, such as skin and soft tissue infections such as abscesses, bullous, furunculosis, and staphylococcal scalded skin syndrome (SSSS) and impetigo; life threatening infections such as bacteremia (presence of bacteria in blood), osteomyelitis (infection of bone), pneumonia, (inflammation of lungs), infections of skin (impetigo, cellulitis and staphylococcal
scalded skin syndrome), endocarditis (infection of the endothelial lining of the heart and valves) and \textit{S. aureus} can also cause food poisoning, the result of enterotoxin production or neonatal TSS like exanthematous disease (NTED). (Tacconelli \textit{et al.} and Takahashi \textit{et al.}, 1998.) \textit{Staphylococcus aureus} remains an important cause of nosocomial infection, especially bloodstream infection and nosocomial pneumonia, surgical wound infection. (Schaberg, 1991). Treatment of infections caused by \textit{S. aureus} has become more problematic since the development of antibiotic resistance. Currently the most important problem is methicillin-resistant \textit{Staphylococcus aureus} (MRSA).

**Methicillin- resistant \textit{Staphylococcus aureus} (MRSA)**

MRSA is isolate of \textit{Staphylococcus aureus} that is resistant to a large group of antibiotics called \textit{Beta-} lactam. Another important feature is its ability to develop resistance to commonly used antibiotics. MRSA is a bacteria responsible for difficult to treat infections in humans. It may also known as multi drug resistant \textit{Staphylococcus aureus}. The development of resistance to penicillin by \textit{S. aureus}, mediated by the production of an enzyme known as penicillinase, a \textit{β}-lactamase, was first reported in 1945. This enzyme is able to break down the \textit{β}-lactam ring, rendering them ineffective. The introduction of new forms of penicillin that are not affected by \textit{β}-lactamase, such as flucloxacillin, methicillin, cloxacillin and initially provided effective control of clinical infections caused by penicillinase-producing isolates of \textit{S. aureus}. By the early 1960s, reports of resistance to these antibiotics began to appear, even before the antibiotics were used in clinical practice. In 1960s MRSA were first emerged as an important clinical problem in the United kingdom shortly after methicillin came into clinical use. (Jevons,1961). The difference between Methicillin resistance \textit{S. aureus} and methicillin susceptible \textit{Staphylococcus aureus} (MSSA) is resistance to \textit{β}- lactamase-stable \textit{β}-lactam antibiotics. Often this is associated with the resistance to multiple other antibiotics which limit the therapeutic options. Now-a-days, increasing prevalence of Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) worldwide is a growing public health concern.

**Why is MRSA particularly important in hospitals?**
In 1960s, MRSA emerged as a nosocomial pathogen. No antibiotic resistance marker has distinguished a species more than methicillin resistance has for *S. aureus*. The rapidity with which methicillin resistance developed in Europe after the introduction of methicillin and the subsequent spread of the organism throughout the world have created therapeutic problems for nurses, confusion for infection control practitioners, and resource-allocation uncertainties for hospital administrations.

MRSA infections in patients in health care facilities tend to be severe. These staph infections may be in the heart, lungs, urine bloodstream or other organs. Some symptoms of these severe infections are: fatigue, chest pain, cough or shortness of breath, headache, fever and chills, general ill feeling, rash, wounds that do not heal.

MRSA is particularly important in hospitals for four reasons:

- Hospitals contain a large number of patients with weakened immune systems who could become infected with MRSA and develop unwanted symptoms
- Patients who have an intravenous drip or a catheter that creates a wound through which MRSA can enter the body
- In some hospitals, patients are in close proximity to each other, which increases the chances of MRSA infecting patients. However, in others patients stay in separate rooms which helps to lower this risk
- Hospitals offer many opportunities for *Staphylococcus aureus* bacteria to encounter a wide range of antibiotics and, through genetic change and survival, develop resistance to all of them.

*Staphylococcus aureus* is the leading cause of hospital acquired infections worldwide. All methicillin resistant *S. aureus* (MRSA) isolates harboured the mobile element staphylococcal cassette chromosome mec (SCCmec) The SCCmec carries the mec gene complex, encoding methicillin resistance, and the ccr gene complex, encoding recombinases (Katayama, 2000). As determined on the basis of the diversity, five types of SCCmec and their variants have been reported previously (Ito, 2004; Katayama, 2000).

In recent years, the increase in the number of bacterial isolates that show resistance to methicillin (MRSA) has become a serious clinical problem because
resistance to this antibiotic implies resistance to all β-lactam antibiotics. The resistance problem demands that a novel approach be made to seek antibiotics effective against pathogenic bacteria resistant to current antibiotics. For these reasons, there is the need for cumulative antibiograms capturing the susceptibility data over a period of time half yearly or annually. In addition, accuracy and promptness in the detection of methicillin resistance is a key importance to ensure correct antibiotic treatment in the infected patients as well as control of MRSA isolates in hospital environments and to avoid them spreading. (Velasco et al., 2005).

The increased incidence of multidrug resistant S. aureus isolates among nosocomial or hospital acquired (HAI) infections has added a challenging dimension to the S. aureus problem. Several risk factors such as exposure to a health care settings, recent hospitalization, residence in long term care facilities, invasive or surgical procedures and injection drug use, predispose a patient to MRSA acquisition (CDC, 1999). The emergence of MRSA isolates and resistance to other antibiotics has become a major concern, especially in the hospital environment, because of increased mortality due to systemic MRSA infection (Klein et al., 2007). Therefore, there is a need to monitor the development of resistance and to establish empirical therapy.

Antibiogram

The hospital antibiogram is a periodic summary of antibiotic susceptibilities of all bacterial isolates submitted to the clinical microbiology laboratory. The antibiogram helps in monitoring antibiotic resistance trends over different periods. Antibiograms are used by clinicians to assess local susceptibility rates and it helps in selecting empiric antibiotic therapy and in monitoring resistance trends over time within a hospital. The guidelines have been developed by the CLSI to standardise methods used in constructing antibiograms, with the goal of promoting the reporting of reliable and consistent antibiogram data. The current guideline is CLSI M39-A3, entitled "Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. However, the hospital antibiogram cannot be used alone to select the optimal empiric therapy in an individual patient, as specific patient factors need to be considered,
The synergistic effect of nanoparticle(s) on the antibacterial activity of a panel of antibiotics against resistant phenotypes of *Staphylococcus aureus*

including the infecting organism, the type and severity of infection, the past antibiotic use and patient's medical history.

The prevalence of MRSA varies from one geographic region to another and between different institutions in a given area. The prevalence of MRSA differs markedly among European countries (Voss, 1994). The frequencies of outbreaks and infections due to methicillin resistant *S. aureus* have continued to increase. MRSA is often multidrug resistant and therapeutic options are limited. In many cases the only glycopeptide antibiotics such as vancomycin and teicoplanine are the only therapeutic alternatives. However, glycopeptide resistance is expected to become an important problem in the future, because of the reduced susceptibility of *S. aureus* isolates to this group of antibiotics. (Brawns *et al*, 2005; Tenover *et al*, 2004; Appelbaum, 2006).

**Why perform Susceptibility testing:**

Diagnostic tests play an essential role in the diagnosis and monitoring of infection, as well as the surveillance of resistance, and contribute to the prudent use of antibiotics. Antibiotic susceptibility testing is to measure the susceptibility of a bacterial isolate, to one or several antibiotics. In effect, *in vitro* susceptibility is a prerequisite for the *in vitro* efficacy of an antibiotic therapy. The susceptibility test serves first and foremost to orient individual therapeutic decisions.

Secondly, susceptibility is to monitor the evolution of bacterial resistance. It is due this epidemiological follow up by region or country, healthcare establishment, ward that antibiotic clinical spectra regularly revised and empiric therapy can be adapted. Moreover the detection of a large number of patients infected with multidrug resistant bacterial isolates at one time and in the same place can influence some healthcare decisions, such as the implementation of prevention programs in hospitals.

Antibiotic Susceptibility testing Interpretation requires proper Identification because of four reasons:

- Bacterial identification helps the distinction between intrinsic and acquired resistances.
The synergistic effect of nanoparticle(s) on the antibacterial activity of a panel of antibiotics against resistant phenotypes of *Staphylococcus aureus*

- The prediction of the phenotype is linked to the species
- The level of resistance is linked to the species since the same enzyme can produce substantial resistance in one species or limited resistance in another.

The burden of the rapid and accurate detection of antibiotic resistance among *S. aureus* remains a continuous challenge for clinical microbiology laboratories. There are several methods for detecting methicillin resistance including conventional methods like disc diffusion method (Kirby-bauer), screening techniques (chromogenic media), for determining MICs (E test, broth dilution), automated identification and susceptibility (ID/AST) testing systems and methods that detect the *mecA* gene or its protein product, PBP2’protein (Van Leeuwen, 1999; Louie, 2001). The detection of *mecA* gene is only considered as the reference method for determining resistance to methicillin (Chambers, 1997). However, few clinical laboratories throughout the world have the capacity or the experienced staff required to develop molecular techniques for detecting MRSA isolates.

When a pathogen is isolated in a clinical microbiology laboratory, the time taken for its identification and susceptibility testing may delay the administration of the appropriate treatment. For routine testing, the ideal method would be rapid and automated, would have minimum sample preparation, would have availability and inexpensive, would analyse sample directly and reliable. (Goodacre, 1996). Most of these requirements are met by automated ID/AST system, Vitek 2 compact. Molecular tests have recently become available that allow for detection of MRSA in less than 1 hour and possibly an increase in sensitivity of detection. (Huletsky, 2004). Although these tests are more expensive than culture (Ritchie, 2006), modelling studies suggest that reducing the reporting time for a positive MRSA result can reduce both the rate of nosocomial transmission (Raboud, 2005) and the number of patient isolation days. (Bootsma, 2006). However, the phenotypic methods are cost effective, are widely available, easier to perform and are easier to interpret however less discriminatory. The genotypic methods are expensive and technically demanding, however more discriminatory.

**Nanoparticles**
The worldwide escalation of bacterial resistance to conventional medical antibiotics is a serious concern for modern medicine. New improvements in present methods and novel strategies are urgently needed to cope with this problem. Owing to their antibacterial activities, metallic nanoparticles represent an effective solution for overcoming bacterial resistance.

**OBJECTIVES**

Thus keeping in view of the above problem the present study was designed with the following aims and objective.

1. The identification of *Staphylococcus aureus* by different biochemical tests.
2. *In vitro* susceptibility testing of *S. aureus* by Kirby-bauer method and by automated susceptibility system (Vitek 2 compact) for minimum inhibitory concentration (MIC).
3. To investigate the resistance pattern of isolated *S. aureus* to various routinely used antibiotics.
4. To assess the prevalence of methicillin resistant *S. aureus* (MRSA) infection from different clinical samples
5. Comparing antibiogram profile of the isolated MRSA and to find out the current pattern of antibiotic resistance among clinical isolates.
6. To evaluate the performance of chromogenic screening agar, Cefoxitin disc diffusion test and MIC values by Vitek and to compare and contrast their suitability as routine methods for detecting MRSA isolates in clinical microbiology laboratories.
7. To determine the synergistic effect of nanoparticles on the antibacterial activity of different antibiotics.