CHAPTER I

INTRODUCTION
1.1 Staphylococci: Discovery

Fossil evidence suggests that staphylococci have existed on earth for more than a billion years, although it was not until the 19th century that they were actually identified as bacterial pathogens. Staphylococci were first observed and cultured by Louis Pasteur and Robert Koch, but the initial detailed studies of staphylococci were made by Ogston in 1881 and Rosenbach in 1884. (1, 2) The genus *Staphylococcus* was given its name by Ogston in 1881 when he observed grape-like clusters of bacteria (Greek *staphyle*, “a bunch of grapes; kokkos, “grain or berry”) in pus from human abscesses. (1) Three years later, Rosenbach was able to isolate and grow these microorganisms in pure culture. He differentiated the staphylococci on the basis of the pigmentation of the growth. For the staphylococcal colonies with golden appearance, Rosenbach used specific epithet *Staphylococcus aureus* (Latin *aureus*, “golden”), and those with white pigment—*Staphylococcus albus* (Latin *albus*, “white”). (2) Later the *Staphylococcus albus* was known as *Staphylococcus epidermidis*.

1.2 *Staphylococcus aureus* Infections

The bacterium *Staphylococcus aureus* (*S. aureus*) can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), and
septicaemia. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections.

The infections confined to hospitals and other health care environments are termed hospital acquired infections (HAI), or nosocomial infections. Most of the S. aureus isolates causing infections in hospitals are found resistant to methicillin, a penicillinase resistant semisynthetic antibiotic. Such isolates are categorised as methicillin resistant S. aureus (MRSA), and since involved in causing nosocomial infections are named as hospital-acquired or healthcare-associated MRSA (HA-MRSA). The MRSA infections were found confined to hospital until early 1990s. It was mid-1990s when MRSA infection cases were first reported in people who had not been exposed to a healthcare setting. Such MRSA causing infections in community are called community-associated MRSA (CA-MRSA).

Sites where pathogens are maintained as a source of infection are termed reservoirs of infection. MRSA can spread from person to person via direct casual contact or from animals to human. The natural reservoir of S. aureus is humans, infected or carriers, in whom S. aureus are colonized in anterior nares, perineum or skin. Carriage rates vary from 20-50% in the general population. It is higher in diabetics, in patients with intravascular lines, in health care workers, and in drug users. Colonisation may be transient or persistent and can last for years. S. aureus including MRSA has been isolated from various animals both infected and carriers. Transmission to man has been reported from dogs, horses, pigs and cows, as evidenced by phenotyping and genotyping of the isolates. Several inanimate sources (fomites) in the environment have been shown many times to be contaminated by the S. aureus, but the relevance of its presence on fomites to human colonization and infection is not clear. Limited evidence from studies in the health care setting indicates that environmental contamination can lead to
human colonization and disease with MRSA, (10) although much of the data are only suggestive.

1.3 Milestones in the History of Antibiotics

1.3.1 Beginning of antibiotic era

The foundation of antibiotic discovery was laid by the father of chemotherapy, Paul Ehrlich. (16, 17) In 1906, Ehrlich, together with Bertheim, developed hundreds of derivatives of Atoxyl, and finally discovered compound 606, a gold powder. (16-18) In 1909, he found that Compound 606 could cure syphilis-infected rabbits in experiments; it could also improve terminal patients with dementia and cured early stage patients with infected sores. (18) The anti-syphilis compound was publicly released as salvarsan in 1910. (17) Despite the adverse side effects, salvarsan and its derivative neosalvarsan kept the status of the most frequently prescribed drug until the introduction of penicillin in the 1940s. (19)

Though the systematic screening approach introduced by Paul Ehrlich became the cornerstone of drug search strategies in the pharmaceutical industry, the antibiotic era was started from the discovery of penicillin by Sir Alexander Fleming in 1928. (20) Fleming observed the antibacterial property of Penicillium notatum, after an accidental event of contamination of S. aureus pure culture with the environmental mold. However, the first scientist to study the antibiotic properties of the penicillium mold was probably the young French researcher Ernest Duchesne (1874-1912) as part of his PhD research. (21) Although Fleming had named the fungal broth culture filtrate with antibacterial property as “penicillin”, it took more than a decade that the agent in pure form was successfully extracted in 1939 by Howard Walter Florey and Ernst Boris Chain (1906-1979). (22) Though earlier clinical use of penicillin seems to be in 1930 when one of
Fleming’s former students, Cecile Paine tested raw penicillin extracts on few patients with some success, but the cases were not published by him. The pure extract of penicillin was first reported to be used clinically in 1941.\(^{(23)}\)

**Figure 1.1.** Upper left; Alexander Fleming (1881–1955) in his laboratory. Upper right; World War II poster. Lower left; Production of penicillin in bedpans during the first years of the World War II. Lower right; Production of penicillin at the end of the World War II.
During World War II (1939-1945), British and U.S. pharmaceutical companies developed the appropriate technology for mass production of benzylpenicillin (penicillin G). (24) Penicillin proved clinically effective in the treatment of infections caused by many bacterial species such as staphylococci, streptococci, gonococci, meningococci, pneumococci, and the diphtheria bacteria. (24) Furthermore, penicillin was also successfully used in the treatment of anthrax and syphilis. (24) Penicillin worked well without toxic effects and without impairing the ability of white blood cells to defeat the infection. In particular, it was its effectiveness in the treatment of sexually transmitted diseases (STDs) and surgical sepsis in wounded allied soldiers that in short time penicillin earned the reputation as a ‘magical bullet’ or a ‘miracle drug’. (24) Penicillin saved millions of lives by the end of World War II, with a great reduction in mortality rate than that in World War I (Figure 1.1). Its availability was first restricted to military uses, and it did not reach civilian medicine until the end of the war. After the war, penicillins other than penicillin G were developed by adding certain precursors to the fermentation process. However, it was not until 1954 that phenoxymethylpenicillin (penicillin V) was recognized for its acid stability, implying that this drug could be taken by mouth. During the 1950s, semisynthetic penicillins, ampicillin and amoxicillin, were developed in the UK, which both had broader antimicrobial spectrums than penicillin G. During the next two decades, a period often referred to as the antibiotic revolution or the antibiotic era, penicillin was followed by a large number of new antibiotics. (24)

1.3.2 Antibiotic resistance

The first report of penicillinase-producing S. aureus was published in 1940 surprisingly almost a year before penicillin was marketed for clinical use. (25-27) By 1943, penicillin was widely available and demand for the drug grew. The antibiotic was seen as miracle drug, and patients developed a generalized expectation for a rapid cure. Even minor
illnesses, previously handled by the body’s own defences, were treated with these miracle drugs. Unfortunately, widespread use of the antibiotics led to a selection of resistant bacteria. Resistance to penicillin emerged and was linked to patient deaths in the early 1950s. (28) Interestingly, Alexander Fleming predicted this event in 1945. (29)

“But I would like to sound one note of warning.... It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body.”

(Alexander Fleming)

Treatment of infections due to penicillin resistant *S. aureus* & other bacterial pathogens became an increasing challenge and worrisome issue for the healthcare professionals. Methicillin was produced at Beecham Research Laboratories as first semi-synthetic penicillin to treat infections caused by penicillin-resistant *Staphylococcus aureus*. (30) On the development of methicillin for penicillin resistant bacteria, many believed that this was the end of the resistant staphylococcus and Ernst Chain was the one scientist who said “no more resistance problems, methicillin is the answer”. (30) After the first clinical use of methicillin in 1959, the methicillin resistant *Staphylococcus aureus* (MRSA) strains were detected in 1961. (31-34) MRSA became a clinical and therapeutical problem with the occurrence of ‘multi-resistant’ strains in the late 1970s. (35, 36) Because of the resistance, toxicity and many side effects, methicillin was discontinued from therapeutic use, and its production was replaced with more stable and similar compounds such as oxacillin, flucloxacillin, and dicloxacillin.

Vancomycin is the potential glycopeptides drug that can reliably treat MRSA infections. (37) However, the massive use of vancomycin for treating MRSA has caused the emergence of vancomycin resistant *S. aureus* (VRSA) and vancomycin intermediate *S.
The first strain of *S. aureus* with reduced susceptibility to vancomycin was isolated in 1996 from a Japanese patient. The first clinical isolate of VRSA was reported from United States in 2002. Emergence of VISA and VRSA has now become a global issue including India.

The therapeutic and life-saving option for VRSA and VISA infections remains linezolid, first antimicrobial of oxazolidinone group available since 2000. The first case of linezolid-resistant staphylococci appeared within 1 year after linezolid was approved for therapeutic use. Although linezolid resistance in *S. aureus* is uncommon, emergence has been shown from some parts of the world. From India, first case report of linezolid resistance was published in 2011 from Kashmir.

### 1.4 Impact of Increasing Antibiotic Resistance

Antimicrobial resistance causes the ineffectiveness of antibiotics or a delay in therapy and presents a threat to the successful treatment of bacterial infections with subsequent adverse consequences of increase morbidity, mortality, length of hospital stay, and health care costs. Factors that contribute to the development of resistance include antibiotic usage to treat infectious diseases in animals and plants, unnecessary antibiotic use in people, incorrect dosing regimens, and failure to complete antibiotic treatment courses; exposure to antimicrobial soaps and solutions.

*S. aureus* is a leading cause of hospital acquired infections associated with significant increase in morbidity, mortality and hospital cost. Increasing resistance in the organism to various antimicrobial agents is a worldwide problem and challenging threat to the healthcare professionals. The mortality rate from a *S. aureus* infection was as high as 82 percent in the preantibiotics era but fell dramatically after the introduction of penicillin in market in 1941. Presently, about >80% *S. aureus* are reported resistant to
Resistance to methicillin in *S. aureus* is associated with the resistance to multiple drug resistance (MDR). MRSA with MDR leaves a limited choice of antibiotics for treatment, and cause difficult-to-treat infections. (57) This in turn not only affects the rates of morbidity and mortality, but also the hospital cost, and thus the economy of a country. Several studies have estimated that antimicrobial drug–resistant infections increase death, illness, and direct costs by 30%–100%. (58)

A study by Klein et al. (54) estimated that in U.S.A. during 1999 through 2005, the number of *S. aureus*–related hospitalizations increased 62%, from 294,570 to 477,927, and the number of MRSA related hospitalizations more than doubled from 127,036 to 278,203, and also that the *S. aureus*-related deaths averaged ≈ 10,800 per year (range 7,440–13,676) and MRSA-related deaths averaged ≈ 5,500 per year.

In U.S.A., the estimates of the excess cost of an infection with MRSA compared with an infection with methicillin-sensitive *S. aureus* range from ≈ $3,000 to $35,000. (59-61) Cassell and Mekalanos (62) estimated the U.S. hospital costs in 1997 caused only by resistant *S. aureus* to be $122 million, whereas the total costs for all nosocomial infections were $4.5 billion.

Antibiotic resistance has now been 72 years old issue affecting public health worldwide. During this period, bacterial pathogens of human have developed resistance against the several antimicrobials in almost all the known classes of antibiotics. It was a 12 years gap from the penicillin discovery (20, 29) to its extraction in pure form, (22) whereas it is very amazing that the *S. aureus* developed resistance to penicillin in just 12 months. (25-27) On the development of methicillin for penicillin resistant bacteria, it was believed to be the end of the resistant staphylococcus. (30) The 20 years distance from first report of penicillinase-producing *S. aureus* (25-27) to the first entry of methicillin in market was
spoiled again in just 1 year with the appearance of MRSA in 1961. (31-34) The fast increase in resistance to even newer antimicrobial agents provides the first glimpse of medical outcomes in a post-antibiotics era. (63, 64)

1.5 Antimicrobial Resistance Surveillance

Antibiotic sensitivity patterns vary geographically; therefore, empiric therapy should be based on local summary tables, known as antibiograms. (52, 65) Most hospitals report cumulative susceptibility testing in these tables. A typical antibiogram displays the total number of bacterial isolates tested against a range of antimicrobials and includes the percentage of bacterial isolates susceptible to or resistant to each antimicrobial agent tested. (52) Antibiograms that include larger numbers of isolates for a particular bacterial species provide a more accurate assessment of antibiotic susceptibility because the impact of unusual isolates is minimized. (66) These antibiograms should be used by clinicians both in hospital and in community practice to optimize initial antibiotic choices and improve patient outcomes. (52)

The Centers for Disease Control and Prevention (CDC) and Clinical and Laboratory Standards Institute (CLSI), along with accreditation organizations and regulatory agencies, emphasize the need for an accurate institutional antibiogram that is standardized and can guide empiric therapy and prevent antibiotic resistance. Annual guidelines are published by CLSI for performance and interpretation of disk diffusion and MIC tests. (67) Clinical laboratories should use one of the CLSI reference methods or a commercial test system that has been cleared by the US Food and Drug Administration (FDA) for testing clinical isolates. (68)
1.6 Tribal Region Bastar

1.6.1 Geographic location

Bastar is a district of Chhattisgarh state in central India located in the geographical coordinates between 19.07° North latitude and 82.03° East longitude (Figure 1.2). The headquarter of the district is Jagdalpur. The district has an area of 8755.79 km². Bastar district is bounded on the northwest by Rajnandgaon district, on the north by Kanker district, on the northeast by Dhamtari district, on the east by Nabarangpur and Koraput districts of Orissa state, on the south and southwest by Dantewada district, and on the west by Gadchiroli District of Maharashtra state.

1.6.2 History

Bastar and Dantewada districts were formerly part of the princely state of Bastar. Earlier studies of the region were conducted by Verrier Elwin a colonial anthropologist. After Indian independence in 1947, the princely states of Bastar and Kanker acceded to the Government of India, and were merged to form Bastar District of Madhya Pradesh state. The district, which had an area of 39,114 km², was one of the largest in India. In 1999, the district was divided into the present-day districts of Bastar, Dantewada, and Kanker, which constitute Bastar Division. In 2000, Bastar was one of the 16 Madhya Pradesh districts that formed the new state of Chhattisgarh. Presently, the district is divided into four tehsils; Jagdalpur, Kondagaon, Keshkal, and Bastar. The district has two municipalities; Jagdalpur and Kondgaon. A beautiful city, Jagdalpur is the administrative headquarter of the district having population of about 1.5 lakhs (150,000).
Figure 1.2. Geographic location of Bastar in map of India.
1.6.3 Demographics

According to the 2011 census, (71) Bastar district has a population of 1,411,644, (69) roughly equal to the nation of Swaziland (72) or the US state of Hawaii. (73) This gives it a ranking of 348th in India (out of a total of 640). (71) The district has a population density of 140 inhabitants per square kilometre (360/sq mi). (71) Its population growth rate over the decade 2001-2011 was 17.8%. (71) Bastar has a sex ratio of 1024 females for every 1000 males, and a literacy rate of 54.9%. (71) Languages spoken include Bhatri, which falls within the Oriya language group but only shares about 60% lexical similarity with Oriya, spoken by about 600000. (74)

1.6.4 Health status

More than half of the world's tribal population lives in India. (75) There are more than 533 tribes comprising 8 percent of the total population of India. Largest concentrations of tribals (15.4 million) are found in Madhya Pradesh. Bastar district of Chattisgarh state (formarly in Madhya Pradesh) has one of the largest (67.4 %) congregations of tribal population. Poverty, illiteracy, malnutrition, poor sanitary conditions and scarcity of safe drinking water have contributed to the poor health status of these people all over the country. (75) Lack of infrastructures, inaccessibility to health institutions and affordability are some of the main problems contributing to their poor health status. (75) The available information on health status of tribes is grossly inadequate, though studies have reported anaemia, diarrhoea and malaria as the common health problems in tribal women and children in various parts of the country.

An earlier study by Chopra et al. (75) revealed the following common health problems encountered by the tribal community in Bastar District:
• Fever (72.5%), cough and cold (56.4%), skin problems (22.0%) and diarrhoeal diseases (32.9%).

• Anaemia, malnutrition, diarrhoeal disease and skin infection among mothers and children.

• Vaginal discharge and excessive bleeding during menstruation in mothers only.

There is unhygienicity, poor socio-economic status, lack of awareness about infection among the tribes of Bastar, and scarcity in the culture and sensitivity facility in the available private pathology/diagnostic laboratories. Most of the patients are being treated empirically at primary health centers (PHC). Chopra et al. gathered information that the majority of Bastar population prefers to avail PHC and government institutions for their health issue and only 16 percent used services from private practitioners including traditional healing methods (sirha or gunia).