1.1 PRELUDE

Microbial diseases are responsible for an estimated 17.8% of all deaths world-wide, amounting to almost 10 million deaths per year. Infectious diseases have an important impact on children and young adults, particularly in countries with scarce economical resources. In India, one in every six children dies before reaching the age of five years due to malnutrition and infectious diseases are the main killers (Global Hunger Index, 2011). However, it is notoriously difficult to assess which diseases contribute most to the suffering and death in the world because proper diagnostic tools are not available in large areas of the world and because many patients suffer from more than one condition at the time. Particularly, many children dying with infectious diseases are also suffering from severe microbial infections and it may be difficult to say which is contributing most to the suffering and death of the patients. Similarly, people dying with HIV infection almost invariably have one or more other conditions such as tuberculosis, other bacterial and parasitic infections and cancers. Thus, microbial infections with Multi drug resistant organisms immunesuppression by HIV or other causes, all contribute to the complex picture of infectious diseases in the developing world. The World Health Organization (WHO) rank the major causes of mortality in children younger than five years in India as neonatal causes (26%, among which the entity “sepsis or pneumonia” contributes a quarter), pneumonia (21%), malaria (18%) diarrhea (16%) and HIV-infection (6%) (www.http://en.wikipedia.org/wiki/ Infectious_disease).
The bacterial infections which contribute most to human disease are also those in which emerging and microbial resistance is most evident, urinary tract infections, diarrheal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital-acquired infections. Some important examples include penicillin-resistant *Streptococcus pneumoniae*, Vancomycin-resistant Enterococci, Methicillin-resistant *Staphylococcus aureus*, Multi Drug Resistant *Salmonellae*, and multi-resistant *Mycobacterium tuberculosis* (Daszak *et al.*, 2000, Krauss *et al.*, 2003, Rachel *et al.*, 2005, Gaynor *et al.*, 2005).

Infections caused by resistant microbes fail to respond to treatment, resulting in prolonged illness and greater risk of death. Treatment failures also lead to longer periods of infectivity, which increase the numbers of infected people moving in the community and thus expose the general population to the risk of contracting a resistant strain of infection (Odonkor and Addo, 2011).

When infections become resistant to first-line antimicrobials, treatment has to be switched to second and third-line drugs, which are nearly always much more expensive and sometimes more toxic as well, e.g. the drugs needed to treat multidrug-resistant forms of tuberculosis are over 100 times more expensive than the first-line drugs used to treat non-resistant forms. In many countries, the high cost of such replacement drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread. Most alarming of all are diseases where resistance is developing for virtually all currently available drugs, thus raising the spectrum of a post-antibiotic era. Even if the pharmaceutical industry were to step up efforts to develop new replacement drugs immediately, current trends
suggest that some diseases will have no effective therapies within the next ten years (Laxminarayan, 2003; Zuccato et al., 2005; Hulscher et al., 2010).

1.2 NEGLECTED INTESTINAL PARASITIC INFECTIONS IN ASSAM

Intestinal parasites and protozoan infections are amongst the most common infections worldwide. It is estimated that some 3.5 billion people are affected, and that 450 million are ill as a result of these infections, the majority being children (WHO, 2004). These infections are documented as serious public health problems as they cause iron deficiency anemia, growth retardation in children and other physical and mental health problems (Evans and Stephenson, 1995). Typically the parasites involved include *Ascaris lumbricoides*, *Trichuris trichiura*, hook worm and *Strongyloides stercoralis*. Worm transmission is enhanced by poor socio economic conditions, deficiencies in sanitary facilities, improper disposal of human faces, insufficient supplies of potable water, poor personal hygiene, substandard housing and lack of education (WHO, 2002).

These intestinal parasites might cause severe conditions like dysentery, dehydration, haemorrhage, anemia, appendicitis, which sometimes lead to death. Ascariasis interferes with protein digestion in children and in combination with hookworm infection or other intestinal parasites could cause, accompany or accentuate kwashiorkor. *Schistosoma haematobium* causes dysuria, increased frequency of micro nutrition deficiency and haematuria (urine with blood) conditions which can eventually lead to kidney failure if untreated. The parasites’ eggs can cause a substantial damage to the liver, intestines, bladder and kidney and cause death in some cases. Endemic hookworm infection keeps affected populations mired in a cycle of destitution and despair by affecting child growth and development,
pregnancy outcome, and worker productivity (Hotez and Ferris, 2006). These factors also account for the many disability-adjusted life years lost because of hookworm (Hotez et al., 2004 & 2005).

The remote humid environment, traditional ways of life, contaminated potable water and limited health services, contribute to the transmission and persistence of fecal parasites (Chandrasekhar and Nagesha, 2003). Prevalence of enteric parasites in the rural population was 51.7% in India (Chandrashekar et al., 2005). Parasites identified included A. lumbricoides, Ancylostoma duodenale, T. trichuria. Enterobius vermicularis, Blastocystis hominis, Entamoeba coli, Entamoeba histolytica, Giardia lamblia and S. stercoralis. The prevalence was significantly increased in women, people aged between 15 to 19 years and over 80 years, the rural farm population, farmers, preschool and primary school groups, residents of a hilly rather than mountainous or plains terrain, and in intermediate income groups (Ning Tang and Nian Ji Luo, 2003).

1.3 NEGLECTED FUNGAL INFECTIONS

There are many different fungal floras around the world. Only few of them are considered as pathogenic. These variations are caused by different climatic conditions, life styles, working conditions and socio economic factors (Vander Straten et al., 2003). These pathogenic fungi cause infections in animals and human beings. Annually they affect millions of individuals. Dermatophytes are one of the most common sources of human fungal infections. Dermatophytic infections have a worldwide distribution with geographical differences in the incidence and prevalence (Campbell et al., 2006; Nweze, 2010). Some dermatophytes spread directly from one person to another (anthropophilic dermatophytes). Geophilic dermatophytes inhabit in
soil and transmitted to humans and still others spread to humans from animal hosts (zoophilic dermatophytes) (Adetosoye, 1977; Abdel Rahman et al., 2010).

Invasive fungal infections remain a life-threatening disease. The development of invasive fungal disease is dependent on multiple factors, such us climate, personal hygiene, environmental factors, malnutrition and poor host immune response. Prevalence of fungal infections was higher in the patient group comprising hematological disorders (19.9%) than in other patient groups (2.9%). Odds ratio was 18.4 for Mucormycosis and 10.0 for Aspergillosis (Shimodaira et al., 2012).

Fungal corneal ulcer in Assam was the predominant fungal infections. Most of the fungal infection cases come from rural areas including the tea garden. The commonest predisposing factor was corneal injury, diabetes, mixed bacterial and fungal infections, poor socio economic factors and unhygienic conditions (Nath et al., 2011).

Fungal infection is predominant disease in tropical countries due to its factors like heat and humidity (Bharathi et al., 2003). This tropical part of Assam receives heavy rainfall during the monsoon months and the humidity remains high almost throughout the year. The high humidity and temperature provides a fertile ground for the abundant growth of fungus. It has been reported from different parts of India (Chander and Sharma, 1994; Basak et al., 2005; Chowdhury and Singh, 2005). But no reports were made from North East part of Assam. In this part, the rural dwellers and tribes are believed to be at higher risk of zoophilic and geophilic dermatophytic infections. Their prime occupation is farming and tea garden work. Hence they are having continuous contact with soil, and moreover all of their houses are built and floored with soil. In addition to that, they are in regular contact with farm animals,
wild animals (rats, mice, etc.) which could also be the source of fungal infection. Inadequacy of data, regarding the prevalence of fungal infections in tropical regions, motivated us to assess the frequency of fungal infections among rural dwellers.

1.4 NATURAL REMEDY AND EMERGING DRUG RESISTANT MICROBES

The incessant and indiscriminate use of antibiotics because of poverty leads to the development of microbial resurgence and acquired resistance by the bacterial pathogens in community level especially in developing countries.

Plants have a long history of use in most communities throughout the world. The plant world comprise of a rich storehouse of secondary metabolites endowed with potential antifungal, antibacterial, antihelminthic and antiviral properties (Kasamota et al., 1995; Sunday et al., 2001; Ameh et al., 2010). These are either the end products of aberrant biosynthetic pathway or the excretory products (Balandrin et al., 1985). They act as phytomedicine, example, Alkaloids, Furanococumarins and Flavanoids (Komaraiah et al., 2003; Juengwatanatrakul Thaweesak et al., 2011). Antimicrobial compound of plant origin are systemic, inexpensive and without any side effects (Marjorie Murphy Cowan, 1999). Several in vitro investigations have proved that the plant products which exhibited a strongest toxicity against bacterial pathogens are superior to synthetic antifungal compounds (Iwu et al., 1999; Evans et al., 2002; Karaman et al., 2003; Syedhidayathulla et al., 2011).

Cow urine has amazing germicidal properties to kill varieties of germs. All germs generate disease are thus destroyed. This residue causes disease. Cow urine destroys the poisonous effect of chemotherapeutic residues. The nutrients, which are less available in food, are compensated by nutrients present in Cow urine and thus help to regain health. Cow urine is elixir and stops aging process. Cow urine and cow
dung possess antibacterial properties, Animal urine has antiseptic and mild antifungal properties. Cow urine mixed with water controls virus, fungus and bacterium (Dhama et al., 2005; Awale, 2006). Botanicals with Cow urine extract do not develop resistance in pathogens and do not affect the normal flora. A perusal of literature proved that there is no report available on antibacterial properties of this formulation especially against the Multi Drug-Resistant Organisms (MDR).

Emergence of Multi drug resistant pathogens are quite common all over the world especially in North East part of Assam due to poverty, malnutrition, self-medication, insufficient medical practitioner and Primary health care. Multi drug resistant microbes fail to respond the treatment, resulting in prolonged illness and greater risk of death. Longer period of infectivity leads to the exposure of the disease to the general population. When first line antibiotics fail to control the infections second and third line drugs which are much more expensive and more toxic must play the role of controlling the microbes. (Example-the drugs needed to treat multidrug-resistant forms of tuberculosis are over 100 times more expensive than the first-line drugs used to treat non resistant forms.)

Alternative, cost effective and secured form of control measure for these Multi drug resistant pathogens is the need of the hour. Data on prevalence of bacterial, fungal and intestinal parasitic infections are not available in this population yet. Worldwide, emergence of Multi Drug Resistance is more common in bacteria than fungus and intestinal parasites. Hence the present work is focused on the prevalence of bacterial, fungal and intestinal parasites, drug resistance pattern, and molecular characterization of the bacterial isolates. Moreover, as a step towards the search for an alternate medicine, natural formulation consists of cow urine and medicinal plant
viz Azadirachta indica, Leucas aspera and Kappaphycus alvarezii (sea weed) were assayed for their antibacterial activity against the bacterial isolates.

1.5 OBJECTIVE OF THE PRESENT STUDY

1. To determine the prevalence of various pathogenic bacteria, fungi and intestinal parasites as etiological agents in infections in rural dwellers of North East Assam.
2. Isolation and identification of bacterial, fungal and parasites and drug resistant patterns of the isolates.
3. Natural remedy against MDR isolates by using Cow urine extract of medicinal Plant.
4. To assay the antibacterial efficacy of aqueous, Cow urine and organic fractions of selected medicinal plants against the MDR isolates.
5. To analyze the secondary metabolites of extracts, and fractions both qualitatively and quantitatively.
6. To identify and characterize the exact molecule or secondary metabolite responsible for activity.
7. To characterize the active principles isolated from the selected medicinal plants using GC-MS, NMR and FT-IR spectrometry.
8. To characterize the Extended-spectrum beta-lactamase (ESBL) production of the MDR bacterial isolates.
9. To construct the phylogenetic tree using MEGA.4, and find out the phylogeny
10. Identification of, the mode of action of the identified compounds using Molecular docking used by Auto Dock program.