INTRODUCTION

Antibiotic resistance is a coping mechanism in bacteria to evade or nullify the deleterious effect of drugs by evolving genetic or intrinsic means to resist attack. Resistance to β-lactam antibiotics have been reported to be a bacterial defense mechanism observed in many soil bacterium even before penicillin, the first β-lactam antibiotic was discovered. The first β-lactamase identified prior to the release of penicillin in medical practice was in *Escherichia coli* (Abraham & Chain, 1940). Eventually, most of gram-negative bacteria were observed to possess naturally occurring chromosomally mediated β-lactamases; due to the selective pressure exerted by β-lactam producing soil organisms found in the environment (Ghuysen, 1991). The first plasmid mediated β-lactamase was discovered in 1965 in *Escherichia coli* isolated from a patient named Temoniera in Greece and was designated TEM (Datta & Kontomichalou, 1965). The transference of TEM-1 to other bacteria via horizontal gene transfer was a result of its association with transposons which facilitated its spread to other bacteria in a few years time after its first isolation. Indeed TEM-1 has spread worldwide and is now found among different species of the family Enterobacteriaceae (Sougakoff, et al., 1998).

Another common plasmid mediated β-lactamase, SHV-1 was later detected in both *Klebsiella spp* and *Escherichia coli* named after the Sulphydryl-variable active site. Further research into the mechanism of action and the hydrolysis of cephalosporins was first reported in 1983; a *Klebsiella ozaenae* isolate from Germany carried a β-lactamase, SHV-2 which enabled it to efficiently hydrolyze cefotaxime and to a lesser extent ceftazidime (Kilebe, et al., 1985). The discovery of these cephalosporinases were named Expanded Spectrum β-lactamases for their ability to hydrolyze 3rd generation cephalosporins. Ever since, the spread of antibiotic resistant genes in bacteria became very rampant owing to the lack of information of how resistance was transferred across genus and mis-use of antibiotics by practitioners and patients alike. The expanded spectrum β-lactamases evolved with a wider range of cephalosporins it could hydrolyze and were designated by the subtypes, SHV-3, TEM-2 based on their molecular characterization and phenotypic behavior. The designation of these newer subtypes was now Extended Spectrum β-lactamases, or ESBL. A group of enzymes preferentially
INTRODUCTION

hydrolyze cefotaxime over ceftazidime and they also hydrolyze cefepime with high efficiency were described (Ishii, et al., 1995; Bonnet, 2004) and were designated as CTX-M, M for Munich the region where these enzyme were first identified. This type of ESBLs were robust in their action against antibiotics, and today the β-lactamases classification database by Lahey clinic reports 172 CTX-M subtypes. Besides, there are over 193 and 223 SHV and TEM subtypes respectively last modified on October 22nd, 2015. (http://www.lahey.org/Studies/)

This global phenomenon of resistance to antibiotics by infectious bacteria affects medical centers in a manner by prolonging the time of recovery, thereby increasing morbidity and mortality, putting susceptible personal at risk of infection. Besides, the decreasing effectiveness of the first line antibiotics is overcome by more expensive second and third line antibiotics posing a greater challenge in terms of the cost accrued for an otherwise non-complicated bacterial infection by hospitals and patients alike. In developing countries the challenge is severe both in terms of the cost of medicines, availability of these drugs, eliciting a nationwide antibiotic policy and regulations, hygiene conditions, reliable water quality and diseases wherein bacterial infections are opportunistic in behavior, e.g. HIV/AIDS, tuberculosis, etc. As opportunistic infections, bacterial antibiotic resistance becomes severe and complicated due to compromised immune response by the patient. In as such, very little information exists across India, both rural and urban. Most of the data on resistance in developing countries come from tertiary care facilities, typically located in large cities. A pan-India detailed study on antibiotic resistance with patient information for public health reasons will be helpful in the implementation of antibiotic policies specifically for India and in conducting a nation-wide antibiotic surveillance program which could curtail the use of antibiotics. It also becomes imperative to know the type of enterobacterium causing or spreading infection. This causative agent is identified by a combination of various biochemical and molecular diagnostic assays, their antibiotic sensitivity pattern, phenotypic characterization of possible ESBL type indicators and finally the molecular confirmatory tests to detect the various types ESBL genes responsible. Further characterization of the antibiotic resistance genes by sequencing to identify their subtypes help understand the biochemical kinetics of antibiotic resistance pattern.
The emergence of ESBL in clinical bacteria resulted in the administration of more potent class of antibiotics – carbapenems. This group of antibiotics is chemically modified class of β-lactams, with the broadest spectrum of activity against bacteria in- as-such was used as the ‘last resort of antibiotics’ when no other classes relieved patients with chronic infections. Over time this led to development of resistance in commonly circulating infectious agents, its dissemination and thus the evolution of carbapenemases with widespread activity against all available antibiotics. The first of its kind was identified in *E. coli* from a Swedish patient who visited New Delhi; hence this class of enzymes was named New Delhi Metallo-β-lactamase (NDM). Besides, there have been reports of carbapenemases across the world – OXA type, and *Klebsiella pneumoniae* carbapenemases (KPC), a type of enzyme specifically produced by *K. pneumoniae* but detected across genus over time. Both OXA and KPC types are carbapenemases with serine at its active site and are not inhibited by metal chelators like ethylenediaminetetraacetic acid (EDTA). Separate classes of β-lactamases which require metal chelators like Zinc (Zn$^{2+}$) ions for their activity were classified with enzymes like NDM, Verona-integron mediated metalo-β-lactamases (VIM) and imipenemases (IMP) were identified. All of these enzymes were classified under carbapenemases with similar phenotypic trait.

There are several methods which have been suggested for the phenotypic detection of ESBLs (Drieux, et al., 2008) and carbapenemases (Yong, et al., 2002) in clinical isolates which includes disk approximation or double disk synergy, modified double disc test (MDDT), a Clinical and Laboratory Standards Institute (CLSI) phenotypic confirmatory method, E-test ESBL and carbapenem-resistant Enterobacteriaceae (CRE) strips, three dimensional test and Vitek® system. However, none of these tests have proved to detect all known types of ESBLs and carbapenemases. Here, the double disk synergy test (DDST) for ESBL detection and modified Hodge test (MHT) for carbapenemase detection are the well explored routinely carried out method of detection in laboratories in India (Kader, et al., 2006). While, newly developed methods like Combined Double Disk test (CDDT) prove helpful in detection of carbapenemases. Laboratories in Europe and the United States of America propose detection of the minimum inhibitory concentration (MIC) using E-test
strips to be a better indication towards antibiotic resistance in bacteria. The problem associated with this is in the selection of which antibiotic to test because of the different levels of activity exhibited by various ESBLs against cephalosporins making it difficult for detection. For example, one enzyme may actively hydrolyze ceftazidime, resulting in MIC for ceftazidime to be 256 µg/ml but have poor activity on cefotaxime, producing MIC of only 4 µg/ml, as pointed out by the CDC in the FAQs about ESBLs (CDC, 2010). Thus, if an ESBL is detected all penicillins and cephalosporins, including aztreonam are reported as resistant even if in vitro test results indicate susceptibility (CLSI, 2011). There are a number of molecular assay kits that detect the presence of ESBL/carbapenemase that can detect new and emerging β-lactam resistant genes as well as resistance targets to other antibiotic classes to give a comprehensive picture of multi-drug resistant bacteria. Detection of resistance genes expressed at low levels is also easily achieved with molecular assays (Gazin, et al., 2012).

**Purpose of Study**

Recent reports suggest the emergence of plasmid mediated antibiotic resistance amongst clinical isolates from North-East India which include ESBL and NDM-1 producers (Sarma, et al., 2011). However, genetic basis of antimicrobial resistance amongst circulating pathogens from this region is yet to be understood. In addition, genetic diversity and evolutionary origin of such strains from this region is not known. A statistically significant number of each of these bacterial pathogens would be subjected to phenotypic characterization including antibiotic susceptibility testing and genotypic characterization including screening for the presence of various antibiotic resistance genes, looking at evolutionary origin by Multi Locus Sequence Typing.

Infection caused by *E. coli*, and *K. pneumoniae* are important public health problems in Assam. Antibiotic resistance has been identified as one of the major public health problems of current time. High resistance to commonly used antibiotics is reported from various parts of the world in *E. coli* and *K. pneumoniae*. Emergence of antibiotic resistance amongst clinically important bacterial pathogens has recently been documented in Assam (Sarma & Ahmed, 2010; Sarma, et al., 2011). However, genetic characterization of such important pathogens from this region is yet to be done.