1. INTRODUCTION

The word *Pseudomonad* has been derived from the Greek word *pseudo* (‘false’) and *monas* (‘a single unit’). The term “monad” was used in the early history of Microbiology to denote single celled organisms. *Pseudomonas aeruginosa* is a gram negative rod, usually 1.5-5µm in length and 0.5 to 1.0 µm in width and belongs to the family *Pseudomonadaceae*. There are around 150 described species in the genus *Pseudomonas* (Parte, 2014). The members of this genus have been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals (Lederberg *et al*., 2000). They remain as saprophytes in warm moist situations in the human environment including sinks, drains, respirators, humidifiers and disinfectant solutions. They have multiple polar flagella that assist in the movement. Most strains are fimbriate. Although most species are noncapsulated, yet some strains produce an alginate capsule. This organism is a strict aerobe, although nitrate can be used as an electron acceptor to permit anaerobic growth.

On nutrient agar colonies of *P. aeruginosa* are surrounded by bluish green coloration. On blood agar, it produces β-hemolysis whereas on MacConkey agar pale yellow colonies i.e. non lactose fermenting colonies are produced. Cetermide agar and Pseudomonas isolation agar are selective media for this organism. Clinical isolates of *P. aeruginosa* are oxidase, catalase positive and can utilize citrate as the sole source of carbon. This organism does not ferment lactose but some strains oxidize glucose with the production of acid only. Biochemical tests such as indole, methyl red, Voges-Proskauer and H₂S tests are negative in case of *P. aeruginosa* (Brenner *et al*., 2005). This organism produces a number of pigments, one of the important being the fluorescent pigment pyoverdin which imparts a yellow/green fluorescence to the bacterial colonies. Another pigment is pyocyanin, which is a bluish phenazine pigment. This pigment is known to kill mammalian and bacterial cells through the generation of reactive oxygen intermediates. A derivative of pyocyanin, called pyorubrin, is a siderophore which is also produced in low iron environments. Yet another pigment which is dark brown in colour, pyomelanin is rarely produced by *P. aeruginosa*. About 10 - 15% strains of this organism do not produce any pigment (Ogunnariwo and Hamilton-Miller, 1975). *P. aeruginosa* has the genome size of about 5.2 to 7 million base pairs (Mbp) with a G + C content of 65%.
genome of this organism is in the form of a single and supercoiled circular chromosome in the cytoplasm (Fick, 1993).

*P. aeruginosa* is an increasingly prevalent opportunistic human pathogen and the most common gram negative bacterium found in nosocomial infections. *P. aeruginosa* is the fourth most commonly isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections according to the Centre for Disease Control and Prevention (CDC) (Todar, 2009). The pathogenesis of *P. aeruginosa* infections is multifactorial as this organism has wide array of virulence determinants such as pili, lipopolysaccharide (LPS), flagella, elastase, alkaline protease, siderophores, siderophore uptake systems and extracellular protein toxins (exoenzyme S and exotoxin A) (Todar, 2009). These factors result in increased tissue damage and protect *P. aeruginosa* against the recognition by the immune system as well as protect them against the action of antibiotics (Cevahir et al., 2008). Infections due to this organism are seldom encountered in healthy adults; but this bacterium has been implicated in hospitalized patients of cancers, cystic fibrosis and burns etc. during last two decades. It is one of the main agents of hospital acquired infections such as pneumonia, urinary tract infections (UTIs) and bacteremia. The Center for Disease Control and Prevention (CDC) has estimated the overall prevalence of *P. aeruginosa* infections in US hospitals at approximately 4 per 1000 discharged patients (0.4%). The fatality rate in immunocompromised patients is near 50 percent (Todar, 2009). In the United States, *P. aeruginosa* is among the most common hospital pathogens and is the second most common pathogen isolated from patients with ventilator associated pneumonia (Hidron et al., 2008). This organism is the second most common bacterium isolated in intensive care unit (ICU) (12.2%) (Streit et al., 2004). The most common *P. aeruginosa* infections are: urinary tract infections (UTIs) (16%-35%), respiratory tract infections (18-34%), bacteremia (3.4%-13%), surgical site infections and wounds (9.5-25%) (Neuhsauser et al., 2003, Gaynes et al., 2005, Yetkin et al., 2006). *P. aeruginosa* can cause meningitis following trauma or surgery, malignant otitis externa in diabetics, endocarditis or osteomyelitis in intravenous drug users, pneumonia in people with chronic obstructive pulmonary disease, and peritonitis (Blondel-Hill et al., 2007). Also, systemic infections caused by *P. aeruginosa* in patients with major burn injuries are
common and are associated with higher mortality than non \textit{P. aeruginosa} infections (McPhee and Papadakis, 2009). As many as 60\% of people with cystic fibrosis have chronic infections due to \textit{P. aeruginosa}, and it is considered the most prevalent infection in patients with cystic fibrosis (Blondel-Hill \textit{et al.}, 2007).

Despite improvements in antibiotic therapy, \textit{P. aeruginosa} is intrinsically resistant to a number of antimicrobial agents (de Freitas and Barth, 2002). The main anti-pseudomonal antimicrobial groups are: penicillin-\(\beta\)-lactamase inhibitor combinations (cefoperazone-sulbactam, piperacillin-tazobactam), cephalosporins (cefoperazone, ceftazidime), monobactam (aztreonam), fluoroquinolones (ciprofloxacin, levofloxacin), fosfomycin, carbapenems (meropenem, imipenem, doripenem) and aminoglycosides (amikacin, gentamicin, tobramycin) (Gill \textit{et al.}, 2011). \textit{P. aeruginosa} develops resistance against almost all antibiotics. The resistance to three or more classes of anti-\textit{Pseudomonas} agents is used as a criterion for regarding an isolate as multi drug resistant (MDR), extensively drug resistant (XDR) isolates describing a resistance profile which compromise most standard antimicrobial regimens and pan drug resistant (PDR) isolates are resistant to all approved antimicrobial agents (Tam \textit{et al.}, 2010). The selection of MDR has been taking place since the 1940s. The evolution and spread of resistance are relatively recent and have occurred mainly during past five decades. The first case of MDR \textit{P. aeruginosa} strain was observed in the hematologic unit in 1992 (Tacconelli, 2002). The strains of MDR \textit{P. aeruginosa} have been isolated all over the world from clinical specimens with increasing frequency (Deptula and Gospodarek, 2010). Increasing frequencies of such strains in nosocomial infections all over the world requires determination of correlation between the resistance to antibiotics and virulence of a pathogen implicated. In general, this would depend on the interactions between the multiple factors associated with bacteria and their environments. The ultimate effect of the association between bacterial virulence and antimicrobial resistance depends mainly on four factors; i. bacterial species involved, some microorganisms acquire antibiotic resistance mechanisms readily and evolve rapidly in response to antibiotic pressure (e.g. \textit{P. aeruginosa}), ii. specific virulence and resistance mechanisms, iii. the environment or ecological niche, iv. the immune system of the host. \textit{Pseudomonads} are more versatile than members of \textit{Enterobacteriaceae} in acquiring drug resistance by various
mechanisms. These mechanisms of resistance are: efflux pumps, biofilm formation, beta lactamases, aminoglycoside modifying enzymes, and mutations in various chromosomal genes. Moreover, exposure to broad spectrum antibiotics and patient to patient spread has added to the rapid increase in the spread of resistant strains.

The need for antimicrobial susceptibility testing is increasing day by day with rising emergence of multidrug resistant microorganisms especially *P. aeruginosa* (Nalini and Sumathi, 2012). Beta-lactam antibiotics are commonly used to treat bacterial infections. The groups of antibiotics in this category include: penicillins, cephalosporins, carbapenems and monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of β-lactamases mediated bacterial resistance, which subsequently led to the development of extended spectrum beta lactamase (ESBL) producing bacteria. ESBLs are enzymes that mediate resistance to extended spectrum e.g. third generation cephalosporins (cefotaxime (CTX), ceftriaxone (CTR), ceftazidime (CAZ) as well as monobactams such as aztreonam) (Clinical Laboratory Standard Institute, 2010). These enzymes do not hydrolyze carbapenems and are inhibited *in vitro* by β-lactamase inhibitors (clavulanic acid, tazobactam and sulbactam).

These enzymes contain serine in their active site and belong to class A or D of the Ambler classification and to group 2be of the Bush-Jacoby classification. These enzymes catalyze the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs are encoded by different genes located either on chromosome or plasmids. ESBLs have been reported worldwide in many different genera of *Enterobacteriaceae* and *P. aeruginosa* (Friedman et al., 2005). However, these are most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli* (Aggrawal et al., 2008). Initially ESBL producing organisms were isolated from nosocomial infections but they are being isolated from the communities (Pitout and Laupland, 2008). Early detection of occurrence and types of multiple β-lactamase enzymes is therefore crucial for the implementation of proper antibiotic therapy and infection control strategy (Upadhyay et al., 2010). Various tests have been developed and followed for detecting β-lactamases. The present study aims at detecting the different β-lactamase producing *P. aeruginosa* isolates recovered from pus, blood, urine, sputum specimens of human
patients suffering from various disease conditions at Indira Gandhi Medical College, Shimla, Himachal Pradesh during one year period by various phenotypic tests. The most resistant phenotypes involve further characterized at molecular level. The present study, therefore, has been proposed with following objectives.

1.1 Objectives of the Research

- Confirmation of clinical isolates of *P. aeruginosa*.
- Antibiotyping of *P. aeruginosa* and selection of multi drug resistant isolates (MDRs) for further characterization.
- Identification of ESBL producing isolates of *P. aeruginosa*.
- Sequencing of the specified *bla* genes.