1.0 INTRODUCTION

It was the beginning of the 20th century, when infections are the main cause of death in the world and from the medical point of view; human history can be seen as a struggle against these infections. This fight is going on since very long time as both human and microbes keep trying to discover new defence to overcome each other.

Antibiotics, which were a prime weapon against these infections, have a history of more than 70 years, during which they have saved millions of lives of individuals. Once Discovery of penicillin by Alexander Fleming in 1929, the first introduction of sulfa drugs by Domagk in 1932, the number of new antimicrobials has in one accelerated pace between 1940 and 1960. "This age of antibiotics led to optimism to the 1970 is that infectious diseases can be controlled and prevented and humanity was confident that modern medicine would prevail. However, infections are still the second most important cause of death in the world and responsible for every year millions of deaths. This is due to the new diseases that remerged from diseases that were previously controlled and development of antimicrobial resistance (Yoneyama & Katsumata, 2006).

However, the golden period of so called magic bullets is over. Microorganisms keep on evolving new method of resistance to current antibiotics and mankind keep on looking new antibiotics. But it is regularly seen that microorganisms are slowly and steadily getting supremacy in their method of evolving resistance and mankind is behind in time in the discovery of new antibiotics (Ghafur, 2010). This worldwide problem of antibiotic resistance is affecting all the patients and medical persons. It is an problem of unknown consequence and, unlike global warming and has no sudden solution. The large scale of antibiotic use known, but conversely entrenched prescription practices are extremely difficult (Sarkar & Gould, 2006). In the initial time, this problem of

antimicrobial drug resistance was solved by the discovery of new classes of drugs as well as by modifying previously existing drugs chemically. Unfortunately there is no guarantee that the development of new antimicrobial drugs can keep pace with the ability of bacterial pathogens to develop resistance (Gould, 2007)**.** The antibiotic source is scarce, with no new classes of antimicrobial agents expected to be in use for treatment purpose in the next 20 years (Lindsay & Holden, 2006).

Now-antibiotic resistance is spreading to almost every class of antibiotics particularly in Gram negative microbes fear has rippled that we may go back enter to pre-antibiotic period again. Besides this great problem, also concern about the types of Gram Negative bacilli that received this resistance or developed.

Resistance among Gram-negative bacilli is not a new phenomenon. The first enzymes in Escherichia coli that can inactivate penicillin were soon after introduction of the drug in the 1940s (Abraham & Chain, 1988). Microorganisms such as Pseudomonas aeruginosa always have a unique ability to overcome new antimicrobial therapies and develop resistance; However, where resistance has generally developed at least one some other therapeutic or treatment options were available, but now P. aeruginosa and Acinetobacter resistant to multiple drugs, which are defined as resistant to three or more of the following classes of antibiotics: β-lactams, including penicillins, cephalosporins and monobactams; carbapenems; fluoroquinolones; and aminoglycosides.

Even more problematic is the fact that these bacteria pool can be drug resistant, that is, they are resistant to all available antibiotic options. In addition, since they are easily treatable organisms such as the species of Escherichia coli and Klebsiella now maintain resistance mechanisms that make them almost pan resistant (Manikal et al., 2000).

Recently, Gram-negative bacilli were broad-resistant documents in hospitals around the world, from the Infectious Diseases Society of "Bad Bugs of America, No Drug

campaign ". In the next 10 years are not expected new antimicrobial classes designed to target some of these multidrug-resistant Gram-negative bacilli" (Talbot et al., 2006). But the rate at which resistance against carbapenems is increasing in Gram-negatives is a very big concern.

Gram-negative bacilli may develop resistance to most antibiotics, including carbapenem by four general methods: production of enzymes that destroy the integrity of the antibiotic; Mutations at the binding site, causing some antibiotics to be avoided difficult to connect; Negative regulation of the outer membrane proteins, thereby preventing the antibiotic entry in the periplasmic space; and efflux pumps, which pump out antibiotic efficiently of the cell (Livermore & Woodford, 2006). Often, for multiple drugs and pan-drug resistance several mechanisms of action are included in the same organism.

The production of β-lactamases is more common and clinically significant mechanisms of resistance showed all Gram-negative bacilli. β-lactamases are enzymes with the β-lactam chemical structure that hydrolyzes and inactivates the drug. They are either classified by Ambler's classification or the Bush-Jacoby-Medeiros classification (Bush's Ambler 1980 et al., 1995). The classification of Ambler based on similarity in amino acid graded β- lactamases into four classes. Classes A, C and D are serine β-lactamases, and the enzymes of class B are zinc-β-lactamases while Bush Jacoby-Medeiros based on substrate and inhibitors profiles divided β-lactamases in four groups and several subgroups against.

Production of acquired carbapenemase makes the choice of antibiotic regimen for the infections caused by Gram-negative bacteria very limited. The horizontal transfer of carbapenemase - genes by genetic elements such as plasmids, transposes, etc. The additional resistance elements contribute to the various groups of antibiotics leads to multidrug resistance and pandrug resistance (Kumaraswamy et al., 2010). In vitro

susceptibility to colistin, tigecycline and aminoglycosides is generally preserved, but the effect these antibiotics in vivo are still uncertain and resistance to these drugs also increases and mortality rates remain high despite treatment in accordance with the results of resistance tests (Falagas et al., 2014).

However, there is no equal substitute for carbapenem-antibiotics in treatment of severe infections caused by multi-resistant Gram-negative bacteria. Carbapenem resistance mediated by β-lactamases, or carbapenemases, has spread worldwide and wirth no new effective antimicrobials available or in development, focus of the world has shifted to timely detection and proper infection control measures.

The reliable, accurate and timely detection of carbapenem-resistant Gram-negative bacteria is also very necessary in outbreak detection and for the institution of appropriate treatment options (Stuart & Leverstein, 2010; Kaase et al., 2012). Many tests especially at the phenotypic level can be used as the indicators of resistance mechanisms, thereafter molecular analysis may be performed for confirmation of these resistance mechanisms. It is still debatable as to which of the test either phenotypic of genotypic is most effective (Nordman et al., 2009; Thomson, 2010). However, production of different carbapenemases is linked to the transfer of plasmid-mediated genes encoding the carbapenemases within and between species (Dipersio & Dowzicky, 2007). These enzymes are normally produced in assoication with several other β-lactamases, hence making it difficult to identify carbapenemases using simple methods at the phenotypic level (Bush et al., 2013). As there is not a single best method for the carbapenemase detection but at least we can have a standard logical approach towards their detection.

So the main aim of this study was to screen carbapenem resistant Gram-negative bacillary by means of susceptibility testing and minimum inhibitory concentration

determination. Additionally, screening for carbapenemases was done with different phenotypic and genotypic methods.

The objectives of the study:

1. To identify carbapenem resistant Gram-negative bacilli isolated at Himalayan Hospital
2. To characterize their resistance to different antimicrobials
3. To characterize mechanisms of carbapenem resistance by phenotypic and genotypic methods