Growth, Morphology, Cross Stress Resistance and Antibiotic Susceptibility of *K. pneumoniae* Under Simulated Microgravity

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**Abstract**

Spaceflights result in the reduction of immune status of human beings and increase in the virulence of microorganisms, especially gram negative bacteria. The growth of *Klebsiella pneumoniae* is enhanced by catecholamines and during spaceflight, elevation in the levels of cortisols occurs. So it is necessary to know the changes in physiology, virulence, antibiotic resistance and gene expression of *K. pneumoniae* under microgravity conditions. The present study was undertaken to study effect of simulated microgravity on growth, morphology, antibiotic resistance and cross stress resistance of *K. pneumoniae* to various stresses. The susceptibility of simulated microgravity grown *K. pneumoniae* to ampicillin, penicillin, streptomycin, kanamycin, hygromycin and rifampicin were evaluated. The growth of bacteria was found to be fast compared with normal gravity grown bacteria and no significant changes in the antibiotic resistance were found. The bacteria cultured under microgravity conferred cross stress resistance to acid, temperature and osmotic stress higher than the normal gravity cultured bacteria but the vice versa was found in case of oxidative stress.

**Key Words**: Simulated microgravity, *K. pneumoniae*, growth analysis, antibiotic sensitivity, TEM, SEM

1. Introduction

Long term spaceflights affects not only the various functions of human systems like demineralization of bones, skeletal atrophy, anaemia, renal lithiasis and suppression of immune systems but also leads to occurrence of diseases by the microorganisms (Guegionou et al., 2009). It is important for microorganisms to sense the various environmental changes and produce appropriate cellular responses in order to survive. Microorganisms survive even under extreme conditions and they are able to adapt to various physical stresses like pH, temperature, oxygen levels, osmotic pressure and nutrient availability by sensing the changes through sensors and receptors (Nickerson et al., 2004). Loss of anaerobic, gain in aerobic microorganisms was observed in Apollo and Skylab crews and opportunistic pathogens like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacteriaceae* were isolated from cosmonauts (Taylor, 1974). *Citrobacter*, *Enterobacter*, and *Klebsiella* sp., were found to be the frequent isolates isolated from crew members after post-flight. It was also found the flight travel leads to bifido and lactoflora deficiency leading to disbacteriosis (Pierson, 1993; Castro et al., 2004; Ilyin, 2005). *Staphylococcus, Bacillus, Micrococcus, Pseudomonas, Enterobacter, Klebsiella, Enterococcus, Haemophilus*
and many other genus were found in Mir environment (Novikova, 2004).

Microgravity is a unique environment and the impact of microgravity on physiology, virulence and gene expression on microorganisms can be effectively studied using ground based models with the high aspect rotating vessel. Previous studies by Nickerson et al., (2000) revealed that the microgravity induces changes in virulence, stress resistance, protein expression of *Salmonella enterica* serovar typhimurium compared with the *Salmonella* grown under normal gravity. The growth was found to be higher and changes in gene expression were observed with *Escherichia coli* under the conditions of modeled reduced gravity (Vukanti, 2008). *Staphylococcus aureus* grown under simulated microgravity decreased the production of proteins and virulence determinants (Rosado et al., 2010). An increase in alginate production and increased heat and oxidative stress resistance was noticed with the *Pseudomonas aeruginosa* PAO1 to low shear modeled microgravity (Crabbe et al., 2010).

*Klebsiella pneumoniae* is a gram-negative, lactose fermenting, facultative anaerobe and it is found in mouth, skin and intestines of the human beings. *K. pneumoniae* is responsible for a wide range of infections like pneumonia, urinary tract infections, cholecystitis, diarrhoea, upper respiratory tract infection and osteomyelitis. *Klebsiella* infections are found commonly in the immuno compromised people. As the immune systems of humans are compromised in space and *K. pneumoniae* was found to thrive in the space, the present study was undertaken to analyze physiological changes and stress responses of bacteria *K. pneumoniae* cultured under simulated microgravity. The growth of bacteria under normal gravity and simulated microgravity were measured and Transmission Electron Microscopy and Scanning Electron Microscopy were performed to analyze changes in the morphology of bacteria. The antibiotic resistance was found to be increased in both gram-positive and gram negative bacteria based on the experiments conducted on Soviet and American Vessels (Tixador et al., 1985; Lapchine et al., 1985). The antibiotic sensitivity assay was performed using five different antibiotics with cultures cultured under normal and simulated microgravity. The ability of bacteria *K. pneumoniae* to resist various other stresses like acid, thermal, osmotic and oxidative stress were examined using the cultures from simulated microgravity and compared with the control normal gravity grown cells.

2. Materials and methods

2.1. Bacterial strain and growth conditions

All experiments were performed using *Klebsiella pneumoniae* subsp. *pneumoniae* (KACC 11402) obtained from Korean Agricultural Culture Collection. Pure cultures were maintained on petriplates for the experiments. Bacterial cultures were cultured overnight in the nutrient broth at 37°C in a shaking incubator shaking at 150 rpm. The cultures were diluted to 1:100 and then inoculated into High Aspect Ratio Vessel (HARV) reactor and same dilution was used for culturing cells under normal gravity. HARV reactor creates reduced sedimentation, low shear and low turbulence conditions similar to the environment conditions exhibited during space flight. The HARV reactor was completely filled with nutrient broth and care was taken to ensure the complete removal of air bubbles. The incubation temperature for both normal and microgravity was maintained at 37°C and a rotation of 25 rpm. All the experiments were performed in triplicates.

2.2. Growth kinetics

The cell density was measured at a regular interval of 4 hours upto 24 hours by using UV-Vis Spectrophotometer at optical density 600 nm. 1 ml of