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MICROBIOLOGICAL EXAMINATIONS OF ULTRA-DRUG RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM HIGHLY INFECTION-PRONE AREAS.

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Abstract: Soil samples from highly infection-prone areas like patient wards in city hospitals, river side's and marine coastal areas were taken and pre-treated in presence of various antibiotics at high concentrations, ensuring the high resistivity and survival of only multi drug resistant microbes into the sample. Sample was serially diluted and inoculated over sterile Petri dish containing sterile solid culture media. The most prevalent microbes were sub cultured up to pure culture and were identified as *Acinetobacter baumannii* as described by Mishra *et. al*. (2011) in their earlier reports. Various microbiological examinations such as growth rate, media optimization and effect of different elicitors were studied for this microorganism.

Keywords: *Acinetobacter baumannii*, MDR, drug-sensitivity, *E. coli.*

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INTRODUCTION

Now days various bacteria came in existence that metabolize the antibiotics or show resistance, these bacteria called Multi-drug resistance bacteria becoming a big threat to clinical therapeutics **(Chandel, *et. al*., 2010)**. The effect of these multi drug resistance microbes are very lethal sometimes leads to death. *Acinetobacter baumannii* is one of the most efficient multidrug resistance bacteria that become biggest threat to clinic and hospitals ICUs **(Karageorgopoulos & Falagas, 2008; Narisawa, *et. al*., 2008)**. *Acinetobacter baumannii* is a species of non-fermentative gram-negative bacteria commonly found in water and soil. The genus *Acinetobacter baumannii* is classified under the family Moraxllaceae and comprises strictly aerobic, Gram- negative, non-motile, non-lactose-fermenting, oxidase negative, catalase-positive cocco-bacilli **(Poole, 2003)**. It has now become a major cause of hospital-acquired infection worldwide due to its remarkable propensity to rapidly acquire resistant determinants to a wide range of antibacterial agents. *Acinetobacter baumannii* exhibits a remarkable ability to rapidly develop antibiotic resistant that led to multi drug resistance (MDR) within a few decades. *Acinetobacter baumannii* infection is typically encountered in hospitalised patients, particularly critically ill ones **(Garnacho-Montero *et. al*., 2003; Mishra, *et. al*., 2011)**. Specific characteristics of affected patients include advanced age, presence of serious underlying disease, immune suppression, major trauma or burn injuries, performance of invasive procedure, as well as presence of indwelling catheters, support with mechanical ventilation, extended hospital stay, and previous administration of antibiotics. The main clinical syndromes reported include pneumonia and bacteraemia, along with surgical site infection, skin and soft tissue infection, and urinary tract infection **(Ahmad & Beg, 2001; Dent, *et. al*., 2010; Eijkelkamp, 2011)**. People who become infected with drug-resistant microorganism usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and it less effective, more toxic, and more expensive.

Materials and Methods:

Soil samples were collected from contaminated sites as the best samples of higher probability of finding a large number of drug resistance pathogens and its abundance such as from Dr. Ram Manohar Lohia Hospital, Civil Hospital, Gomti River water, Lucknow and Marine sea water, Mumbai.

The samples were incubated in two flasks-one containing 10% antibiotic supplemented by 50 ml nutrient media and other containing only 10% antibiotic (50 ml). Both flasks were incubated for one week at 55 ^{0}C to remove drug and heat sensitive microbes, also to ensure maximum number of drug resistance microbes. After above treatment of samples, it transferred to sterile nutrient

agar media after serial dilution to several test tubes, followed by 3-4 days incubation at 37 ^{0}C. The NA plates containing different colonies obtained after serial dilution was subculture to get pure colony.

The most prevalent colonies were selected and identified for further study after confirmation of drug resistivity by agar well diffusion method **(Aneja, 2003; Mishra, *et. al*. 2011)**.

Growth kinetic study was performed by recording indirect growth of the microbe in respect of turbidity into sterile liquid culture media after recording optical density of inoculum at repeated intervals. Then pH optimization tests was at different pH, different media components, metal ions and different incubation time at 37 ^{0}C.

Multi drug resistant microbes were isolated by serial dilution of soil pre-treated with the various antibiotics. Obtained microbes that grow on media were found multi drug resistant. After the primary and secondary screening, obtained four types of bacteria labelled as MRAR 1201, MRAR 1202, MRAR 1203, MRAR 1204, were maintained throughout the study. On the basis of inhibition zone one isolate MRAR 1203 was selected, shown in fig. 1.

Optical density of broth culture of isolate was recorded at the difference of 1 hr incubation time and data has been shown in figure 1. We plotted a graph between time and O.D. obtained sigmoid growth curve, showing the four typical phases of growth *i e.* A) Lag phase, B) Exponential phase, C) Stationary phase, D) Death phase.

Results:

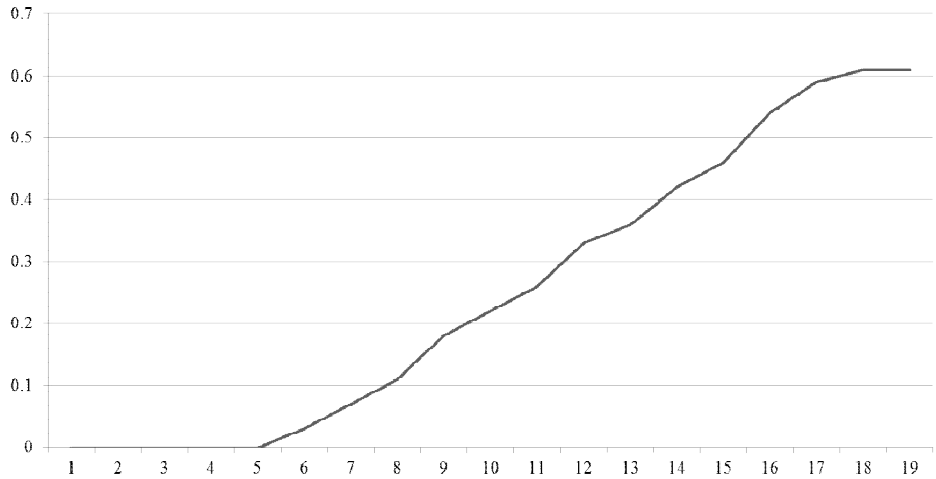

Fig. 1: Graph showing growth pattern of *A. baumannii* at different time. **X**-axis shows time (in hrs.) while **Y**-axis shows optical density (at 600 nm) of the strain.

Fig. No. (I)

We evaluated nutritional requirements for MRAR 1203 & observed that maximum growth in yeast extract (Fig. 2).

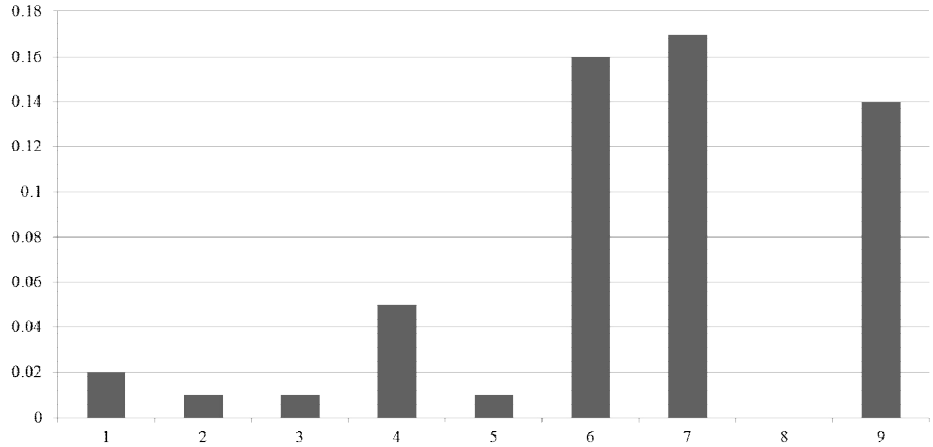

Fig. 2: Graph showing growth pattern of *A. baumannii* in different media components (1% w/v in DW). **X**-axis showing madia components as, 1. Lactose, 2.Glucose, 3.Starch, 4.Tryptone, 5. Glycerol, 6. Beef extract, 7. Yeast extract, 8. Galactose, 9. Peptone and **Y**-axis showing OD (at 600 nm).

Fig. No. (II)

We evaluated media optimization at different concentration, observed that maximum growth occurred at 2% yeast extract, at 5% beef extract, at 5% peptone & at 10% Tryptone (Fig. 3).

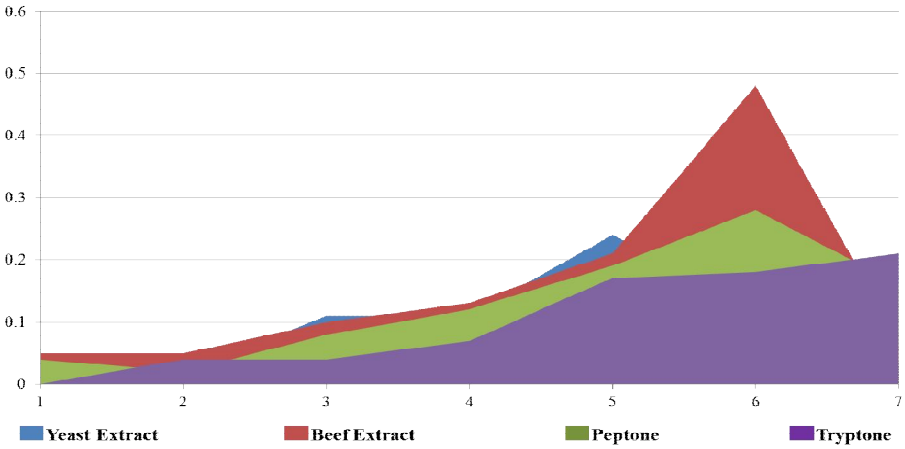

Fig. 3: Graph showing the effect of different concentrations of media components onto growth of *A. baumannii*. 1. 0.1%, 2. 0.2%, 3. 0.5%, 4. 1.0%, 5. 2.0%, 6. 5.0% and 7. 10.0%

Fig. No. (III)

We recorded the effect of pH on growth of microbes & observed that maximum growth occurred at pH 7, shown in figure 4.

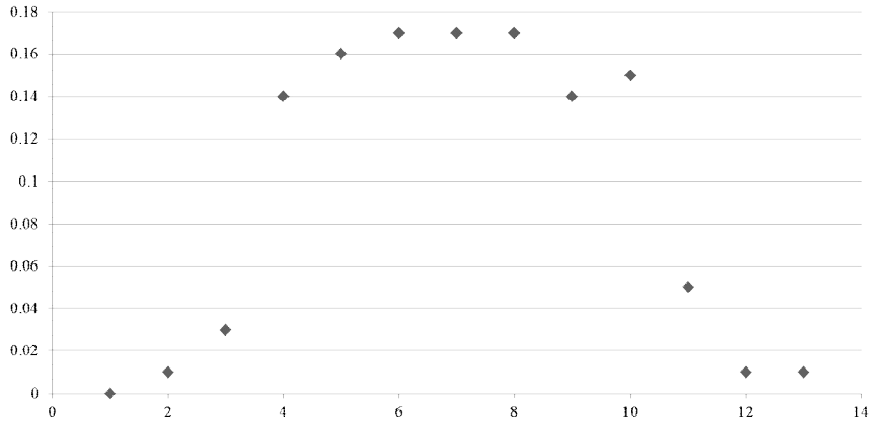

Fig. 4: Graph showing growth pattern of A. baumannii at different pH. Y-axis showing OD (at 600 nm) while X-axis showing pH; 1.) 4.0, 2) 4.5, 3) 5.0, 4) 5.5, 5) 6.0, 6) 6.5, 7) 7.0, 8) 7.5, 9) 8.0, 10) 8.5, 11) 9.0, 12) 9.5 & 13) 10.0

Fig. No. (IV)

In presence of metal ions, maximum growth occurred in Magnesium & zinc (Fig. 5).

The effect of different metal ions (Mg^{+2}, Zn^{+2}, Ca^{+2} , Fe^{+3} & Cu^{+2}) at different concentration, we observed maximum optical density at 0.01% for Cu^{+2} , at 0.1% for Mg^{+2}, at 0.5% for Zn^{+2}, at 0.1% for Ca^{+2} & at 0.01% for Fe^{+3}.

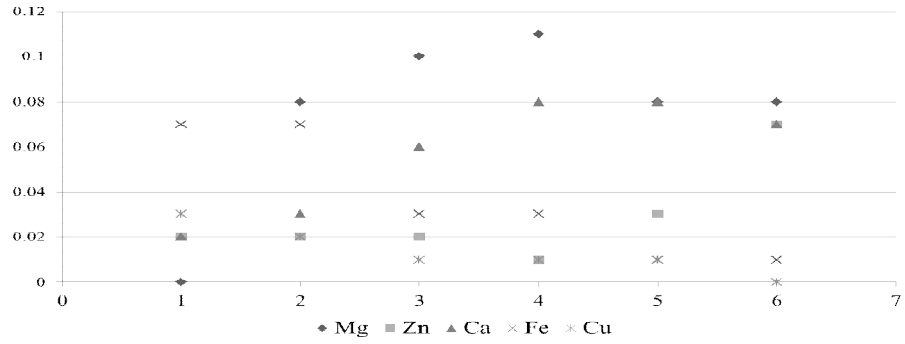

Fig. 5: Graph showing effect of various metal ions over growth of *A. baumannii*. X-axis showing OD (at 600 nm) of the culture and Y-axis is showing different concentrations of metal ions; 1) 0.01%, 2) 0.02%, 3) 0.05%, 4) 0.10%, 5) 0.20% & 6) 0.50%

Fig. No. (V)

Discussions:

The genus known as *Acinetobacter* has gone significant taxonomical modification over the last 30 years. Its most important representative, *Acinetobacter baumannii,* has emerged as one of the most troublesome pathogens for health care institutions globally. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to up regulate or require resistance determinants, making it one of the organisms threatening the current antibiotic era **(Peleg *et. al.*, 2008)**. *Acinetobacter baumannii* is non-fermentative gram-negative, aerobic, non-motile, non-lactose-fermenting, oxidase negative, catalase-positive cocco-bacilli bacteria commonly found in water and soil **(Yoon, *et. al*., 2004; Waters, *et. al*., 2011)**. *A. baumannii* have a unique quality to acquire new environment and become more resistant then before. It shows variation in its properties like pigmentation, resistance, texture *etc* **(Uraban, *et. al*., 2003; Tenover, 2006; Tak-chiu, 2011)**.

During our experimental findings, *Acinetobacter baumannii* was found to exhibit a strong drug resistance for almost all of the antibiotics selected in the study even at their higher concentrations, ensuring highest drug tolerance against the drugs, resembling the previous data **(Dent *el. al*., 2010)**. Ultra pathogen was found to have a strong growth potential after three to four hour hours to the introduction into the media. The complex natural media components such as yeast extract or beef extract were found more growth supportive in comparison to simple defined media components like starch and glucose as it supports a limited growth of the organism. Our several experimental data regarding the growth conditions of the pathogen suggests that instead of being a highly drug resistant pathogen, still its growth is influenced by a moderate change in normal growth conditions like change in pH, media compositions and temperature. A detailed study on culture conditions is still needed to understand how the organism is influenced under stress conditions and this data will help us to prevent the spread of inoculum of this highly dangerous pathogen. There is an urgent need to search for alternative to synthetic antibiotics. By controlled culturing of this bacterium under strict observations can lead to an innovative antimicrobial component that could be used as health care drug in near future **(Mishra, *et. al*., 2011)**.

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