

Study of Culture Conditions and Antimicrobial Drug Production Properties of *Acinetobacter baumannii*.

*R P Mishra¹, Deepak², Surya Pratap Singh² & Raheel Anwar²

¹R & D Division, MRD LifeSciences, Lucknow, Uttar Pradesh, India.

²Stani Memorial P. G. College, Jaipur, Rajasthan, India.

Abstract: Infections caused by drug resistant microorganisms result in significant increase in mortality, morbidity and cost related to prolonged treatments. To check the virulence and sensitivity towards drugs, a brief knowledge of culture conditions and nutritional requirements are needed. This study includes the culture optimization and nutritional evaluation of the *Acinetobacter baumannii* under different pH, media components and also the effect of different drugs. Intra & extracellular extracts obtained from the culture proved very good response against other virulent pathogens, thus a controlled culturing under strict observations can lead to an innovative antimicrobial components to be used as health care drug in future.

Keywords: *Acinetobacter baumannii*, antimicrobial activity, MIC, *E. coli*, *S. aureus*, *C. albicans* MRSRA 1102 MH.

Introduction:

We may soon be facing the end of the “antibiotic era”. The initial and seemingly unstoppable success of antibiotics, the fruit of human ingenuity, has been countered by an escalation of resistance mechanisms in bacteria. This crisis has been described as an “unwinnable war”. The statistics compiled as a result of surveillance efforts illustrate the emergency of many genera of bacteria that are resistant to antibiotics [5, 6]. The genus *Acinetobacter* epitomizes this trend and deserves close attention. *Acinetobacter* spp. display mechanisms of resistance to all existing antibiotic classes as well as a prodigious capacity to acquire new determinants of resistance [2]. *A. baumannii* is a non-fermentative, Gram negative, non motile, oxidase negative bacillus, whose natural reservoir, still remains to be determined. Nevertheless, it is found in many health care environments and is a very effective human colonizer in the hospital. The combination of its environmental resilience and its wide range of resistance determinants renders it a successful nosocomial pathogen [15]. There are reports of MDR *A. baumannii* from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan, and Korea and from areas as remote as Tahiti in South Pacific [1, 7, 10-14, 16, 18-19]. These MDR strains often spread to cause outbreaks throughout entire cities, countries and continents [1, 3, 9, 17]. It is often difficult to distinguish between infection and colonization with *A. baumannii* [8].

Materials and Methods:

Preserved pure culture of MRSRA 1102 MH was obtained from MRD LifeSciences, Lucknow and maintained by repeated sub culturing of the culture.

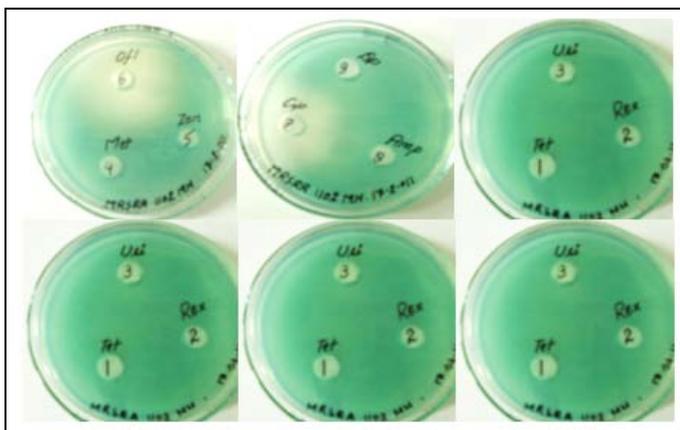
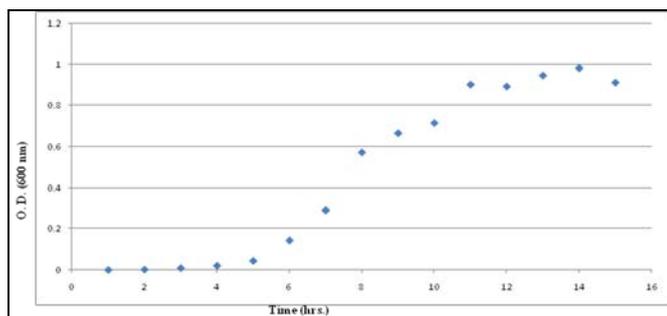
For study of growth kinetics of the culture, an indirect measurement of growth was performed by recording optical density (600 nm) of the culture at every hour after

inoculating in sterile nutrient broth. Culture conditions of the isolate MRSRA 1102 MH was optimized under different pH, metal ions and media components. To see the effect of different elicitors, isolate was inoculated in separate (20 ml each) test tubes having sterile nutrient broth supplemented with varying concentrations of elicitors and different pH. Growth was recorded in terms of turbidity in the culture media after incubation.

For screening of bioactive antimicrobial compounds, intracellular (methanol) and extracellular (chloroform) extracts were selected against *E. coli*, *P. aeruginosa* and *S. aureus*. Extracts were obtained by solvent extraction, concentration of the compounds followed by dissolving in DMSO. Obtained compounds were loaded (30 µl each) into wells of plates containing test microorganisms along with positive and negative control. Further, extraction of antimicrobial compounds was performed at different pH and the compound recovered was screened against the pathogens. For screening of the compound at different parameters, before loading into wells, extracts were treated for 30 minutes at different temperatures (*i.e.* 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, and 121 °C). Minimum inhibitory concentration of the compound was determined by agar plate gradient method against all the pathogens.

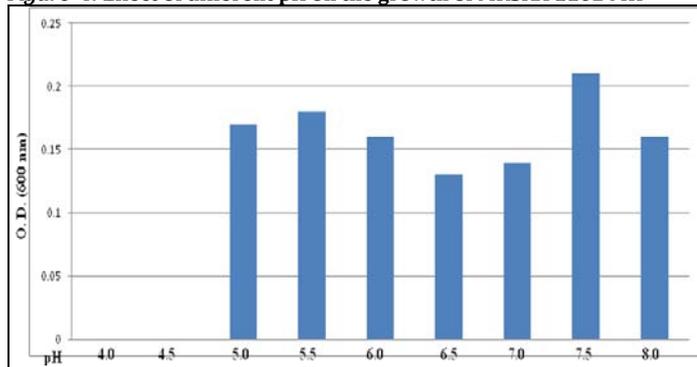
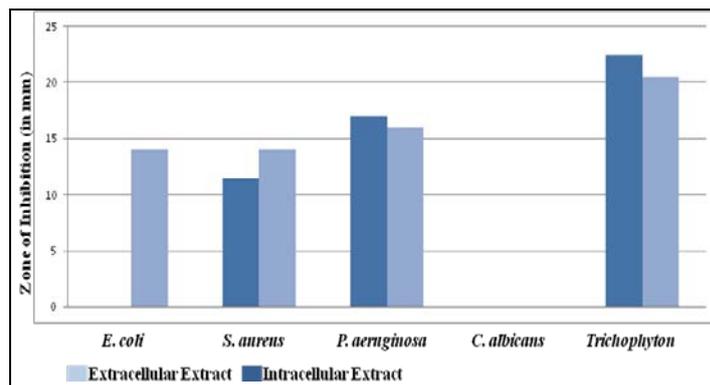
Results:

Multi drug resistant isolate MRSRA 1102 MH colonies were smooth, elevated with entire margins. Colonies appear in Greenish to bluish in color on nutrient agar medium. After staining and observing under optical microscope, bacterial cells were found coccobacilli, which is a characteristic of *Acinetobacter* species. Growth Kinetics of the culture was observed and after 6 hrs. of inoculation, a sharp increase in growth was recorded and it was stabilized after 14 hrs.

Figure 1: Plate containing pure colonies of MRSRA 1102 MH.**Figure 2:** Screening of MRSRA 1102 MH against various drugs.**Figure 3:** Growth kinetics of culture MRSRA 1102 MH.

When pure colonies were inoculated in sterile media having different pH, maximum growth was recorded when pH was slightly alkaline.

During optimization of the culture conditions of the isolate, various media components, elicitors were selected and tested. Among all following parameters was found optimum for the better growth and higher pigmentation of the culture.

Figure 4: Effect of different pH on the growth of MRSRA 1102 MH**Figure 5:** Antimicrobial activity of intra & extra cellular extracts of MRSRA 1102 MH against various pathogens.

Discussions:

Acinetobacter baumannii is an opportunistic human pathogen that presents a potential risk of severe infections for patients who are compromised or suffer from polymicrobial infections. Thus, the simple presence of this bacterium is not sufficient for establishment of an infection, which is thus related to the clinical status of colonized host. Furthermore, the nature of the factors affecting the virulence of this bacterium is not fully understood [4]. A greenish or bluish pigmentation was observed when the isolate was grown on nutrient agar medium (Fig. 1 & 2). At different pH conditions the optimum growth was pH 7.5 and no growth was obtained at pH 4.0 & 4.5 (Fig. 4). Bioactive compound (intracellular and extracellular extract) from bacteria was screened against different test organisms. Data revealed showed that intracellular extract was much better against all the pathogens than that of extracellular extracts (Fig. 5). The MIC obtained against *E. coli*, *S. aureus*, and *P. aeruginosa* are 105.7µg/ml, 100µg/ml and 94.2µg/ml respectively.

Conclusion:

Morphological, physiological and biochemical characteristics of our culture MRSRA 1102 MH resembles with a super resistant pathogen *Acinetobacter baumannii* which is a opportunistic enemy for those people suffering from prolonged treatments. Our data reveals how to cultivate and resist the pathogen under biotic and abiotic conditions. Also, this pathogen proved a strong antimicrobial activity against various bacterial and fungal pathogens even in its very low concentrations. Another very effective drug could be obtained under strictly monitored conditions, from this isolate that will be highly effective against most of the pathogens.

References:

- 1.Barbolla RE, Centron D, Di Martino A, Malmone S, Salgueira C, Famiglietti A, Vay C & Catalano M. Identification of an epidemic carbapenem-resistant *Acinetobacter baumannii* strains at hospitals in Buenos Aires City. *Diagn. Microbiol. Infect. Dis.* 2003. 45: 261-64.
- 2.Bergogne-Berezin E & Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clin. Microbiol. Rev.* 1996. 9: 148-65.
- 3.Da Silva GJ, Quinteira S, Bertolo E, Sousa JC, Gallego L, Duarte A, & Peixe L. Long-term dissemination of an OXA-40 carbapenemase- producing *Acinetobacter baumannii*

- clone in the Iberian Peninsula. J. Antimicrob Chemother. 2004. 54:255–58.
- 4.Echenique JR, Arienti H, Tolmasky ME, Read RR, Staneloni RJ, Crosa JH & Actis LA. Characterization of high affinity iron transport system in *Acinetobacter baumannii*. J. Bact. 1992. 174(23): 7670-79.
- 5.Gaynes R & Edwards JR. Overview of nosocomial infections caused by Gram negative bacilli. Clin. Infect. Dis. 2005. 36: 1111-1118.
- 6.Goossens H. European status of resistance in nosocomial infection. Chemotherapy. 2005. 51: 177-81.
- 7.Houang ET, Chu YW, Leung CM, Chu KY, Berlau J, Ng KC & Cheng AF. Epidemiology and infection control implications of *Acinetobacter* spp. in Hong Kong. J. Clin. Microbiol. 2001. 39: 228-34.
- 8.Joly-Guillou ML. Clinical impact and pathogenicity of *Acinetobacter*. Clin. Microbiol. Infect. 2005. 11:868–73.
- 9.Landman D, Quale JM, Mayorga D, Adedeji A, Vangala K, Ravishankar J, Flores C, & Brooks S. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, N.Y.: the preantibiotic era has returned. Arch. Intern. Med. 2002. 162:1515–20.
- 10.Lee K, Ha GY, Shin BM, Kim JJ, Kang JO, Jang SJ, Yong D & Chong Y. Metallo-beta lactamase –producing Gram negative in Korean Nationwide Surveillance of Antimicrobial resistance group hospitals in 2003; continued prevalence of VIM-producing *Pseudomonas* spp. and increase of IMP-producing *Acinetobacter* spp. Diagn. Microbiol. Infect. Dis. 2004. 50: 51-58.
- 11.Levin AS, Mendes CM, Sinto SI, Sader HS, Scarpitta CR, Rodrigues E, Sauer N & Boulos M. An outbreak of multiresistant *Acinetobacter baumannii* in a University hospital in Sao Paulo, Brazil. Infect. Control Hosp. Epidemiol. 1996. 17: 366-68.
- 12.Liu SY, Lin JY, Chu C, Su LH, Lin TY & Chiu CH. Integron-associated imipenem resistance in *Acinetobacter baumannii* isolated from a regional hospital in Taiwan. Int. J. Antimicrob. Agents 2006. 27:81–84.
- 13.Naas T, Levy M, Hirschauer C, Marchandin H, & Nordmann P. Outbreak of arbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. J. Clin. Microbiol. 2005. 43:4826–29.
- 14.Nishio H, Komatsu M, Shibata N, Shimakawa K, Sueyoshi N, Ura T, Satoh K, Toyokawa M, Nakamura T, Wada Y, Orita T, Kofuku T, Yamasaki K, Sakamoto M, Kinoshita S, Aihara M & Arakawa Y. Metallo-β-lactamase-producing gram-negative bacilli: laboratory-based surveillance in cooperation with 13 clinical laboratories in the Kinki region of Japan. J. Clin. Microbiol. 2004. 42:5256–63.
- 15.Nordmann P. *Acinetobacter baumannii*, the nosocomial pathogen par excellence. Pathol. Boil. (Paris) 2004. 52: 301-03.
- 16.Quale J, Bratu S, Landman D, & Heddurshetti R. Molecular epidemiology and mechanisms of carbapenem resistance in *Acinetobacter baumannii* endemic in New York City. Clin. Infect. Dis. 2003. 37:214–20.
- 17.Van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Paauw A, van den Broek P Verhoef J, & Brisse S. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res. Microbiol. 2004. 155:105–12.
- 18.Van Looveren M & Goossens H. Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin. Microbiol. Infect. 2004. 10:684–704.
- 19.Yu YS, Yang Q, Xu XW, Kong HS, Xu GY & Zhong BY. Typing and characterization of carbapenem-resistant *Acinetobacter calcoaceticus- baumannii* complex in a Chinese hospital. J. Med. Microbiol. 2004. 53:653–56.

Source of Funding: - R&D Division, MRD LifeSciences, Lucknow, INDIA.

Conflict of Interest: - Not declared

***Corresponding Author:-**

R P Mishra,
R & D Division, MRD LifeSciences, Lucknow,
Uttar Pradesh, India.



Quick response code (QR-Code) for mobile user to Assess JPBS website electronically.