

# Carbapenem resistant *Acinetobacter spp* restricted the Therapeutic alternatives

Naheed Afshan, Benish Nawaz and Maliha Hamid

Department of Microbiology, Jinnah University for Women, Karachi, Pakistan

**Abstract:** Carbapenem resistant *Acinetobacter* has appeared an organism of uncertain resistivity towards antimicrobial agents. Among non-fermenting bacterium *Acinetobacter* is the second-most-commonly-isolated organisms in human. The fast intensify of their resistance to antibiotics, especially global emergence and extend of *Acinetobacter* strains resistant to carbapenem more restricted the therapeutic alternatives. The importation of *A. baumannii* and subsequent presence in hospitals has been well documented. In this study we evaluate the resistivity of *Acinetobacter* against carbapenem antibiotics at Jinnah University for Women, Karachi. Total 439 isolates of *Acinetobacter* were collected from different clinical samples of hospitalized patients, identified by standard microbiological methods. AntibioGrams were done on Mueller-Hinton agar plates with disk diffusion method (Kirby Bauer method). Disc tested: Meropenem (10µg/disk). Among 439 samples, 300 (68.3%) samples were resistant to Meropenem and the remaining that is 139 (31.7%) showed sensitivity to the drugs. In developing countries including Pakistan the contentment of multi drug resistance and their dissemination in *Acinetobacter* species is not a simple task. While multiple drug resistance is increasing in this pathogen and Carbapenem conflict is quickly spreading which may become a major threat in future.

**Keywords:** AntibioGrams, Carbapenem, Resistant, Outbreaks

## INTRODUCTION

Species of genus *Acinetobacter* are gram-negative belongs to the class Gammaproteobacteria. Species are non-motile, coccobacilli in shape appear in pairs, oxidase-negative (Peleg *et al.*, 2008), glucose non-fermentative aerobic bacteria (Looveren *et al.* 2004) *Acinetobacter* consist of 27 authentically named and 11 unknown (genomic) species. *A. baumannii* is characterized by a single circular chromosome that contains 3,976,747 base pairs in which 3,454 are used for protein coding (Fournier *et al.*, 2006) One strain of *A. baumannii* called AYE contains an 86kb resistance island, called AbaR1, that is made up of 45 resistance genes and is currently the largest island known to date. *A. baumannii* is able to survive on various surfaces in the hospital that are abiotic, wet, or dry (Houang *et al.* 2005) One strain of *A. baumannii*, 19606 is capable of forming biofilm on glass and plastic surfaces via pili formation. (Tomaras *et al.*, 2003). Widely dispersed in environment they can be alive on dry and moist surfaces, as well as in hospital surroundings (Maragakis and Perl, 2008). *Acinetobacter* mostly affect patients which needs mechanical ventilation in ICU (Intensive Care Unit) (Bassetti *et al.*, 2008). Also some strains have been found from foodstuffs, water, and associate with low occurrence of allergies. Several species of *Acinetobacter* cause serious infections in immunocompromised patients (Peleg *et al.*, 2008). They also show comparatively wide scale of antibiotic resistance (Towner, 2009). Infections together with skin and wound added complication as well as bacteremia and

meningitis (Choi *et al.*, 2008). The bacteria have the ability to colonize in medical utensils e.g. catheters (Doughari *et al.*, 2011). Plasmid-borne markers which made the *Acinetobacter* resistant to various antibiotics are capable of transferring to new pathogenic bacteria through horizontal gene transfer (Juni, 1978). 'MDR *Acinetobacter spp.* Defined as the isolate resistant to at least three classes of antimicrobial agents all penicillin and cephalosporin (including inhibitor combinations), fluoroquinolones and amino glycosides. XDR *Acinetobacter spp.* That is resistant to the three classes of antimicrobials described above (MDR) and shall also be resistant to carbapenem (Kurcik-Trajkowska, 2009). In the early 1970s *Acinetobacter spp.* were susceptible to many drugs like gentamycin, minocycline, nalidixic acid, or carbapenem either used singly or in combination therapy (Bergogne-Berezin and Towner, 1996). But, since 1975, growing resistance started to appear in almost all groups of drugs including the first and second-generation cephalosporin. Primarily, they retained at least partial susceptibility against the third and fourth generation cephalosporin, fluoroquinolones, semi synthetic amino glycosides, and carbapenem, with nearly 100% isolates holding susceptibility to imipenem (Vila *et al.*, 2007). Global rise and spread of *Acinetobacter* resistant to imipenem further restricted the therapeutic alternatives. By the late 1990s, the only useful agents that fight many severe infections caused by *Acinetobacter sp.* were carbapenem (Camp and Tatum, 2010). The aim of the study is to determine the antimicrobial resistance, distribution of carbapenem resistance and the clonality of the *Acinetobacter* isolates from different clinical samples of hospitalized patients thus provided that valuable

\*Corresponding author: e-mail: naheedafshan7@hotmail.com

information concerning this significant nosocomial pathogen in this region.

## MATERIALS AND METHODS

### Bacterial isolates

A total of 439 isolates were included in this study. These isolates were taken from different clinical samples of patients of different hospitals in the period of Jan 2013-December 2013. The isolates were initially processed to purify and confirm for their species identification. Briefly, *Acinetobacter* isolates were streaked on MacConkey Agar, Sheep Blood Agar (SBA) and Chocolate agar plates for isolated colonies & incubated for 24hrs at 37°C. Morphological, biochemical and cultural characteristics were also tested. Morphology was determined through gram staining. Catalase test, IMViC Tests and TSI reaction was performed to check the typical reaction of *Acinetobacter* strains.

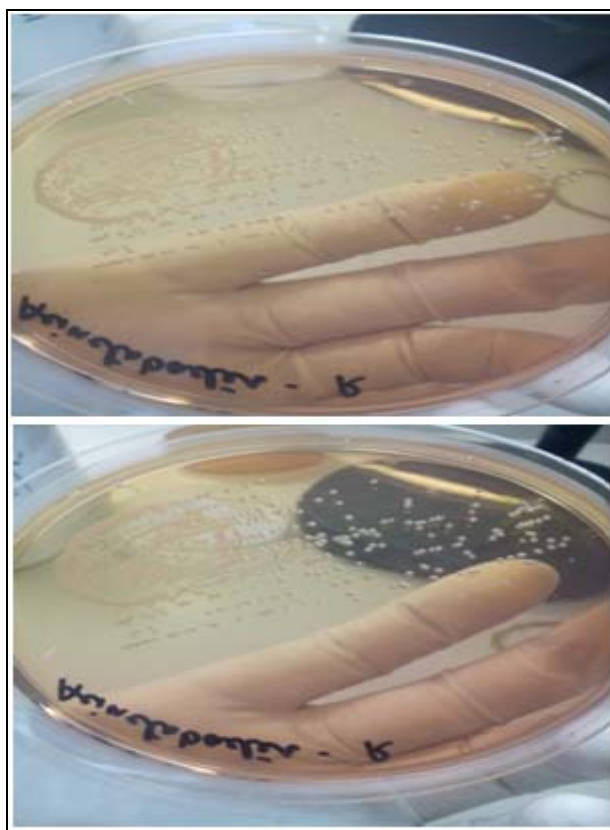


Fig. 1: Growth of acenetobacter on MacConkey media

### Antibiotic susceptibility testing

Antibiograms were done on a MHA plates with disk diffusion method according to Kirby Bauer method (Bauer *et al*, 1996). Briefly, sterile swab was placed into the broth culture of *Acinetobacter* and streaked on MHA plates to form a bacterial lawn. Plates were allowed to dry for 5 minutes. Antibiotic disk including meropenem (10 µg/disk) (Oxoid Ltd., England) dispenser was used to

dispense disks containing Antibiotic onto the plate. Disk was pressed gently to the agar plate by sterilized forceps. Plates were incubated for 24 hours at 37°C. The results was interpreted according to the Guidelines of the Clinical and Laboratory Standards Institute (CLSI).

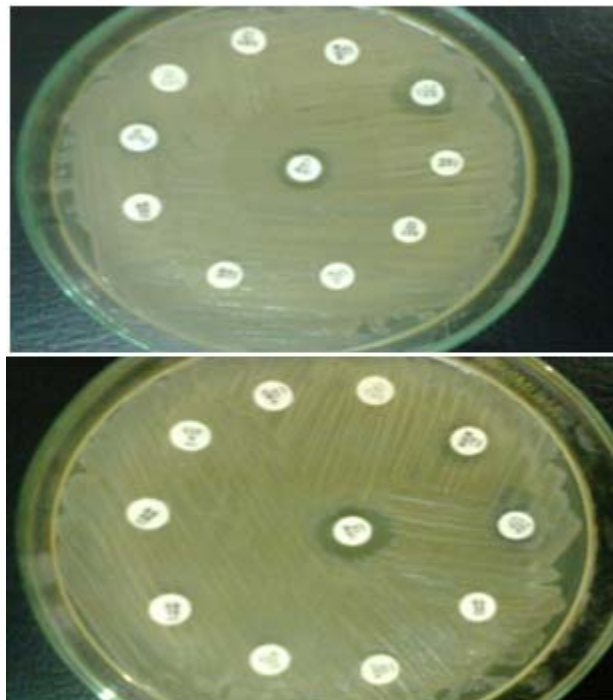


Fig. 2: Resistant pattern of antibiotics including meropenem.

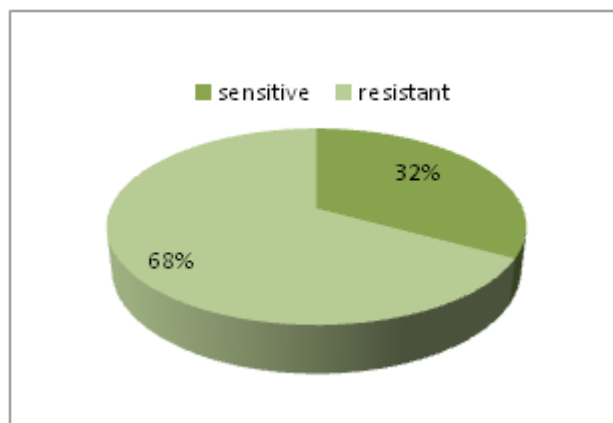
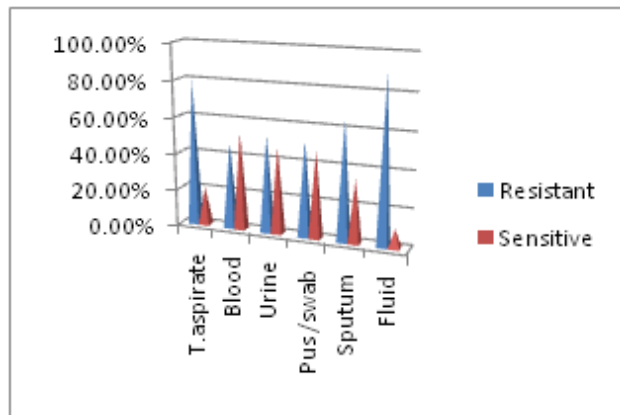


Fig. 3: Sensitivity & Resistivity % of Meropenem of Total Samples

## RESULTS

It was observed that 300 *Acinetobacter* isolates out of 439 (68%) were Resistant while remaining 139 (32%) were Sensitive to Meropenem (As shows in the fig. 3). Among 439 isolates of *Acinetobacter*, 154 out of 195 (79%) of Tracheal Aspirates, 16 out of 34 (47%) of Blood sample, 16 out of 30 (53%) of Urine sample, 48 out of 101 (47%) of Pus and Swabs, 27 out of 41 (65%) of Sputum sample

and 34 out of 38 (89%) of Fluid sample show Resistance against Meropenem (Represent in fig. 4).



**Fig. 4:** Sensitivity & Resistivity % of Meropenem among different clinical samples

## DISCUSSION

This increased resistivity of *Acinetobacter* is considered as an important health problem due to considerable clinical impact of this resistivity on the management of health care associated infections. Infections due to *Acinetobacter* frequently involve organ systems that have a high fluid content (e.g., respiratory tract, CSF, peritoneal fluid, urinary tract), revealing as nosocomial pneumonia, related by constant ambulatory peritoneal dialysis (CAPD), or catheter-associated bacteremia. The most common infections caused by *Acinetobacter* in Intensive care units are ventilator associated pneumonia (VAP) and bacteremia with the increase rate of mortality and morbidity (Sartelli, 2010). Strains of *Acinetobacter* cause broad spectrum of illnesses and their pathogenicity depends on number of effective virulence factors. These factors comprise the attachment to and persistence on solid and dry surfaces, capable to capture essential nutrients, such as iron, sticking to and destroy the cells, harm the host tissues by producing their enzymes gelatinases and proteinases (Camp and Tatum, 2010). Numerous studies have been conducted throughout the world on Carbapenem Resistant *Acinetobacter*. In a recent study, 90 isolates of *Acinetobacter* was taken. Of which 59 (65%) were resistant to Carbapenem (Hasan et al., 2014). Comparing to these results, our study shows that these resistant strains are the alarming sign to health authorities and is responsible for many outbreaks. The emerging problem is getting complicated and new clones with Carbapenem resistant are come to play. This danger has caused a noticeable and alarming decline in the available chemotherapeutic resources that includes drugs like, carbapenem antibiotics which inhibits the biosynthesis of peptidoglycan and was taken as the best standard for the treatment of *Acinetobacter* (Camp and Tatum, 2010)

## CONCLUSION

The infections of *Acinetobacter* are clearly associated with considerable morbidity and mortality. Our study suggests that contentment of multi drug resistance and their dissemination in *Acinetobacter* spp. is not a simple task. While multiple drug resistance is increasing in this pathogen, and Carbapenem conflict is quickly spreading which become a major threats in future. This increased resistivity indicates that proper attention, prevention and cure should be followed. The increased resistivity of the pattern of the organism has become a serious threat for health.

## REFERENCES

- Bassetti M, Righi E, Esposito S, Petrosillo N and Nicolini L (2008). Drug treatment for multidrug-resistant *Acinetobacter baumannii* infections.
- Bergogne-Berezin E and Towner KJ (1996). *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clin. Microbiol. Rev.*, **9**: 148-65.
- Camp C and Tatum OL (2010). A review of *Acinetobacter baumannii* as a highly successful pathogen in times of war. *Lab. Medicine*, **41**: 649-657.
- Choi CH, Lee JS, Lee YC, Park TI and Lee JC (2008). *Acinetobacter baumannii* invades epithelial cells and outer membrane protein A mediates interactions with epithelial cells. *BMC. Microbiol.*, **8**: 1471-2180.
- Doughari HJ, Ndakidemi PA, Human IS and Benade S (2011). The ecology, biology and pathogenesis of *Acinetobacter* spp: An overview. *Microbes Environ*, **26**: 101-12.
- Hasan B, Perveen K, Olsen B and Zahra R 2014. Emergence of carbapenem-resistant *Acinetobacter baumannii* in hospitals in Pakistan. *Journal of Medical Microbiology*, **63**: 50-55.
- Juni E (1978). Genetics and physiology of *Acinetobacter*. *Annu. Rev. Microbiol.*, **32**: 349-371.
- Jane C Davies and Bruce K Rubin (2007). Emerging and Unusual Gram-Negative Infections in Cystic Fibrosis. *Semin. Respir. Crit. Care. Med.*, **28**(3): 312-321.
- Kurcuk-Trajkovska B (2009). *Acinetobacter* spp. -- A Serious enemy threatening hospitals worldwide. *Macedonian Journal of Medical Sciences*, **2**: 157-162.
- Maragakis LL and Perl TM (2008). *Acinetobacter baumannii*: Epidemiology, antimicrobial resistance and treatment options. *Clin. Infect Dis.*, **46**: 1254-63.
- M Van Looveren and H Goossens (2004). The ARPAC Steering Group. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clinical Microbiology and Infection*, **10**(8): 684-704.
- Peleg AY, Seifert H and Paterson DL (2008). *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clin. Microbiol. Rev.*, **21**: 538-582.

- Sartelli M (2010). A focus on intra-abdominal infections. *World J. Emerg. Surg.*, **5**: 2-20.
- Towner KJ (2009). Acinetobacter: An old friend, but a new enemy. *J. Hosp. Infect.*, **73**: 355-363.
- Vila J, Marti S and Sanchez-Cespedes J (2007). Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. *J. Antimicrob. Chemother.*, **59**: 1210-1215.
- Xiao YH *et al.* (2011). Epidemiology and characteristics of antimicrobial resistance in China. Drug resistance updates: *Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*, **14**(4-5): 236-250.