Emergence Of Multidrug Resistant Acinetobacter Baumannii In An ICU

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Abstract: Background and objectives: Acinetobacter *baumannii* is emerging as an important pathogen causing hospital acquired infections. The present study was directed to find out the incidence, antibiotic susceptibility and metallo β lactamases production of *Acinetobacter baumannii* isolated from various clinical samples, in an intensive care unit. Methods: Isolation of *Acinetobacter baumannii* from various clinical samples was done. The isolates were tested for antibiotic sensitivity as per conventional methods. Imipenem resistant isolates were further tested for MBL production by double disk synergy test and MBL E test. Results: Total number of *Acinetobacter baumannii* isolates from clinical samples was 48. Maximum number of isolates were from blood (31.25%) followed by endotracheal aspiration (25%). Total 10 (20.83%) isolates were imipenem resistant, among which, 9(18.75%) were metallo β lactamases producers. MBL producers were more resistant to commonly used antibiotics than its non MBL producing counter parts. All isolates were susceptible to colistin (10 µg), polymyxin B (300 µg) and tigecycline (15µg).Conclusions: Multidrug resistant, metallo β lactamases producing Acinetobacter baumannii infection was not uncommon in our intensive care unit. Colistin, polymyxin B and tigecycline were very effective against such isolates. [Bose S et al NJIRM 2013; 4(2) : 11-15]

Key Words: *Acinetobacter baumannii,* Metallo β lactamases, Multidrug resistant

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Introduction: *Acinetobacter species was previously considered as non pathogenic organism. It colonizes* normal human skin, respiratory tract and vagina. *Acinetobacter baumannii (A. baumannii)* is emerging as an important organism causing hospital acquired infection. This organism can readily colonize and survive on abiotic surfaces, forms biofilms and is resistant to desiccation and disinfectants.[1,2,3] The mortality rate of Nosocomial infection caused by *A.baumannii* is relatively high, i.e.,25 to 30 % for bacteremia and 40 – 80% for pneumonia.[4]

Emergence of metallo –β- lactamases (MBL) producing multidrug resistant (MDR) *A.baumannii* is a matter of concern in an intensive care unit (ICU). This reflects increased use of carbapenems, use of medical devices and prolonged hospital stay. Uses of broad spectrum antibiotics are very high in ICUs, which results replacement of normal flora by other MDR organisms. MBL producing *Acinetobacter* species is a therapeutic challenge as it hydrolyzes higher generation of cephalosporin. MBL genes from such organisms can spread rapidly to other gram negative bacilli, making them resistant to other antibiotics.[5] The objective of this study was to isolate A.baumannii from various clinical samples in an ICU and to find out in vitro antimicrobial activity against the isolates. Imipenem resistant isolates were further tested for MBL production.

Material and Methods: Approval of institutional ethical committee was taken for this study. The study was conducted in the microbiology department of a tertiary care rural hospital. Study period was 12 months. Study population – Patients admitted in an ICU of a tertiary care rural hospital, irrespective of age, sex or antibiotic therapy.

Specimens, such as, blood, endotracheal aspiration, urine, pus, wound swab and catheter tips sent in the microbiology laboratory were processed as per conventional methods.[6] Blood was collected in blood culture bottles containing Brain heart infusion broth. Subcultures were done on blood agar and MacConkey's agar and incubated aerobically at 37° C for 24 hours. Isolates were identified by colony morphology, Gram's staining, motility, Oxidase test, sugar fermentation and other biochemical tests. [7]

Antibiotic susceptibility tests of the isolates were done by Kirby Bauer disk diffusion method following clinical and laboratory standard institute (CLSI) guidelines.[8] Following antibiotic disks were used for the study – imipenem (10 µg), piperacillin/tazobactam (100/10µg), netilmycin

(30µg), ticarcillin (75 µg), amikacin (30µg), ceftazidime (30µg), ciprofloxacin (5µg), colistin (10µg), cefepime (30µg), polymyxin B (300 units) and tigecycline (15 µg). All antibiotic disks were procured from Hi Media Pvt Ltd, India. Minimum inhibitory concentrations (MIC) of imipenem against imipenem resistant organisms were evaluated by E test (available from AB BioMerieux).Presence of MBL was further detected by double disk synergy test and MBL E strips (obtained from AB BioMerieux). This strip contains increasing concentration of imipenem at one end and imipenem plus ethylene diamine tetra acetic acid (EDTA) at the other end. ATCC 27853 *P. aeruginosa* was used as negative control. [9]

Result: Over a period of 12 months, total 48 *A. baumannii* were isolated from an ICU of a tertiary care rural hospital. Maximum number of *A. baumannii* was isolated from blood samples, i.e. 15 (31.25%), followed by endotracheal aspiration, 12(25%), urine, 11(22.91%), wound swab, 4 (8.33%), catheter tip & pus, 3 (6.25%) each. (Table – 1)

Table 1: Number and Percentage (%) of *A. baumannii* isolates from various clinical samples. (n = 48)

Samples	Number (%)
Blood	15 (31.25)
Endotracheal secretion	12 (25)
Urine	11 (22.91)
Wound swab	4 (8.33)
Catheter tip	3 (6.25)
Pus	3 (6.25)

Out of 48 *A.baumannii* isolates, 11 were imipenem resistant by disk diffusion method. However one of the imipenem resistant isolates by disk diffusion method showed susceptibility to the same drug by MIC detection. In our study, total number of imipenem resistant strains (by disk diffusion and MIC detection) was 10 (20.83%). Total number of MBL producing strains was 9(18.75%). We found DDST and MBL E test equally sensitive for detection of MBL. Table 2

All the isolates were sensitive to polymyxin B, colistin and tigecycline. MBL producers were resistant to most of the antibiotics used. Table 3

Table 2: Results of DDST & MBL E test (n = 48).

No. of imipenem resistant isolates	DDST positive	MBL E-test positive
10 (20.83%)	9 (18.75%)	9(18.75%)

Table 3: **Antimicrobial susceptibility pattern of both MBL producer & MBL non producer A. baumannii isolates.**

Antibiotics	No. & % of MBL producer	No. & % non MBL producers
	(n = 10)	(n = 38)
Imipenem	0 (0%)	37 (97.36%)
Piperacillin/tazobactam	0 (0%)	17 (44.37%)
Netilmycin	2 (20%)	5 (13.15%)
Ticarcillin	0 (0 %)	2 (5.26%)
Amikacin	1 (10%)	8 (21.05%)
Ceftazidime	0 (0%)	2 (5.26%)
Ciprofloxacin	0 (0%)	0 (0%)
Colistin	10 (100%)	38(100%)
Cefepime	0 (0%)	2 (5.26%)
Polymyxin B	10 (100%)	38 (100%)
Tigecycline	10 (100%)	38 (100%)

Discussion: The medically important species of genus *Acinetobacter (A)* are *A.baumannii, A. lowffi* and *A. haemolyticus.* They are combinedly known as *A. calcoaceticus – baumannii complex*. *A.baumannii* is glucose oxidizing and non haemolytic. *A. lowffi* is glucose non fermenter and non haemolytic. *A. haemolyticus* shows haemolysis. [10] The organism is a saprophyte found in soil, water, sewage etc. Occasionally they colonize over moist areas of human skin. The organism is sturdy and survives well in health facilities, especially in ICU. This may be due to high antibiotic selective pressure and increased use of life saving medical devices.[11, 12] It can cause serious infection, such as, meningitis, pneumonia and septicaemia, predominantly in immunocompromised patients.[13] Some

researchers observed that mortality due to *A.baumannii* was more, i.e. 68% than *P. aeruginosa*, i.e. 47%.[14]

Over the period of twelve months, we isolated 48 *A.baumannii* from clinical specimens of patients, admitted in an ICU. Irfan et al [15] isolated 100 *Acinetobacter* species from critical care patients in 6 months. In our study, maximum numbers of isolates were from blood, i.e. (31.25%), followed by endotracheal secretion (25%). Anuradha de et al [4] found maximum no of isolates from endotracheal secretion, i.e. 53.84%, followed by blood (46.15%). *A.baumannii* from various clinical samples were 100% susceptible to colistin, polymyxin B and tigecycline.

We used Kirby Bauer disk diffusion method for antibiotic susceptibility of isolates. Imipenem resistant isolates were further tested by E test for MIC detection to imipenem. One of our clinical isolates of *A.baumannii* was resistant to imipenem by disk diffusion method. However, it was found sensitive to the same drug by doing MIC detection by E test. Sinha et al [16] also reported high percentage of meropenem resistant *Acinetobacter* isolates by disk diffusion test, which were found to be sensitive to imipenem by MIC detection.

We observed multidrug resistance among *Acinetobacter* isolates. High prevalence of *Acinetobacter* species are attributed to various factors, such as, enzyme inactivation of antibiotics, efflux pumps, altered porin and target modifications. [17, 18]

Out of 48 *A.baumannii* isolates, 10 were imipenem resistant (by both disk diffusion method and MIC detection by E strip). Carbapenem group of antibiotics are widely used against MDR isolates. These drugs have got broad spectrum activity and they are stable to hydrolysis by most of the β lactamases including extended spectrum β lactamases. Recently MBL producing, carbapenem resistant *Acinetobacter* species is emerging fast as a virulent organism, especially in ICU. [5, 15] MBL belongs to molecular class B and can hydrolyze both imipenem and ceftazidime. Some workers found carbapenemase gene bla_{IMP}, in clinical isolates of imipenem and ceftazidime resistant *A.baumannii* strains. They also observed that it could disseminate among other Gram negative pathogens. [19]

In our study we adopted DDST and MBL E strip for detection of MBL producers. We found both the tests equally sensitive, i.e., 9 out of 10 imipenem resistant isolates each. E test MBL strips contain increasing concentrations of imipenem (IP) at one end and imipenem plus EDTA (IPI) at other end. Function of EDTA is to chelate the zinc ions required by MBLs to catalyze hydrolysis of carbapenems. The ratio of IP/IPI≥8 is interpreted as MBL production by the organism. Anuradha De et al[4] also suggested routine detection of MBL in clinical isolates by DDST or MBL E test. However, Heidi et al [9] suggested that results obtained with MBL E test strips should be interpreted cautiously against *A.baumannii*, which also produce oxacillinases. They observed that carbapenem resistance in *A.baumannii* might be due to either MBL or oxacillinase production. He also suggested carrying out Polymerase chain reaction for detection of MBL or *oxa* genes.

In our study, we found that all the isolates were 100% susceptible against colistin, polymyxin B and tigecycline. Others also observed that colistin with rifampin and/or tigecycline were useful against carbapenem resistant strains. [17] Colistin and polymyxin B are very useful for MDR *A.baumannii* infections, but not without side effects, such as, renal toxicity (27 to 58%). Tigecycline has good in vitro bacteriostatic activity against *A.baumannii*, including strains resistant to imipenem. [20] In such cases, Tigecycline can be successfully used. Tigecycline (Gar 936) is a new glycylcycline derivative of tetracycline. It can be equally effective against Gram positive as well as Gram negative organisms. [21] However reports regarding resistance to tigecycline have been reported by some workers. [22]

Conclusion: This study documents that MDR *A.baumannii* is emerging as an important pathogen in an ICU. We also observed relatively high number

of MBL producers among *A.baumannii* isolates. MBL producers were more antibiotic resistant than non MBL producers. Therefore, all clinical microbiology laboratories should routinely test for MBL production. We found that DDST and MBL E test both were equally sensitive and easy to perform. Strict safety precaution, such as, use of gowns and gloves, hand washing before and after each patient check up should be followed. Antibiotic selective pressure is one of the important causes of emergence of MDR *Acinetobacter* infection. A strict antibiotic policy should be implemented in each health care facility. Infection control practices and antibiotic resistance surveillance should be carried out regularly. Polymyxin B, colistin and tigecycline are very useful against MDR *Acinetobacter* infections. Therefore judicious use of antibiotics to treat such patients should be done to avoid further development of resistance to these drugs.

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