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Original Research Article

Emergence of Multidrug Resistant Acinetobacter baumannii in the Intensive Care Unit of a Tertiary Care Hospital

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Abstract: Acinetobacter baumannii is emerging as an important pathogen causing hospital acquired infections. It has been reported as the cause of serious infectious diseases involving mostly patients with impaired host defences, especially in intensive care units (ICUs). Emergence of metallo –β- lactamases (MBL) producing multidrug resistant (MDR) A. baumannii is a matter of concern in an intensive care unit (ICU). 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. Material and Methods in this study Isolation of Acinetobacter baumannii from various clinical samples was done over a period of 1 year. 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. In Results the Total number of Acinetobacter baumannii isolates from clinical samples was 42. Maximum number of A. baumannii were isolated from endotracheal aspirates, i.e. 20 (47.6%), followed by blood, 11(26.19%). All 42(100%) isolates were multidrug resistant. A total of 35(83.3%) isolates were Imipenem resistant, among which, 31(73.8%) were MBL 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). In Conclusion Multidrug resistant, metallo β lactamases producing Acinetobacter baumannii infections are on the rise in intensive care units. Colistin is very effective against such isolates. The analysis of susceptibility patterns will be useful in understanding the epidemiology and help in treatment and control of the spread of resistant isolates.
Keywords: Acinetobacter baumannii, Metallo β lactamases, Multidrug resistant, ICU, Nosocomial infections

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is emerging as an important organism causing hospital acquired infections [1]. This organism can readily colonize and survive on abiotic surfaces, forms biofilms and is resistant to desiccation and disinfectants [2, 3]. A. baumannii causes epidemic outbreaks or endemic occurrence with documented high mortality rates [4, 5]. A. baumannii outbreaks have also been reported from India [5]. The mortality rate of nosocomial infections caused by A. baumannii is relatively high, i.e. 25 to 30 % for bacteremia and 40 – 80% for pneumonia [6].

A.species had been reported as the cause of serious infectious diseases such as ventilator associated pneumonia, bacteraemia, urinary tract infections, burn wound infections, endocarditis, secondary meningitis, and septicaemia, involving mostly patients with impaired host defences, especially in intensive care units (ICUs) [7,8]. Predisposing factors for Acinetobacter infections include endotracheal intubation, intravenous (I.V.) catheters, the presence of prosthesis and prior antibiotic therapy in a seriously ill-patient in hospital.[9] Such infections are often extremely difficult to treat because of the widespread resistance to major groups of antibiotics and long-term survival of these bacteria in the hospital environment [10].

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.[11] The drug-resistant nature of the pathogen, its unusual and unpredictable susceptibility patterns and poor clinical understanding of significant sepsis, make empirical and therapeutic decisions even more difficult.[12] 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.

MATERIALS AND METHODS

Study setting & duration:

The study was conducted in the Microbiology department of a tertiary care hospital in Nizamabad for a period of 12 months from March 2015 to February 2016. Approval of institutional ethical committee was taken for this study.

Study design:

Prospective, Cross sectional study

Study population:

Patients admitted in the ICU of a tertiary care hospital, irrespective of age, sex or antibiotic therapy. No specific exclusion criteria were envisaged.

Specimens, such as, blood, endotracheal aspirates, urine, pus, wound swab, cerebrospinal fluid, and catheter tips were collected under standard aseptic precautions. These were processed as per conventional methods [9]. All specimens were inoculated on 10% sheep blood agar and MacConkey agar and incubated aerobically at 37°C for 18-24 h. Blood was collected in blood culture bottles containing Brain heart infusion broth and then subcultures were done. Non-fermenters were initially separated and further identified as Acinetobacter spp. In Gram stain of direct smears Acinetobacter appeared as tiny, Gram-negative cocco-bacillary cells. Colonies on blood agar were 0.5-2 mm diameter, translucent to opaque, convex and entire. On Mac Conkey agar a faint pink tint was produced. After Gram stain, catalase, oxidase and motility tests were performed. Acinetobacter are Gram-negative coccobacilli, non-motile, strictly aerobic, catalase positive and oxidase negative. Rapid utilization of 10% glucose was seen with O-F medium [11].

Antibiotic susceptibility tests of the isolates were done by Kirby Bauer disk diffusion method following Clinical and Laboratory Standard Institute (CLSI 2015) guidelines. Following antibiotic disks were used for the study – Imipenem (10 µg), piperacillin/tazobactam (100/10µg), gentamicin (10µg), tetracycline (10µg), ampicillin-sulbactam (10/10µg), ticarcillin (75 µg), amikacin (30µg), ceftazidime (30µg), ciprofloxacin (5µg), colistin (10µg), cefepime (30µg), and trimethoprim-sulfamethoxazole 1.25/23.75µg) [13].

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 [14].

RESULTS

Over a period of 12 months, total 42 A. baumannii were isolated from an ICU of a tertiary care hospital. These infections were more common in males (57.14%) as compared with females (42.8%). The age range was range between 6 to 71 years. Maximum number of A. baumannii were isolated from endotracheal aspirates, i.e. 20 (47.6%), followed by blood-11(26.19%), wound swab - 4 (9.5%), CSF - 3 (7.14%), pus -2(4.76%) and urine - 2(4.76%).

Table 1: Number & Percentage of A. baumannii isolated from various clinical samples.(n=42)

S.No.	Sample	No. of A.baumannii isolated
1	Endotracheal aspirates	20 (47.6%)
2	Blood	11 (26.19%)
3	Wound swab	4 (9.5%)
4	CSF	3 (7.14%)
5	Pus	2 (4.76%)
6	Urine	2 (4.76%)

Table 2: Antimicrobial susceptibility pattern of Acinetobacter baumannii isolates

S.No.	Antibiotic	Sensitive	Resistant
1	Ticarcillin	0 (0%)	42(100%)
2	Ampicillin-Sulbactam	9(22.5%)	33 (78.5%)
3	Piperacillin/Tazobactam	4(9.6%)	38(90.4%)
4	Ceftazidime	0(0%)	42(100%)
5	Cefepime	0(0%)	42(100%)
6	Trimethoprim-sulfamethoxazole	0(0%)	42(100%)
7	Gentamicin	4(9.6%)	38(90.4%)
8	Amikacin	7(16.7%)	35(83.3%)
9	Tetracycline,	0(0%)	42(100%)
10	Ciprofloxacin	2(4.8%)	40(95.2%)
11	Imipenem	7(16.7%)	35(83.3%)
12	Colistin	42(100%)	0(0%)

DISCUSSION

Multiresistant Acinetobacter spp. has become established as "alert" pathogens, particularly in ICUs and are associated with outbreaks of infection [15]. A. baumannii , a clinically important species has a tendency toward cross-transmission in ICUs, where numerous outbreaks are encountered. Their ubiquitous nature in the ICU environment and inadequate infection control practice has continuously raised the incidence of Acinetobacter infections over the past two decades. The understanding and recognition of Acinetobacter infections in the ICU is critically needed [16].

Occurrence of Acinetobacter is contributed by several factors including immunosuppressed hosts, patients with severe underlying disease, previous use of antibiotics, duration of hospital stay and more frequent use of antibiotics in ICU. Patients in ICU are sicker and require more invasive monitoring and therapeutic procedures to survive. ICU environmental contamination appears to be another important source of Acinetobacter infection [16]. The development of ICU-acquired infections is strongly related to prolonged ICU stay and is associated with worse outcomes including increased morbidity and mortality [17]. Acinetobacter ICU-acquired infections during the last decade represent a growing concern among clinicians and researchers. It can cause serious infections like meningitis, pneumonia and septicaemia, predominantly in immuno-compromised patients. Some researchers observed that mortality due to A.baumannii was more, i.e. 68% than P. aeruginosa, i.e. 47% [18].

Over the period of twelve months, we isolated 42 A.baumannii from clinical specimens of patients, admitted in an ICU. Irfan *et al.;* [19] isolated 100 Acinetobacter species from critical care patients in 6 months. In our study, maximum numbers of isolates were from endotracheal aspirates, i.e. 20 (47.6%), followed by blood, 11(26.19%). Anuradha de *et al.;* [20] also found maximum no of isolates from endotracheal aspirates i.e. 53.84%, followed by blood (46.15%). Bennani *et al.;* [21] reported 68.18% VAP ranging from 9% to 68% Acinetobacter infections.

In the present study, Acinetobacter infections were more common in males (57.14%) as compared with females. This may be due to the fact that the males report more frequently to the hospitals compared with females. Prashanth and Badrinath [22] also reported the infections to be more common in males (58.00%) compared with females (42.00%).

A majority of our isolates showed resistance to other important groups of antibiotics including third generation cephalosporin, amino glycoside and quinolone, which is a characteristic of majority of metallo- β-lactamases producing, isolates [23].

In our study, all isolates had multiple drug resistance. In a study by Sadeghifard *et al.;* [24], 100% of isolates had multiple drug resistance, which is agreement with our results. The most probable explanation for this increasing trend is the incorrect use of antibiotics to treat viral infections, incorrect diseases identifying, incorrect doses of antibiotics, inappropriate treatment duration (less or more than been recommended time), arbitrary use of antibiotics, and prescription of antibiotics by unaware persons, inappropriate formulation and low quality of some of antibiotics [24].

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. We observed multidrug resistance among Acinetobacter isolates. High prevalence of MDR Acinetobacter species are attributed to various factors, such as, enzyme inactivation of antibiotics, efflux pumps, altered porin and target modifications [25, 26].

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.[19] Anuradha De *et al.;* also suggested routine detection of MBL in clinical isolates by DDST or MBL E test [20].

In our study, we found that all the isolates were 100% susceptible to colistin. Others also observed that colistin with rifampin and/or tigecycline were useful against carbapenem resistant strains. Colistin and polymyxin B are very useful for MDR A.baumannii infections, but not without side effects, such as, renal toxicity (27 to 58%) [27].

For MBLs, limited treatment options are available and the only therapeutic option may be polymyxins, but it should not be used as monotherapy [28]. Imipenem or meropenem combined with ampicillin-sulbactam is active against carbapenem-resistant as well as MBLpositive strains of Acinetobacter species [29].

Currently at least 31 Acinetobacter genomo species have been described. Acinetobacter johnsonii, Acinetobacter lwoffii and Acinetobacter radio resistant seem to be natural inhabitants of human skin and commensals in human oropharynx and vagina. The digestive tract of patients within ICUs often serves as reservoirs for multiresistant A. baumannii strains involved in hospital outbreaks. The most common site for A. baumannii infection is the respiratory tract and the most common manifestation is VAP and bloodstream infections [30].

The ability of Acinetobacter strains to adhere to surfaces is an important mechanism in the pathogenicity. It frequently causes infections associated with medical devices, e.g., vascular catheters, cerebrospinal fluid shunts or Foley catheters. Biofilm formation is a well-known pathogenic mechanism in such infections. Biofilms have clinical and therapeutic implications, because biofilms preserve bacteria from the action of host's defensive mechanisms and antimicrobial activity against bacteria in biofilms might be substantially diminished [31].

As noted by the Infectious Disease Society of America, Acinetobacter is "a prime example of mismatch between unmet medical need and the current antimicrobial research and development pipeline." Acinetobacter spp. is notorious for their ability to acquire antibiotic resistance [32].

Antimicrobial resistance among Acinetobacter spp. has increased substantially in the past decade and has created a major public health dilemma. The most potent antibiotic drug class currently available are the carbapenems, but resistant strains have emerged [33].

CONCLUSION

MDR Acinetobacter infections are on the rise and this is a matter of concern in an intensive care unit (ICU). MBL producers are more antibiotic resistant than non MBL producers. 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. Timely implementation of strict infection control practices and antibiotic resistance surveillance programs should be carried out from time to time. Detection of MBLs by either DDST or MDST should be routinely performed in all microbiology laboratories for all imipenem-resistant isolates, which will help to reduce morbidity and mortality in these patients. There is a need for surveillance studies about the spread of antibiotic-resistance genes of A. species in local clinical settings. Further research should include the determination of genetic relatedness based on circulating A. species which is isolated to study transmission dynamics. This would serve to identify the sources of these strains and introduce intervention strategies to interrupt the transmission chains. Colistin is 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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