Emerging antimicrobial resistance and clinical relevance of *Acinetobacter* isolates in a tertiary care hospital of rural area of Punjab, India

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ABSTRACT

Objective: To carry out a study on *Acinetobacter* isolates from various clinical samples in a tertiary care hospital in India. To evaluate the prevalence of *A. baumannii* isolates, their antibiograms and clinical significance. Their contribution as causative agents of nosocomial infections has also been evaluated.

Methods: The clinical specimens over a period of 12 months from January 2013 to December 2013 were analyzed and the *A. baumannii* isolates obtained were further studied. Their antibiograms were studied and a clinical correlation was made to assess their pathogenic status.

Results: *A. baumannii* was isolated in 417 samples out of 2116 gram negative isolates (19.7% prevalence) from the entire hospital. Maximum isolates were from exudates & abscesses (41.5%) followed by respiratory secretions (28%). Overall resistance of *A .baumannii* towards carbapenems was 25.65% from all hospital isolates. ICU isolates showed higher resistance (62.6%) as compared to Inpatient Department (35.5%) and Out-patient Department (1.86%).

Conclusions: In this study, *A. baumannii* isolates showed pathogenic potential, however a majority were found to be carbapenem sensitive. There was significant correlation between the carbapenem resistant strains isolated from the intensive care units, in-patient and out-patient departments of the hospital.

KEY WORDS: **Emerging infection, Antimicrobial resistance *A. baumannii***

Introduction

Acinetobacter baumannii can be found in the natural microbial flora of the human skin. Members of the genus Acinetobacter have emerged from organisms of questionable pathogenicity to multidrug-resistant nosocomial pathogens worldwide in the past two or three decades, especially since 2005-2006 [1]. There are more than 30 genomic types of Acinetobacter identified so far, of which more than two third of Acinetobacter infections are due to *Acinetobacter baumannii*. *A. baumannii* colonizes healthy humans transiently at a low density on the warm and moist skin of axilla, groin, between toes, throat, nares and intestinal tract but it generally does not cause infection [2]. Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to up-regulate or acquires resistance determinants, making it one of the organisms threatening the current antibiotic era. Acting in synergy with this emerging resistance profile is the ability of *A. baumannii* to survive for prolonged periods throughout a hospital environment, thus potentiating its ability for nosocomial spread.

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In the hospital environment, *A. baumannii* can colonize the respiratory, urinary, gastrointestinal tract and wounds of the patients and cause infections in burn, trauma, mechanically ventilated and immunocompromised patients. It shows a special predilection for the ICUs [3]. The epidemiological, clinical, prognostic, and therapeutic characteristics of *A. baumannii* isolated from infected patients have been studied widely in the last decade [4]. During this period it was noted that *A. baumannii* has attained

resistance to most classes of known antibiotics posing a great threat to our containment of the infection. *A. baumannii* can rapidly modify transmembrane proteins and efflux pumps to prevent current antibiotics from penetrating its inner membrane and executing their mechanism of action. Furthermore, the enhanced ability of *A. baumannii* to obtain DNA from the external environment has allowed the species to obtain novel drug and heavy metal ion resistance genes. With resistance documented to all known classes of antibiotics, as well as cellular mechanisms that prevent desiccation and the action of antimicrobials, the world is in great need of new antimicrobials that can eliminate this dangerous pathogen. The most active agents in vitro against the multidrug resistant *A. baumannii* arepolymyxin B, polymyxin E (Colistin) and tigecycline [5].
In this study, we report the prevalence of *A. baumannii* isolates, their antibiograms and their clinical significance.

Materials and methods

This retrospective study was carried out in a tertiary care hospital over a period of 12 months from Jan to Dec 2013. Informed consent was taken from the patients. Samples collected and processed during the course of routine diagnostic work up from patients in the intensive care units (ICUs), wards and outpatient department (OPD) of the hospital for the identification of pathogens using routine microbiological techniques were analyzed and consecutive *A. baumannii* isolates were picked up for further studies. The specimens studied were urine, respiratory samples (sputum, and endo-tracheal aspirate), blood, pus, body fluids (pleural fluid, peritoneal & cerebrospinal fluid etc). Specimens were plated using appropriate culture media. All Gram negative were processed for identification and antibiotic sensitivity tests were performed by standard culture methods following CLSI guidelines [6]. Inclusion criteria: All gram negative coccobacilli, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria were taken as Acinetobacter isolates. Exclusion criteria: Gram negative bacilli, facultative anaerobes, fermenters, non-fastidious, non-motile/motile, catalase-positive, oxidase-negative as well as Gram-negative bacilli, strictly aerobic, non-fermenting, non-fastidious, motile, catalase-positive and oxidase-positive bacteria were excluded from the study.

The Acinetobacter isolates, thus identified were studied for their antibiotic sensitivity patterns as shown in table1. The antibiotics tested against the organism were amikacin, gentamicin, netilmicin, ciprofloxacin, levofloxacin, chloramphenicol, ceftazidime, cefotaxime, ceftriaxone, ceftazidime-clavulanic acid, piperacillin-tazobactam, imipenem, colistin, polymyxin B and tigecycline. Chi-square test was used to analyse the significance of presence of carbapenem resistant isolates in ICU, IPD and OPDs of the hospital. The role of *A. baumannii* as a pathogen or a colonizer in the respective infectious cases was determined by clinical correlation involving discussion with clinicians in order to assess the pathogenic status of the isolate.

Statistical analysis

Chi-square test was used to analyses the results. $P<0.05$was considered to be statically significant.

Results

The culture samples from the entire hospital processed during the 12 month study period from Jan to Dec 2013 were 11452. The antibiotic sensitivity patterns as shown in table1. Of the total cultures processed, the number of pathogenic bacterial isolates was 2966 (25.9%) which constituted 2116 (71.3 %) gram negative and 850 (28.6%) gram positive organisms. Overall, *A. baumannii* isolates constituted 19.7% of the total gram negative load (417 out of 2116). The majority of *A. baumannii* isolates were from exudates & abscess (41.5%) followed by respiratory secretions (28%), 49 in blood (11.7%), 72 in urine (17.2%) and 6 in fluids as shown in Table 2. The carbapenem resistance in these *A. baumannii* isolates was seen the most in respiratory isolates (35.5%, Suction tips 32/90 R to imipenem) as compared to non-respiratory isolates. Of the 417 isolates of *A. baumannii* from the entire hospital, 298 belonged to the ICU (71.5%). The inpatient department (IPD) and the outpatient department (OPD) contributed to 19.6% (82 out of 417) and 8.8% (37 out of 417) of the total *A. baumannii* isolates respectively. Overall resistance of *A. baumannii* towards carbapenems was 25.65% from all hospital isolates. ICU isolates showed higher resistance as compared to Inpatient Department and Outpatient Department as shown in Table 3.

Table 1. Percentage of Acinetobacter strains sensitive to various antibiotics.

ANTIBIOTICS	JAN–MAR (75)	APR-JUN (78)	JUL-SEP (160)	OCT-DEC (104)
AMIKACIN	58	57	54	31.5
GENTAMICIN	35.7	33.9	31	16.4
NETILMICIN	57.9	55	54	52
CIPROFLOXACIN	7.7	7.5	17	13.7
LEVOFLOXACIN	13	13.3	17.4	41
CEFTRIAXONE	7.2	7.5	17	12
CEFTAZIDIME	13	13.2	12	8.2
CEFTAZIDIME-CLAVULANIC ACID	23	21.5	34	16.4
PIPRACILLIN/ TAZOBACTUM	70	68	48	12.3
IMIPENEM	93	87	75	67
TIGECYCLINE	100	100	99.6	98.3
COLISTIN	100	100	100	98.6
POLYMYXIN B	100	100	100	100

Table 2. Prevalence of Acinetobacter isolates in different clinical specimens.

Specimens	Total isolates	Isolates from ICU	Colonisers from ICU
PUS	173	110 (36.9%)	12 (10.9%)
RESPIRATORY	117 (28%)	112 (37.6 %)	18 (16.07%)
URINE	72 (17.2%)	29 (9.7%)	05 (17.24%)
BLOOD	49 (11.7%)	41 (13.8%)	12 (29.3%)
FLUIDS	06 (1.43%)	06 (2%)	01 (16.6%)
TOTAL	417	298 (71.5%)	48 (16.1%)

Table 3. Prevalence of Acinetobacter isolates & carbapenem resistance at different places in hospital.

SITE OF ISOLATION	NUMBER OF ISOLATES	PERCENTAGE OF CARBAPENEM RESISTANT ISOLATES
ICU	298 (71.5%)	62.6%
IPD	82 (19.6%)	35.5%
OPD	37 (8.8%)	1.86%
TOTAL	417	25.65%

To assess whether *A. baumannii* was actually causing clinical infection or was an innocent bystander, a clinical correlation was done in the 298 isolates of *A. baumannii* in the ICU. Of the total 298 isolates of *A. baumannii* from the ICU, 250 (83.8%) proved to be pathogenic. Of the 112 isolates from respiratory samples in the ICU, 18 (16.07%) appeared to be colonizers without contributing to the signs and symptoms of infection and 94 (83.9%) contributed to the infection. 90 out of 112 (80.3%) were clinically proven ventilator associated pneumonia (VAP) cases and 4 were admitted with community acquired infections. Of these 90 isolates, 53 (58.8%) were proven as having been acquired from our tertiary care hospital and 37 (41.1%) were brought in at the time of admission from other hospitals. Of the 41 *A. baumannii* isolates from the blood, 29 (70.7%) were proven for their pathogenic status and in the remaining 12 patients who showed no symptoms of blood stream infection (BSI), the culture positivity may have been due to contamination with *A. baumannii* colonized on skin during sample collection . Of the samples isolated from pus and drain fluid (110 isolates), 98 (89%) isolates were proven as pathogens and rest of the 12 (11%) were skin colonizers. Out of the 98 pathogens, 50 were attributed to having been hospital acquired from our tertiary care centre, and 48 were brought in from the community or other hospitals. Of the 29 isolates from urine, 24 (82.7%) were found pathogenic. Out of 24, 17 were observed as acquired from our tertiary care hospital and the other 7 were community acquired. There existed significant ($P<0.05$) correlation between the presence of carbapenem resistant strains of Acinetobacter in the ICU, OPD and IPD.

Discussion

A. baumannii was isolated in 417 consecutive samples forming 19.7% of the total gram-negatives. This corresponds to similar study carried out by H Siau et al [7] where figures of *A. baumannii* isolates were 11% of the total gram negative isolates. Of the total 417 isolates of *A. baumannii*, a maximum relative percentage (41.5%) was obtained in exudates & abscesses followed by respiratory secretions (28%). However in this study the ICU showed the maximum yield of *A. baumannii* from the respiratory samples (37.6 %) followed by pus (36.9%) & blood (13.8%). Siau et al [7] reported in their ICU isolates that respiratory tract was the most common site from which Acinetobacter was isolated. Villers et al [8] have also reported a predominance of A. baumannii in tracheobronchial secretions as 24.8% to 48.8% and Suri et al [9] as

45.6% respectively in their studies. An attempt was made in this study to distinguish clinical infection from colonization. Of the total 298 isolates of *A. baumannii* from the ICU, 250 proved to be pathogenic (83.8%) This was done by correlating various clinical and lab parameters and discussion with the clinician. An analysis was also done of the pathogenic potential of *A. baumannii* in various samples like respiratory secretions, blood, pus and body fluids and urine specimens. Of the 112 isolates of *A. baumannii* from respiratory secretions, 94 (83.9%) were recognized as pathogens and rest were colonizers. Since this organism is a fast colonizer of the respiratory tract, its percentage can increase from 7% to 45% in a healthy subject to those on ventilators respectively [10]. Of the *A. baumannii* isolates from blood 70.7% were found to be pathogenic and rest were the contaminants. As Acinetobacter cannot exist as a colonizer in blood, it would have a higher pathogenic potential at this site. We did isolate *A. baumannii* as contaminants in 29.3% cases. However, Lahiri et al [11] have reported 33% of A. baumannii isolates from blood as skin contaminants. Typically, patients with A. *baumannii* infections have had prolonged ICU stays [12], sources of bloodstream infection are usually line related or attributed to underlying pneumonia, UTI, or wound infection. Pus and fluids analysis showed 89% of *A. baumannii* as pathogens. Sengupta et al reported a lower isolation rate of 11.5% of *A. baumannii* from wounds [13]. High isolation rate in our hospital could be because of severely ill patients coming into a tertiary care centre. This study also showed seasonality in the occurrence of *A. baumannii* infection during the one-year period, with a significantly greater number of infections occurring from July through September (160) .The reason for this seasonality is unknown. The antibiograms of the isolates of *A. baumannii* from the entire hospital showed 25.65% carbapenem resistance in our study. When the isolates in the ICU were studied, the resistance to carbapenems rose to 62.6% whereas in IPD it was 35.5%. However, a comparatively very low carbapenem resistance of 1.86% was observed *in A. baumannii* in the community as represented by the OPD isolates. Antibiotic resistance in *A. baumannii* is increasing at an alarming rate leading to increased morbidity, mortality and treatment costs in ICU settings as revealed by surveillance studies from Europe, the Asia Pacific region, Latin America and North America over the last 3-5 years [14]. However, lower rates of carbapenem resistance have been reported in studies carried out by Knam Soo Koo et al as 8.3%. This could be explained by their stringent antibiotic policies and judicious use of carbapenems in their countries [14]. Earlier studies in India have also reported lower resistance rates (9.8-18.5%) in *A. baumannii*. This study shows the carbapenem resistant isolates are circulating among ICU, OPD & IPD areas of hospital, to prevent this there is a need of enforcing strict infection control guidelines. This study also brings up an important aspect of increasing resistance in *A. baumannii* towards carbapenems [15]. The antibiotic susceptibility patterns clearly showed the increasing resistance of *A. baumannii* to various antibiotics as compared to other gram negatives. Colistin (Polymyxin E) is one agent which is active against *A. baumannii*. In the present study, colistin resistance has been reported as 1.4% but all strains were sensitive to Polymyxin B as shown in table1. A recent study of clinical isolates from the Western Pacific region showed 3.3% resistance of *A. baumannii* to colistin [16]. Heteroresistance to colistin among *A. baumannii* isolates has also been described in earlier reports [14]. In a study in Korea, there was high resistance to colistin (30.6%) and polymyxin (18.1%) [14]. However, as the resistance against colistin/polymyxin is not very high in our country, it can still be used as the drug of choice against multidrug resistant strains of *A. baumannii*. *A. baumannii* was sensitive to tigecycline in 99.47% cases in present study which correlates well with the study by Yilmaz F. F. et al., where 3.57% isolates were resistant to tigecycline [17]. However studies by Behara B. et al., in India have shown only 42% susceptibility in *A. baumannii* isolates to tigecycline [18]. Since therapeutic options are limited for multidrug-resistant Acinetobacter infection, the development or discovery of new therapies, well-controlled clinical trials of existing antimicrobial regimens and combinations, and greater emphasis on the prevention of health care-associated transmission of multidrug-resistant Acinetobacter infection are essential. In 2006, the CDC released a report describing guidelines to prevent the transmission of MDR organisms. The steps the CDC recommends all health care facilities take include improvement of hand hygiene, use of contact precautions until the patient tests culture-negative for the target organism, active surveillance cultures, education of hospital personnel,

improved environmental cleaning, and better communication about patients with these infections to not just personnel within the facility but also between facilities[19]. While *A. baumannii* may not be particularly virulent, it can cause unnecessary disease and expense in the critically ill patients affected by it, and the transmission of such a pathogen should be limited. Measures to prevent the inter- and intra-hospital transmission of *A. baumannii* must be established in health care settings. The resistance patterns detected in Acinetobacter could reflect the antibiotic misuse and lack of regulations on the over the counter sale. Our study suggested that due to the increasing resistance of *A. baumannii*, we should judiciously use antibiotics by making an attempt to distinguish colonization from infections and treatment should be only given to the clinically confirmed Acinetobacter infections.

Conflict of Interest

We declare that we have no conflict of interest.

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