International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 4, Issue 1, pp: (406-411), Month: April 2016 - September 2016, Available at: www.researchpublish.com

Effect of Carbapenems on Clinical Isolates of *Acinetobacter baumannii spp.* in Tertiary Care Hospital of North India

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Abstract: ***A. baumannii*** **has been designated as a "red alert" human pathogen, generating among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum. Carbapenem resistance in *Acinetobacter* is now observed increasingly, and constitutes a sentinel event for emerging antimicrobial resistance. The production of carbapenem hydrolyzing beta-lactamases is the most common mechanism responsible for carbapenem resistance. Design: This prospective study was conducted in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak from December 2012-November 2013, on 100 isolates of *Acinetobacter baumannii.* The specimens were process for bacteriological cultures, biochemical reactions and antigenic sensitivity against Antibiotic [Imipenem, Meropenem and Doripenem (Carbapenem group)] by kirby-bauer method. P value <0.05 was statistically significant. Results: Out of 42141 samples 855 (7.75%) specimen shows *A. baumannii* growth. Male to female ratio was 1.70:1. Patients belonged to age 51-60 years (29%) and 76% with ICU. The endo-tracheal aspirate isolates were 66%. Isolates were sensitive to Carbapenem group followed by imipenem (81%), Meropenem (39%) and doripenem (40%). On comparing the resistance to carbapenems P value were significant in imipenem, meropenem and Doripenem, i.e. <0.05. Conclusion: Treatment of *Acinetobacter* infection should be prescribed on the basis of antimicrobial sensitivity reports and judicious way to decrease the emergence of drug resistant isolate. Sharing of expertise and collaboration between the clinicians using antibiotic therapy and microbiologist may be the simplest health measure to optimise the use of antibiotics.**

Keywords: **Carbapenems, *Acinetobacter baumannii,* ICU, UTI, BAL, ETA, ET, Lower respiratory tract sample.**

1. INTRODUCTION

During the last three decades, outbreaks of infections caused by *Acinetobacter* spp. have capable of causing a broad range of clinical disease syndrome especially in critically ill patients with recent intensive care unit stay, surgical procedure or long hospitalization. *Acinetobacter baumannii* is a gram-negative, non-fermenting, oxidase-negative and non-motile cocco-bacillus, it can survive in water, soil, surface of skin and hospital environment, so that causes a series of opportunistic infections such as, ventilator associated pneumonia (VAP), skin and soft tissue infections, surgical site infections, bactermia, meningitis, urinary tract infections and septicemia in immuno-compromised patients.[1]

A. baumannii accounts for as many as 10% of all infections caused by gram-negative bacteria seen in intensive care units (ICUs), and in the United States, it accounts for only 2.5% infections. Additionally, *A. baumannii* is now days recognized as an important cause of community-acquired pneumonia, with a high mortality rate of 40% to 64%. About 3 to 5 % of nosocomial pneumonias are caused by *Acinetobacter* spp.[2] In large surveillance studies from the United States, between 5 and 10% of cases of ICU-acquired pneumonia were due to *A. baumannii*.[3] *A. baumannii* is an occasional cause of UTI, being responsible for just 1.6% of ICU-acquired UTIs in one study.[4] And also small number of case reports of *Acinetobacter* endocarditis exist.[5] In recent years, *A. baumannii* has been designated as a "red alert" human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum. The

mechanism of resistance generally falls into three categories, antimicrobial inactivating enzymes, reduced access to bacterial targets and efflux pump mechanism.

The whole genome sequencing of *Acinetobacter baumannii* clinical strains led to identification of 86kb resistance is land known as AbaR1. Overall 52 resistance genes were identified and 45 (86.5%) of them were localized to AbaR1 island.[6] The increasing trend of carbapenem resistance in *Acinetobacter baumannii* worldwide is a concern since it limits the choice of antimicrobials for the treatment of *Acinetobacter baumannii*. Metallo-β-lactamases (VIM, IMP, SIM) have been reported worldwide, especially in Asia and Western Europe, and confer resistance to all β-lactams except aztreonam. The most widespread β-lactamases with carbapenemase activity in *A. baumannii* are carbapenem-hydrolysing class D β-lactamases (CHDLs) that are mostly specific for *A. bauamnnii*. These enzymes belong to three unrelated groups of clavulanic acid-resistant b-lactamases, represented by OXA-23, OXA-24 and OXA-58, that can be either plasmid- or chromosomally encoded. *A. baumannii* also possesses an intrinsic carbapenem-hydrolysing oxacillinase, the expression of which may vary, that may play a role in carbapenem resistance. In addition to b-lactamases, carbapenem resistance in *A. baumannii* may also result from porin or penicillin-binding protein modifications. Several porins, including the 33-kDa CarO protein, that constitute a pore channel for influx of carbapenems, might be involved in carbapenem resistance.[7] Carbapenem-resistant isolates of *A. baumannii* are usually resistant to all classes of antimicrobials, and show intermediate resistance to rifampin, while usually retaining susceptibility to tigecycline and colistin.[8] Resistance against carbapenems is, in itself, considered sufficient to define an isolate of *A. baumannii* as highly resistant. The resistance of *A. baumannii* to carbapenems can be mediated by one of the resistance mechanisms that are known to occur in bacteria, including enzymatic inactivation, active efflux of drugs, and modification of target sites.[7] The production of carbapenem hydrolyzing beta-lactamases is the most common mechanism responsible for carbapenem resistance in *A. baumannii*. Several carbapenem-hydrolyzing β-lactamases have been identified so far in *A. baumannii*.

Aims and Objectives

To study the Effect of Carbapenems on 100 Clinical Isolates of *Acinetobacter baumannii spp.* in Tertiary Care Hospital at North India.

2. MATERIAL AND METHOD

The present prospective study was conducted in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak over a period of one year (December 2012 - November 2013). A total of 100 isolates of *Acinetobacter baumannii* were obtained from various clinical samples received in Microbiology laboratory from outdoor and indoor patients of the hospital, irrespective of age and sex. The samples included urine, sputum, pus, blood, endo-tracheal aspirates and broncho-alveolar lavage (BAL) and process for the bacteriological cultures. The bacterial isolated were identified using standard methods. After incubation, non-lactose fermenting colonies on MacConkey agar were further processed for identification of *Acinetobacter baumannii*.

The various biochemical reactions were used, as indole test, urease test, citrate test, triple sugar iron. And antigenic sensitivity was measured against Antibiotic [Imipenem, Meropenem and Doripenem (Carbapenem group)] by kirby-bauer method of 100 isolates and takes the results.

Statistical Analysis:

Collected data was entered in MS Excel spreadsheet and coded appropriately in SPSS (Statistical Package for Social Studies) for Windows version 20.0. All tests were performed at 5% level significance, thus an association was significant if the value was less than 0.05 (p value< 0.05).

3. RESULTS

The present study was conducted in the Department of Microbiology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak over a period of one year. A total of 100 *A. baumannii,* isolated from various clinical samples were included in the present study. *A. baumannii* isolates identified on the basis of Gram staining, motility and biochemical reactions were further confirmed by automated BD phoenix system. Antimicrobial susceptibility testing of 100 clinical isolates of *A. baumannii* against three drugs was done by using Kirby-bauers disk diffusion method as per CLSI guidelines. The following observations were made:

From December 2012 to November 2013, 42141 clinical samples received in the laboratory during the study period, 11028 (26.16%) showed bacterial growth, while rest 31113 samples (73.83%) were either culture sterile, or showed the growth of bacterial contaminants or fungal isolates. Total no of *A. baumannii* isolated in study period was 855, so the overall isolation rate of *Acinetobacter baumannii* was 7.75%. out of 855 positive specimen, male to female ratio was 1.70:1. Majority of the patients belonged to age groups 51-60 years (29%), followed by 21-30 (29%), 31-40 (12%), 11-20 (12%) and only 10% belongs than <10 years. The p value was significant in age group 21-30 years.

TABLE 1: Distribution of 885 *A. baumannii* among different clinical samples

Name of specimen	Total culture positive samples	*A. baumannii* isolated	
		Number (n)	Percentage (%)
Pus	1290	118	9.14
Blood	4477	415	9.26
LRTS	1550	268	17.29
Sputum	834	24	2.87
Urine	2877	30	1.04
Total	11028	855	7.75%

LRTS- Lower Respiratory Tract Samples,

Among the culture positive samples, maximum rate of isolation of *A. baumannii* was from lower respiratory tract samples (17.29%) followed by blood (9.26%), pus (9.14%), sputum (2.87%) & urine (1.04%)

TABLE 2: Distribution of 100 isolates of *A. baumannii* among various clinical samples

Name of sample	Number of *A. baumannii* isolates(n)	Percentage (%) of *A. baumannii* isolates
Urine	4	4
Pus	10	10
Blood	9	9
Sputum	3	3
BAL	1	1
Endo-tracheal tip	7	7
Endo-tracheal Aspirate	66	66
Total	100	100.0

The maximum number of *A. baumannii* were isolated from endo-tracheal aspirate (66%), followed by pus samples (10%), blood samples (9%), ET Tip (7%), urine (4%).

TABLE 3: Distribution of *A. baumannii* isolates among patients admitted to ICU and Ward

Name of sample	Ward		ICU		Total		P value
	N	%	N	%	n	%	-
Urine	4	16.66	-	-	4	4	-
Pus	10	41.66	-	-	10	10	-
Blood	2	8.3	7	9.21	9	9	0.096
Sputum	3	12.5	-	-	3	3	-
BAL	0	0	1	1.31	1	1	-
Endo-tracheal	1	4.16	6	7.89	7	7	0.059
Endo-tracheal Aspirate	4	16.66	62	81.57	66	66	<0.001
Total	24	24.0	76	76.0	100	100.0	-

A. baumannii recovered from indoor patients among various samples, these indoor patients were further classified into ICU and wards. It was observed that the infections caused by *A. baumannii* were commonest in the ICU patients 76 (76%). Among the ICU patients, maximum numbers of *A. baumannii* isolates were recovered from endo-tracheal aspirate (81.57%), followed by blood (9.21%). Among ward patients maximum number of *A. baumannii* were recovered from pus (41.66%), ETA (16.66%), urine (16.66%). On comparing the distribution of *A. baumannii* isolates among ICU and ward significant difference in terms of P value was observed in Endo-tracheal aspirate.

TABLE 4: Antimicrobial resistance pattern of clinical isolates of *A. baumannii* against 22 drugs

Drugs	No. of resistant isolates (n)	No. of sensitive isolates (%)	P-value
Carbapenems			
Imipenem	19(19%)	81(81%)	<0.01
Meropenem	61(61%)	39(39%)	<0.028
Doripenem	60(60%)	40(40%)	<0.046

On comparing the resistance to carbapenems P value were significant in imipenem, meropenem and Doripenem, i.e. <0.05.

4. DISCUSSION

The present study was carried out in the Department of Microbiology, Pt. B.D Sharma PGIMS, Rohtak to identify and perform antimicrobial susceptibility of 100 *A. baumannii* strains isolated from various clinical specimens, and the Effect of Carbapenems of all 100 clinical isolates of *Acinetobacter baumannii spp.*

In the present study, the rate of isolation of *A. baumannii* from culture positive specimens was 7.75 % which was lower than the study by Jaggi et al[5] who reported an isolation rate of 9.4% from all culture positive samples. However Siau H et al[9] reported the prevalence rate of 11% from the culture positive samples. Lahiri et al[10] reported isolation rate of 12.9% from all culture positive samples. In our study maximum rate of isolation of *A. baumannii* was from lower respiratory tract samples 17.29%, pus 9.14%, blood 9.26%, followed by urine 1.04%, sputum 2.87%. The results of current study were in accordance with Jaggi et al[5] who observed maximum rate of isolation of *A. baumannii* from lower respiratory samples 56.9%, blood 25.2%, pus 10%, while Prashanth et al[11] reported the distribution of *A. baumannii* isolates among blood, respiratory samples, and urine samples to be 16.27%, 48.8%, 9.3%. Various authors have reported different distribution pattern of *Acinetobacter* isolates among various samples. The present study showed 9% isolation of *A. baumannii* from blood samples, which was in accordance with the study by Lahiri et al[10] done in ICU patients. Other studies have reported more or less isolates from blood samples, ranging from 46.7% as reported by Saleem et al[12] to 0.9 % by Liling et al.[13] In our study 66% isolation of *A. baumannii* was from lower respiratory tract samples, which was in accordance with jaggi et al[5], Saleem et al[12], Liling et al[13] who recovered 56.9%, 45.1%, 78.6% isolates respectively from LRT samples. Liling et al[13] reported much higher (78.6%) isolation of *A. baumannii* from respiratory samples, which could be explained by the fact their study included the hospitalized patient only. In contrast to our study Lahiri et al[105] and Mindolli et al[14] reported 7.8% and 28.5% isolates of *A. baumannii* from LRT samples, Which was lower than in the present study. This could be explained by the fact that the majority of LRT samples in our study were from ICUs, where *Acinetobacter* spp. can cause higher cross contamination by virtue of its presence in ICU environments. The isolation rate of *A. baumannii* in the present study, from pus and urine samples was 10% and 4% respectively which was similar to other studies Viller et al[15], Lahiri et al[10], 17.1 % and 31.0 % and 11.1% and 51.3% respectively.

In our study 76% *Acinetobacter* isolates were recovered from ICU patients and only 24% isolates were from various wards. Our study is in accordance with the study done by Hakima K et al[16] who reported 76% *Acinetobacter* isolates from ICU patients in 2005 and 82% in 2010. In contrast to our study Jaggi et al[5] and Sinha et al reported the 22.6% and 22.4% *Acinetobacter baumannii* from the ICU patients. The high rate of isolation in our ICU corroborates the fact that a lot of risk factors associated with *Acinetobacter* infection exist in the ICU like potential environmental reservoirs, opportunities for cross transmission, sick, immune compromised patients who are colonized, patients having multiple wounds and indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands of health care workers while patient care.

The male to female ratio among patients with *Acinetobacter* infections in the present study was 1.7:1, which was in accordance with the study done by various authors in India who reported male to female ratio ranging from 1.6:1-1.7:1[14,17] In the present study, maximum number of *A. baumannii* were isolated from patients belonging to the age group 21-30 years (29%), followed by >50 years (29%), 11-20 years (12%) and 31-40 years (12%) respectively. The present study was similar to the study conducted by Mindolli et al[14] and Rubina el al[17] who also reported maximum number of *Acinetobacter* isolates from the patients of >50-60 years of age. P-value was statistically significant in age group 21-30 years. In the present study, the significant risk factor for *Acinetobacter baumannii* infection were age group >55 years, in old age patients immune system was weak. Various studies were reported that old age also acts as predisposing factors for *Acinetobacter baumannii* infections.[15]

We studied antimicrobial resistance pattern of clinical isolates of *A. baumannii* against 03 drugs belonging to Carbapenem group. In our study all isolates of *A. baumannii* were 60-61% of the isolates were resistant to doripenem and meropenem whereas, 19% isolates were resistant to imipenem. The studies by various authors viz, Jaggi et al[4] and Tripathi et al[18] reported 89.6% and 43% resistance to imipenem which was higher than the present study whereas, studies done by Rubina et al[17], Aktas et al[19], Oberoi et a[20] Sinha et al[21] reported lower resistance to carbapenems in comparison to the present studies. A surveillance study from MYSTIC (meropenem yearly Susceptibility test information collection) programme 2006 reported resistance rate of 43.4% and 42.5% for meropenem and imipenem respectively. In another large surveillance study, susceptibility of *A. baumannii* to imipenem was lower for isolates in Latin America and in Asia–Pacific region (60.6%, 69.2% respectively) than in those from Europe and North America (85.9% and 88.6% respectively).[22] A recent surveillance study conducted in United States reported only 60.2% susceptibility to imipenem.[23]

It was observed that the infections caused by *A. baumannii* were commonest in the ICU patients (76%), followed by the patients admitted in wards (24%), the maximum no of *A. baumannii* were isolated from endo-tracheal aspirate (66%), pus (10%), blood (9%) and Et tip (7%) and the carbapenems group drugs was most effective drug 81% (imipenem) and sensitive drugs were doripenem 40% and meropenem 39%. P-value was significant in carbapenems.

5. CONCLUSION

This study concludes that the treatment of *Acinetobacter* infection should be prescribed on the basis of antimicrobial sensitivity reports and in judicious way to decrease the emergence of drug resistant isolate. Sharing of expertise, cooperation and collaboration between the clinicians using antibiotic therapy and clinical microbiologist at the regional level may be the simplest and most useful public health measure to optimise the use of antibiotics and manage infectious disease. As efflux mechanism is getting widespread in hospitalized patients, especially in ICU patients, efforts should be aimed at detecting such resistant bacteria for controlling infections caused by them, and finally, providing better alternative therapies against these notorious organisms.

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