Phenotypic and Genotypic Identification of Acinetobacter baumannii with Special Reference to Bla$_{oxa-51}$Like Gene and its Antimicrobial Susceptibility Pattern from Intensive Care Unites in Kanpur

Nidhi Pal[1], R. Sujatha[2], Anil Kumar[3]

ABSTRACT

Introduction: *Acinetobacter baumannii* are important nosocomial pathogens being increasingly reported worldwide in critically ill patients. Resistance to multiple antimicrobial agent are major concern for all clinical settings. So this study was conducted to identify the *Acinetobacter baumannii* from different clinical samples in Kanpur.

Material and Methods: A total of 200 clinical cases were observed for *Acinetobacter baumannii* isolation by the standard phenotypic microbiological procedures. Antimicrobial resistant pattern was analysed by as per CLSI guidelines 2016. And intrinsic gene bla$_{OXA-51}$ like were also detected by PCR.

Results: In this prospective study 34 *Acinetobacter baumannii* were isolated from 200 cases but 10% were found to be infected. The higher percentage of isolates were from respiratory samples 24 (70.58%) followed by central line 5 (14.70%), blood 2 (5.88%), urine 1 (2.9%) and foley's tip 1 (2.9%). Isolates were highly resistant to most of the antimicrobial agents. Antibiotic susceptibility analyses of the isolates revealed that the resistance to penicillin and cephalosporins were most common followed by 85.29% resistance to gentamicin, and 79.41% resistance to imipenem. All isolated acinetobacter were susceptible to tigecycline and polymyxin B.

Conclusion: A high level of antibiotic resistance was observed in our study. Carbapenamase producing gene bla$_{OXA-51}$like gene intrinsically present in *A.baumanni.* Therefore presence of bla$_{OXA-51}$ like gene was found to be confirmation for *A.baumannii* species. Thus judicial use of antimicrobials & adopting strict infection control policies will be useful.

Keywords: Acinetobacter baumannii, Antimicrobial Resistant Pattern, bla$_{OXA-51}$like, Phenotypic, Genotypic

INTRODUCTION

Hospital-acquired infections (HAI) have been recognized for over a century as a critical problem. Intensive care Units are the specialised section of the hospital that provides special care through life supporting devices to the patients and also act as epicenters for HAIs. *Acinetobacter* is a gram negative coccobacilli, ornamented as sophisticated nosocomial pathogen in 21st century. There are more than 25 genomic types of Acinetobacter identified so far, of which more than two third of Acinetobacter infections are due to *Acinetobacter baumannii*.[1, 2] A.baumannii having capacity to tolerate wide range of pH, sanitary, humidity and unique ability to survive on almost all nutrient sources, this habit make this pathogen ubiquitous in the hospital environment.[3, 4] This behaviour is likely source for most outbreaks of hospital infections.

The incidence of *A.baumannii* infections has risen over the past decades[5], and recent studies indicate that this pathogen is more resistant and virulent, and has become a serious nosocomial threat, likely because of patient-to-patient transmission via the hands of health care workers from a common environmental source.[5, 6]

Accurate identification of Acinetobacter baumannii by phenotypic methods is not very reliable. Standard laboratory techniques will identify the genus of Acinetobacter only, not species. Automated methods, which is actually a phenotypic technique, utilizing no. of biochemical test to identify the genus and species of bacteria in short duration. Today's, there are number of analyzer that could be used for early diagnosis. Another methods for earlier and accurate identification of genus as well as species can be analyzed by molecular testing. There are mounting evidence that Acinetobacter baumannii has bla$_{OXA-51}$ like gene. This is naturally occurring gene intrinsically present in this species only.[7, 8] When IS*Aba1* is located 7 bp upstream of the bla$_{OXA-51-}$like genes, it provides a promoter that can increase bla$_{OXA-51-}$like gene expression levels by 50-fold. This increase in expression levels raised the MICs of the carbapenems for the isolate and become resistant to carbapenem.[7]

Acinetobacter is commonly associated with high morbidity and mortality which includes infections, such as respiratory tract infections, bacteraemia, urinary tract infection, meningitis, skin and soft tissue infections especially in patients with severe health conditions.[9, 10] Acinetobacter having excellent capacity to acquire resistance for multiple drugs therefore management of Acinetobacter infection is always difficult.

This study was conducted to isolate, identify phenotypically and genotypically A.baumannii from different clinical samples from ICUs and to determine the antimicrobial resistance pattern of *A. baumannii*.

MATERIAL AND METHODS

In this prospective study clinical samples were collected from six different hospitals of Kanpur during 16 months (Oct 2015 to Jan 2017). Total 200 cases were observed and the studied specimens were respiratory samples (endotracheal aspirates, sputum, suction samples, endotracheal tips, tracheostomy tips, tracheal aspirates), blood samples, urine samples, pus and any

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How to cite this article: Nidhi Pal, R. Sujatha, Anil Kumar. Phenotypic and genotypic identification of acinetobacter baumannii with special reference to bla$_{oxa-51}$Like gene and its antimicrobial susceptibility pattern from intensive care unites in Kanpur. International Journal of Contemporary Medical Research 2017;4 (5):1154-1158.

aspirated fluids collected from ICUs patients. From a patient multiple samples were also collected. Total 315 samples were processed according to standard procedure.

Sample Processing

Collection

For sample collection, the nursing staffs were instructed to collect the sample aseptically. Any types of respiratory aspirates or tips, pus and aspirated fluids were aseptically collected. Urine samples were collected from urinary catheter not from urinary bags. Blood sample was collected aseptically by venous puncture method.

Processing

All the samples were inoculated on appropriate culture media i.e. Blood agar, MacConkey agar or CLED (Cystein lactose electrolyte deficient) agar and incubated at 37 °C. On the bases of gram staining, colony morphology and biochemical test all the isolated microorganisms were identified.

All non-lactose fermentering gram negative bacilli were subjected to phenotypic test mentioned in table 1 for identification of *Acinetobacter baumannii.*[11-13] [Fig 1A, 1B, 1C] All the *Acinetobacter baumannii* isolates were included, while isolates other than *Acinetobacter (Pseudomonas species etc.)* were excluded from the study.

The antimicrobial resistant pattern was performed by Kirby-Bauer disk diffusion technique as per Clinical and Laboratory Standards Institute guidelines (CLSI) 2016.[14] The isolates were tested for Piperacillin (Pi, 100 mcg), ampicillin-sulbactam (A/S, 10/10 mcg), ticarcillin –clavulanic acid (Tc, 75/10 mcg), piperacillin-tazobactam (PIT, 100/10 mcg), cefotaxime (CTX, 30 mcg), ceftraxone (CTR, 30 mcg), Ceftazidime (CAZ, 30 mcg), ceftriaxone (CPM, 30 mcg), Cefipime–tazobactum (CPT, 30/10 mcg), Amikacin (AK, 30 mcg), Gentamicin (GEN, 30 mcg), Tobramicin (TOB, 10 mcg), Netilmycin (NET, 30 mcg), Ciprofloxacin (CIP, 5 mcg), Ofloxacin (OF, 5 mcg), Levofloxacin (Le, 5 mcg), Imipenem (IMP, 10 mcg), Meropenem (MRP, 10 mcg), Polymyxin B (PB, 300 unit) and Tigecycline (TGC, 15 mcg).

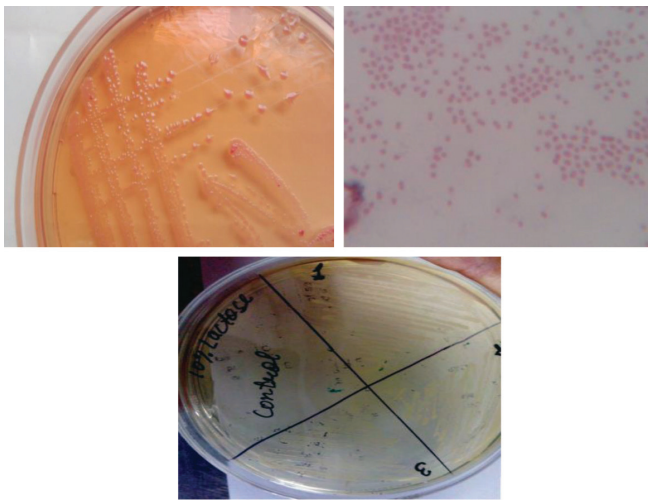

Figure-1: (**A**) Acinetobacter growth on MacConkey Agar, **(B)** Gram Stain show gram negative coccobacilli, **(C)** Growth at 10% Lactose

Genotypic Identification

Acinetobacter baumannii were confirmed by PCR of bla$_{OXA-51}$ like gene. Primer sequences were F 5'TAATGCTTTGATCGGCCT TG 3' and R 5'TGGATTGCACTTCATCTTGG3'. All phenotypically identified Acinetobacter baumannii were subjected to conventional PCR.

DNA extraction

For DNA extraction, pure Acinetobacter broth suspension [1.0 MacFarland turbidity] from nutrient agar culture plate after overnight incubation was used. DNA isolation kit (Qiagen, Germany) was used and processed according to manufacturer's guideline.

PCR reactions

PCR was carried out in 20 µl reaction volume with 10µl mastermix, 5µl DNAse/RNAse free water, 1 µl forward and revers primer each and 3µl DNA sample. Conditions for PCR were initially 94 °c for 3 min and then 34 cycle at 94 °c for 30 sec for denaturation, 55 °c for 30 sec for annealing and 72 °c for 30sec for extention, followed by a final extension at 72 °c for 5 min.

The roles of A.baumannii as a pathogen or a colonizer in the respect of infectious cases were determined by microbiological and clinical correlation.[15]

STATISTICAL ANALYSIS

Microsoft office 2007 was used for the statistical analysis. Descriptive statistics like mean and percentages were used for data interpretation.

RESULTS

In this study 200 cases were studied from October 2015 to January 2017 from six different hospitals of Kanpur. From 200 patients admitted to different ICU, total 315 samples were collected and microbiologically processed. Among these samples total 121 (38.41%) microorganisms were isolated which constitute 99 (81.81%) were gram negative bacilli 20 (16.52%) were gram positive budding yeast cells and 2 (1.6%) were gram positive cocci. Of 200 cases 34 *Acinetobacter baumannii* were isolated. These 34 isolated Acinetobacter baumannii were also gave positive result in PCR with OXA 51 primer. [Fig 2] Acinetobacter baumannii were isolated from various clinical

Gram stain	Oxi	Cat	Ind	MR	VP	Cit	Ure	TSI	Motility
Gram negative coccobacilli	-	+	-	-	-	+	-	K/K	*NM*
Gelatin Liqu.	Growth at 44^0 C	Growth at 10% lactose	Oxidation of				Decarboxylation of		
			Glu	Lac	Mal	Man	Lysin	Ornithine	Arginine
-	+	+	+	-	-	-	-	-	+/-

Oxi= oxidase test, Cat= Catalase test, Ind= Indole Test, MR= Methyl Red test, VP= Voges proskaur test, Cit= Citrate test, Ure= Urease Test, TSI= Triple sugar Iron test, K/K= Alkaline slant/Alkaline butt, NM= non motile, Glu= Glucose, Lac= Lactose, Mal=Maltose, Man=Mannitol.

Table-1: Phenotypic tests for Identification of *Acinetobacter baumannii*

samples as indicated in Table 2. Maximum isolates were from endotracheal tips and secretions followed by tip of central line, blood, urine, pus and tip of foley's catheter. Age wise and gender wise distribution of patients were mentioned in table 3 and 4. On the basis of microbiological and clinical correlation among 34 isolated A.baumannii, 20 were contributed to the infection. Hence the rate of A.baumannii infections were 10% (20 acquired infection out of 200 cases) Antimicrobial resistance pattern of isolated A.baumannii were showed in Fig 2. Only Polymyxins and Tigecyclines showed 100% sensitivity.[Fig 3]

DISCUSSION

In the present study 34 *A.baumannii* was isolated from 315 samples collected from 200 patients admitted to ICUs and all were microbiologically processed according to standard protocol. Among these samples total 121 (38.41%) microorganisms were isolated which constitute 99 (81.81%) were gram negative bacilli, 20 (16.52%) were gram positive budding yeast cells and 2 (1.6%) were gram positive cocci. Fifty out of 121 (41.32%) were non fermenting gram negative bacilli [NFGNB] isolated from ICU. This result was comparable to Bhargava et.al. who reported 29.62% of NFGNB in their study.[16]

Of these isolated microorganism 34 Acinetobacter were isolated and all isolated *A.baumannii* were confirmed by presence of *bla*$_{OXA-51}$ like gene. *bla*$_{OXA-51}$like was present in every strain of *A.baumannii* and a previous studies also reported *bla*$_{OXA-51}$like gene in every *A.baumannii*.[7, 8, 17, 18]

Among the source of the isolates, 34 *A.baumannii* were isolated from various clinical samples and *A.baumannii* frequently isolated from respiratory samples i.e. endotracheal tips (14 out of 83) and secretions (6 out of 11). Lahiri KK et al.[19] were also reported 48.8% of *A. baumannii* from respiratory samples.

The reason behind this was Acinetobacter is normal commensal of upper respiratory tract but because of low immunity, severe illness of patients in ICU gave the best opportunity for the commensal to become a pathogen. With the help of their virulence factors it invades the cells or with the support of invasive devices, bacteria reach the lower respiratory tract or any other favorable region and causes infection. Sometime invasive devices can be contaminated with commensals during extubation or sample collection. Hence the presence of *A.baumannii* in sample without clinical signs and symptoms was considered as colonizer. Some cases were also found that same microorganism were isolated with different antibiogram from different time of samples of the same patients. Microbiologically, growth on culture plate should be significant for considering Acinetobacter as pathogen.[20] That's why clinical as well as microbiological correlations are necessary for reporting infecting pathogen or commensal.[13]

On the basis of microbiological and clinical correlation among 34 isolated A.baumannii, 20 were contributed to the infection. Hence the rate of A.baumannii infections was 10% (20 acquired infections out of 200 cases). Rest of isolated A.baumannii were colonizers, not responsible for infection. Hence all *A.baumannii* isolations doesn't necessarily mean infection and antibiotics should only be given in clinically proven infections.

Correlating with the present study Maryam A et.al.[21] also observed that above 50 years age patients were mostly infected.[22, 23] The main reason was reduced immune status in elder age people. Patients with weak immune system were at the risk to infections and risk of nosocomial infections by opportunistic microorganisms. In ICU admitted patients due to severe illness,

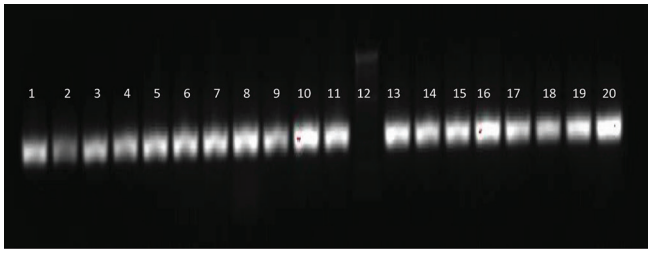

Figure-2: Detection of bla$_{OXA-51}$ like gene
Lane 1 to 11 and 13 to 20- positive for bla$_{OXA-51}$ like gene
Lane 12 –Ladder 100bp

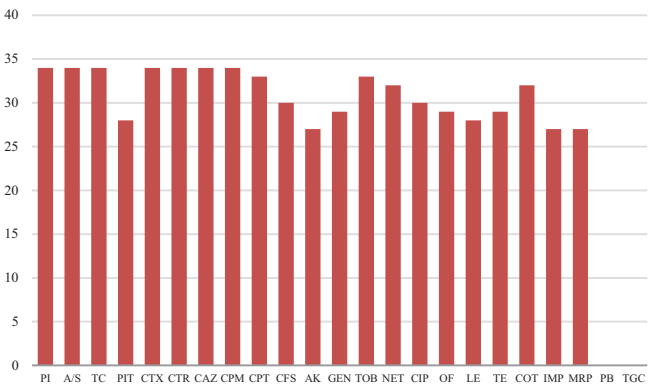

Figure-3: Antimicrobial resistance pattern of isolated A.baumannii

Sample	Total sample	Total Acinetobacter isolate
ET.Tube	83	14
ET aspirate	11	6
Suction tip	17	4
Sputum	4	0
Tracheastomy tip	2	0
Tracheal aspirate	1	0
Central line	27	5
Blood	25	2
Urine	69	1
Foley's tip	65	1
Pus	7	1
Total	315	34

Table-2: Distribution of *A. baumannii* from different clinical sample

Age	No. of Patients
<20	4 (11.8%)
21-40	2 (5.9%)
40-60	15 (44.1%)
<61	13 (38.2%)

Table-3: Age wise distribution of patients with A. baumannii infection/colonization

Gender	No. of Patients
Male	26 (76.4%)
Female	8 (23.5%)

Table-4: Gender wise distribution of patients with A. baumannii infection/colonization

long-term hospitalization associated chronic diseases in these age groups and use of invasive procedures raises the risk for infection.

According to gender wise distribution, there were higher rate of infection among male as compare to female, other studies also in favour of this result but there is no statistical significance.[24, 25] The resistance profile of the *A. baumannii* isolates showed that 100% were resistant to penicillin and cephalosporin group of antibiotics but 82.4% and 88.2% resistant to inhibitor combination antibiotics i.e. piperacilline tazobactam and cefaperazone sulbactam respectively. This is considered high resistance rate when compared to other study conducted by Neetu et al.[23] reported comparatively less resistant to piperacillin (55%), followed by ceftriaxone (46%) and ceftazidime (46%). Rahbar *et al*.[26] also reported 90.9% of resistance for ceftriaxone and piperacillin and 84.1% resistance for ceftazidime. Sohail M et al.[27] reported high resistant for fluoroquinolones (97%) as compare to our study 85% and 88% of resistance were found for ofloxacin and ciprofloxacin respectively. In the study aminoglycosides were also found highly resistant. In this study 97.05% resistance was observed to tobramycin followed by 85.29% and 79.4% resistance to gentamycin and amikacin respectively. While Plege Y et al. reported only 47.5% and 35% and of resistant to gentamycin and amikacin.[28]

In that case only carbapenems have been the drug of choice for treating *Acinetobacter* infections, but unfortunately, carbapenem-resistant *Acinetobacter* spp. due to carbapenemase enzyme is becoming common worldwide.[13] Of the β-lactamases, those with carbapenemase activity are the most concerning for drug resistance. Out of 34 Acinetobacter isolates 27 (79.4%) were carbapenem resistant phenotypically. While Kaur A et al, Lautenbach E 2009 and Gladstone P et al. reported 57%, 23.1% and 14.2% of carbapenem resistant Acinetobacter respectivily.[29-31] The most probable explanation for this increasing trend is incorrect use of antibiotics to treat viral infections, misdiagnosis of diseases, inappropriate doses of antibiotics, inappropriate treatment duration (less or more than been recommended time), arbitrary use of antibiotics, prescription of antibiotics by quaks, inappropriate formulation, and low quality of some of antibiotics. Only Polymyxins and Tigecyclines showed 100% sensitivity.

CONCLUSION

Control of infections is always difficult because of widely distribution in nature of bacteria that is resistant to antimicrobial agent. The reported results of the study is alarming toward the implementation of strict infection control because the emergence and increase in the rate of XDR *A. baumannii.* Continuous surveillance of Acinetobacter especially resistance strain is necessary to control the further spread of resistant strains. Accurate detection of species by PCR provides powerful information not only on identification but also confirms the presence of pathogen and the identification is done rapidly that would be highly useful to clinicians and infection control team to take necessary action.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 02-05-2017; **Accepted:** 30-05-2017; **Published:** 08-06-2017