

Pattern of Acinetobacter Species Isolated from Critically ill Patients of a Tertiary Care Hospital

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ABSTRACT

Objective: to isolate Acinetobacter species and to evaluate its antibiotic susceptibility pattern in all types of clinical specimens of critically ill patients of Jinnah Hospital, Lahore.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Pathology Department, Allama Iqbal Medical College, Lahore from 1st July 2015 to 31st December 2015.

Materials and Methods: One hundred clinical specimens obtained from the critically ill patients admitted in Jinnah Hospital, Lahore, were cultured using standard microbiological techniques and antimicrobial susceptibility pattern was evaluated using modified Kirby Bauer disc diffusion method following CLSI guidelines 2015.

Results: The isolation rate of Acinetobacter baumannii was high as compared to the other species. Most of them were identified from the blood samples followed by the tracheal secretions and pus samples. The isolation rate of overall Acinetobacter species was high in ICU's and surgical wards. Antimicrobial susceptibility pattern revealed resistance rate of Acinetobacter species as co-trimoxazole 96(96%), gentamicin 82(82%) and ciprofloxacin 84(84%). Almost all of the isolates were susceptible to tigecycline and colistin.

Conclusion: Acinetobacter species were resistant to most of the commonly used antibiotics. Only tigecycline has good susceptibility i.e 72% and colistin 100% susceptible to the Acinetobacter isolates from intensive care units.

Key Words: Acinetobacter baumannii, nosocomial pathogen, colistin, tigecycline

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INTRODUCTION

Acinetobacter species is one of the most common cause of nosocomial infections. It was originally given the name as Acinetobacter after the baumann's et al in 1968. Acinetobacter species is gram negative and aerobic coccobacilli¹, non-lactose fermenting, encapsulated gram negative opportunistic pathogen. Acinetobacter species is not fastidious and is pleomorphic.²

Acinetobacter species readily colonizes skin, oropharyngeal secretions and respiratory tract parts.³ There are almost 35 known species of Acinetobacter that has been identified so far.⁴

Multidrug resistant Acinetobacter species has been involved in meningitides, bacteremia, nosocomial pneumonia, skin and soft tissue infections, urinary tract infections and the infections at the sites of trauma and surgery etc.⁵ Acinetobacter baumannii is mostly found on the surface of the medical devices. Acinetobacter species are mostly frequently found in the ICU's of the hospital.⁶ Another unique property of the mutant Acinetobacter strains BD413trpe27 has been observed that is naturally transformable tryptophan auxotrophic to the wild type phenotype.⁷ Acinetobacter species resist the sterilization and disinfection processes because of its ability to form biofilm which along with the resistance towards the disinfection also plays an important role in its attachment to the medical devices.

A recent study also revealed that the colonies of the Acinetobacter species can persist in their mucoid form for at least 6 days increasing its pathogenicity. A recent study conducted by Grace et al in 2016 shows that mutations in two of the genes of the two component system (TCS's) namely AdeR and AdeS which are involved in the formation of adeA, adeB, adeC (RND family) which causes the efflux of the antibiotics out of the cell hence making it multidrug resistance. Acinetobacter species has the ability to produce six different types of QS molecules.⁸ Microbial resistance is

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a natural biological response of the organism towards the antibiotics, heat, environment, food and acquired. Antimicrobial resistance mechanisms include antimicrobial inactivation by the enzymes, reduced access to the bacterial targets and mutations that changes the targets or cellular functions.⁹

Acinetobacter species produces different Ambler classes of enzymes that act as carbapenemases. Ambler class A, extended spectrum β – lactamases (ESBL), includes PER 1, PER 2, VEB 1, TEM 1, TEM 2, SHV, CTX-M.¹⁰ Ambler class B, Metallo β lactamases (MBL) include IMP, VIM, SIM. Ambler class D is the main cause of carbapenem resistance in *Acinetobacter* species. It is also known as OXA carbapenemases. These enzymes acts in the periplasm and convert their monomeric (less active) form into dimeric (more active) form and producing greater amount of carbapenemase activity.¹¹

Acinetobacter species with these altered proteins have 0.5% less permeability as compared to other enterobacteriaceae which plays an important role in the prolonged survival as nosocomial pathogen.¹² *Acinetobacter* species produces plasmids or chromosomal mediated aminoglycosides mediated enzymes (AME's) that causes the modifications of aminoglycosides. *Acinetobacter* species causes the over production of the AdeABC efflux pump which causes the efflux of tetracyclines out of the bacterial cell.¹³ *Acinetobacter* species has the great ability to tolerate the harsh environment, dryness, PH and temperature basically DNA damage and cellular damaging by the production of RecA gene.¹⁴

MATERIALS AND METHODS

This study was carried out in Pathology Department, Allama Iqbal Medical College, Lahore. 100 isolates were included from different clinical specimens of critically ill patients that were admitted in Jinnah Hospital, Lahore. All types of samples were included in the study including urine, blood, pus, pus swabs, tracheal secretions, sputum, CVP tip and body fluids.

The preliminary identification of bacteria was done by standard microbiological procedures including colonial morphology, gram staining, catalase test, oxidase test. *Acinetobacter* isolates were confirmed by using API 20NE (Biomérieux, France), according to the manufacturer's instructions.

Antimicrobial susceptibility of these isolates was performed by Modified Kirby Bauer disc diffusion method using Mueller-Hinton agar (Oxoid UK), bacterial suspension equal to 0.5 McFarland turbidity standard and the plates were lawned and incubated at 35°C for 24 hours. Zone sizes were interpreted according to Clinical Laboratory Standards Institute (CLSI) 2015 guidelines. *Acinetobacter baumannii* ATCC 19606 was used as quality control strain.¹⁵

RESULTS

A total of total 100 *Acinetobacter* isolates from clinical samples of all types were included. All the culture positive isolates showed characteristic non fermenting colonies on MacConkey, Gram staining of all the isolates showed typical gram negative coccobacillary morphology. All the isolates were catalase positive and oxidase negative. API 20NE was used for identification of *A. baumannii* from the other species.

Out of total 100 isolates 25 were isolated from the tracheal secretions, 19 from the blood specimens, 22 from the wound/swabs, 6 from the sputum samples, from the urine, 5 from the CVP line tip, 19 from the pus specimens and 1 from the wound/swabs and pus specimens.

Out of 100 isolates 73(73%) were positive for *A. baumannii* and 27(27%) were other species of *Acinetobacter*. Out of total 100 *A. baumannii*, 54(54%) were found in male and 19(19%) were in females. Total isolates found in males were 68(68%) and among females that were 32(32%).

A total of 26 isolates were from the ICU out of which 21(29%) were *A. baumannii* and 5(18.5%) were *Acinetobacter* species, 17 from the surgical wards out of which 13(18%) were *A. baumannii* and 4(15%) were *Acinetobacter* species, 10 were from orthopedic out of which 9(12.3%) were *A. baumannii* and 1(3.7%) was *Acinetobacter* species, 6 from dermatology out of which 5(7%) were *A. baumannii* and 1(3.7%) was *Acinetobacter* species, 1 from ENT ward and was *Acinetobacter* species, 7 from neurosurgery out of which 5(6.8%) were *A. baumannii* and 2(7.4%) were *Acinetobacter* species, 4 from pulmonology out of which 1(1.4%) was *A. baumannii* and 3(11.1%) were *Acinetobacter* species, 16 from the pediatric ward out of which 4(12.3%) were *A. baumannii*, 7(25.8%) were *Acinetobacter* species and 2 from the gynaecology, out of which 1(1.4%) was *A. baumannii* and 1(3.7%) was *Acinetobacter* species.

Out of total 73 isolates of *A. baumannii*, 73(100%) isolates of *Acinetobacter baumannii* were resistant to piperacillin-tazobactam, 54(74%) were resistant to cefoperazone-sulbactam, 52(71%) were resistant to amikacin, 63(86%) were resistant to gentamicin, 64(88%) were resistant to ciprofloxacin, 54(74%) were resistant to imipenem and meropenem, 67(92%) were resistant to doxycycline, 28(38%) were resistant to tigecycline, 70(96%) were resistant to co-trimoxazole and 73(100%) isolates were susceptible to colistin.

Out of total 27 isolates of *Acinetobacter* species, 26(96%) isolates of *Acinetobacter* species were resistant to Ampicillin 26(96%) to co-amoxycloxacillin, 25(93%) were resistant to ceftriaxone, 21(78%) were resistant to piperacillin-tazobactam, 14(52%) were resistant to gentamicin, 20(74%) were resistant to ciprofloxacin, 11(44%) were resistant to tigecycline,

26(96%) were resistant to co-trimoxazole and 27(100%) isolates were susceptible to colistin.

Table No.1: Acinetobacter species distribution among different wards

Wards	A. baumannii n=73	Acinetobacter species n=27
Surgical wards	13 (18%)	3(11.1%)
Intensive care unit(ICU)	21(29%)	5(18.5%)
Critical care unit(CCU)	2(3%)	0(0%)
Dermatology	5(7%)	1(3.7%)
ENT	0 (0%)	1(3.7%)
Medical wards	7(9.5%)	3(11.1%)
Pediatric ward	9(12.3%)	7(25.9%)
Orthopedic wards	9 (12.3%)	1(3.7%)
Neurosurgery	5(6.8%)	2 (7.4%)
Gynecology Wards	1(1.4%)	1 (3.7%)
Pulmonology	1(1.4%)	3 (11.1%)



Figure No.1: Antibiotic sensitivity of Acinetobacter species on Muller Hinton Agar

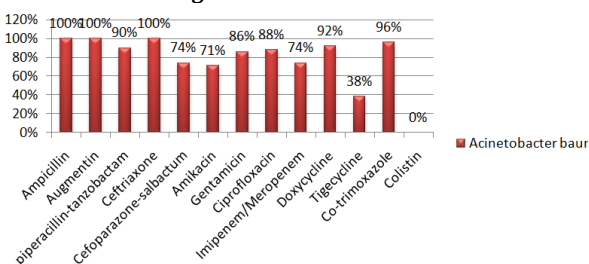


Figure No.2: Antibiotic Resistance Pattern Among Acinetobacter baumannii

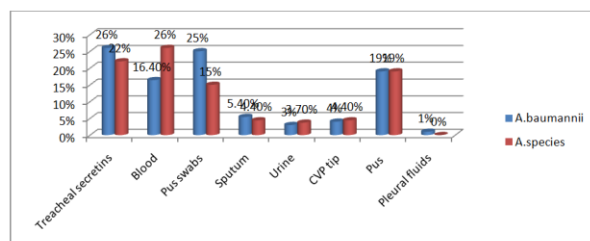


Figure No.3: Antibiotic Resistance Pattern Among Acinetobacter species

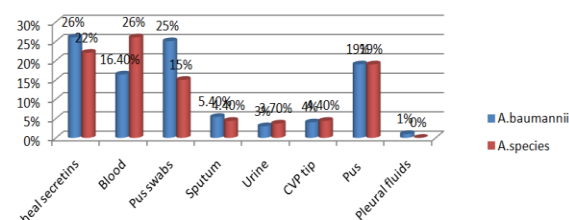


Figure No.4: Specimen wise distribution of Acinetobacter species

DISCUSSION

Acinetobacter is also known as "Iraqibacter", since 1980's uptill now it has been one of the emerging problems in the health care sectors. Many of the factors associated with the mutations and acquired resistance are making it more resistant as a superbug day by day.¹⁶ In this study 100 clinical samples of all types were included. 100% isolates of *Acinetobacter baumannii* showed resistance against ampicillin and co-amoxyclove as compared to 96% and 96% isolates of *Acinetobacter species* respectively. *Acinetobacter baumannii* and *Acinetobacter species* were 100% and 93% resistant to ceftriaxone, 86% and 70% resistant to gentamicin, 71% and 26% resistant to amikacin, 74% and 52% resistant to cefepime-salbutam, 90% and 78% resistant to piperacillin-tazobactam, 88% and 74% resistant to ciprofloxacin, 74% and 56% resistant to meropenem / imipenem, 92% and 93% resistant to doxycycline, 96% and 96% resistant to co-trimoxazole, 38% and 41% resistant to tigecycline respectively. All the species of *Acinetobacter* shows 100% susceptibility against colistin.

A local study conducted at General hospital in 2013 by Sohaila et al showed similar results. In that study 90 isolates were included and 66% *Acinetobacter baumannii* were resistant to meropenem and piperacillin-tazobactam, 50% resistant to ciprofloxacin as compared to our study in which it is 64% resistant. Out of total, 31% isolates were resistant to tetracycline but in our study it resistance is 38%, 50% resistant to gentamicin but in our study it is 86% resistant, ceftriaxone is 44% resistant but in our study it is 100% resistant. Both of the studies include all types of the clinical specimens. There is a marked increasing resistance of ceftriaxone in our study as compared to Sohaila et al.¹⁷

A study carried out in India in 2014 by Tripathi et al included 107 clinical specimens, 74% cultures were positive from the general wards while 11.96% cultures were positive from the ICU's. In that study 57% isolates of *Acinetobacter species* were resistant to imipenem while in our study it is 74%. Out of total, 55% are resistant to amikacin but in our study it is 71% resistant. *Acinetobacter species* were 100% resistant to Piperacillin-tazobactam while in our study it is 90% resistant to piperacillin-tazobactam. Due to the increase

use of piperacillin-tazobactam *Acinetobacter* is also acquiring the resistance against it.¹⁸

A study carried out in a tertiary care hospital at Dhaka by Azizun Naharet al in 2012 included 95 samples from the critically ill patients of ICU's. *Acinetobacter* species were 100% resistant against co-amoxyclove in that as well as in our study. In that study 68.4% isolates were resistant against amikacin while in our study it is 71%. In that study meropenem is 66.7% resistant against *Acinetobacter* species while in our study it is 74% resistant. Gentamicin according to that study is 100% resistant to *Acinetobacter* species while in our study it is 86% resistant. That study revealed the presence of 54.3% *Acinetobacter* species in tracheal secretions while in our study it is 25%. Our study also discloses the highest rate of presence of *Acinetobacter* species in the tracheal secretions as compared to other specimens.⁴ Another study carried out in India in 2015 by Gitanjali et al included 3298 infected samples, 111 (3.36%) were found to be *Acinetobacter* species while in our study 100 isolates of *Acinetobacter* were included. The most predominant species was *Acinetobacter calcoaceticus*-*A. baumannii* (Acb) complex i.e 72% while in our study it is 73%. High incidence of resistance was recorded for Piperacillin-tazobactam (55%) but in our study piperacillin-tazobactam was resistant to 90% of the isolates, followed by ceftriaxone (46%) and ceftazidime (46%) while in our study it is 100%. The studies supports the fact that *Acinetobacter baumannii* is the most commonly occurring nosocomial pathogen as compared to other species of *Acinetobacter*.²

CONCLUSION

Acinetobacter associated hospital acquired infection are rising with passage of time. Antimicrobial resistance is also rising among *Acinetobacter* species.

Author's Contribution:

Concept & Design of Study:	Fizza Khan
Drafting:	Hafiz Haseeb Afsar, Muneeb Afsar
Data Analysis:	Kokab Jabeen, Farhan Rasheed
Revisiting Critically:	Fizza Khan, Hafiz Haseeb Afsar
Final Approval of version:	Fizza Khan

Conflict of Interest: The study has no conflict of interest to declare by any author.

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