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Frequency and Antimicrobial Susceptibility Profile of *Acinetobacter baumannii* Isolated at a Tertiary Care Diagnostic Facility

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ABSTRACT

Objective: To evaluate the frequency and antimicrobial susceptibility pattern of *Acinetobacter baumannii* isolated at a tertiary care setting.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from July to Dec 2018.

Methodology: All the specimens submitted, which yielded the growth of *Acinetobacter baumannii,* were included in the study. Isolates were confirmed biochemically by using api 20NE. Antimicrobial susceptibility was done using standard procedures and as per CLSI's latest guidelines.

Results: Nine thousand nine hundred thirty-one specimens were submitted to the Microbiology Department AFIP for culture and antimicrobial susceptibility testing during the study period. Amongst these, 1633(16.5%) specimens yielded the growth of different pathogens. Of 1633 culture-positive isolates, 101(6.2%) were identified as *Acinetobacter baumannii.* Among these, 34.6% of the isolates were recovered from respiratory specimens, while 29.7% from pus and pus swab specimens. Minocycline was the most sensitive antibiotic with 59.4% sensitivity, followed by Doxycycline, to which 48.5% of isolates were susceptible. Sensitivity to all other antimicrobials was 24% or less. In vitro, sensitivity to Carbapenems is very low.

Conclusion: *Acinetobacter baumannii* was isolated from 6.2% of specimens submitted for culture and susceptibility testing. Minocycline was the most sensitive antibiotic, followed by Doxycycline. Sensitivity to most of the other antimicrobials was 24% or less. An alarming resistant pattern demands strict infection control practices and judicious use of antibiotics.

Keywords: *Acinetobacter bauamnnii*, Antimicrobial susceptibility, Multi-drug resistance,

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INTRODUCTION

Microorganisms included in the genus Acinetobacter are non-fermentative and non-motile. On gram staining, they appear as gram-negative *coccobacilli*.[1] They are catalase-positive while oxidase-negative. Among all the *Acinetobacter species*, *Acinetobacter baumannii* (Ab) is the most common cause of infections and outbreaks.[2] Once, they were considered opportunistic pathogens, but in recent years, they have emerged as an important nosocomial pathogen over.[3] *A. baumannii* is attaining special importance in clinical settings and is reportedly responsible for up to 20% of infections in intensive care units around the globe.[4] Acinetobacter is also one of the six ESKAPE" patho-gens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa,* and *Enterobacter species*) to express that they escape the lethal action of antibiotics.[5]

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This emerging superbug is also moving towards resistance against a large variety of currently used antimicrobial agents and is becoming a real treatment challenge for treating physicians.[6] Multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) is becoming a significant healthcare-associated pathogen, and its global reporting is rising.[7] *A. baumannii* is usually very resistant to the effects of most chemical disinfectants and detergents, ultraviolet radiations and dehydration. These properties enable these MDR pathogens to be extremely hard to eradicate from many hospital settings like intensive therapeutic care units and many catheter-related devices.[8] It is said that no particular procedures exist for removing A. *baumannii* from hospital environments, and the use of routine antimicrobial methods only inhibits their growth. These factors increase the risk of infection, especially for the patients in intensive care units by these antibiotic-resistant A. baumannii.[9,10]

This microorganism's changing susceptibility profile and increasing multi-drug resistance patterns demand constant surveillance in all healthcare settings.

The rationale of this very important experimental study is to record the epidemiology and antimicrobial susceptibility pattern of Acinetobacter bauamnnii in our geographical location and look for any changes in the antimicrobial resistance of this superbug by comparing it with previous studies. This study will also help suggest antimicrobials for empirical treatment based on the antibiogram of the pathogen responsible for causing many infections.

METHODOLOGY:

The cross sectional study was conducted at the Department of Microbiology Armed Forces Institute of Pathology, Rawalpindi Pakistan, from July to December 2018 after IERB Approval letter. The sampling technique was non-probability consecutive sampling.

Inclusion Criteria: All clinical specimens from I ndoor and outdoor patients, which yielded the growth of *Acinetobacter baumannii*,were included.

Exclusion Criteria: Contaminated and duplicate specimens from the same patients were excluded from the study.

A total of 99 *Acinetobacter baumannii* were isolated from different clinical specimens. Details like hospital identity number, age, gender of the patients, type and place of specimen submission were recorded. All the specimens were inoculated on blood, MacConkey, chocolate, CLED and anaerobic agars (one or more types of media were used depending upon the type of specimens) as soon as these were received in the department of microbiology and incubated at 35-37^{0}C for 24 to 48 hours. Direct microscopy from the Gram-stained smears was performed, and any evidence of Gram-negative pathogen in the smear was recorded. Culture plates were evaluated for evidence of growth after 24 and 48 hours of incubation. Any non-fermenting growth on MacConkey agar was provisionally identified by colony morphology noted on culture plates and then by microscopy of a Gram-stained smear of the colony. Further identification of *Acinetobacter baumannii* was made with the help of rapid tests like catalase, oxidase and biochemical reactions on analytical profile index (API) 20 NE.

Antimicrobial susceptibility of the isolate was carried out on Mueller-Hinton (MH) agar (Oxoid, UK) by modified Kirby Bauer disc diffusion technique by inoculating with the test organism (0.5 McFarland standards) to get a semi-confluent growth as per recommendations of Clinical and Laboratory Standards Institute (CLSI).11 Appropriate antibiotic discs were applied to this MH agar. After overnight incubation at 35^{o}C ± 2, zone diameters were measured and interpreted per CLSI guidelines. Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

Nine thousand nine hundred thirty-one specimens were submitted to the microbiology department for culture and drug susceptibility testing during the study period. Amongst these, 1633(16.5%) specimens showed growth of different microorganisms. Of these culture-positive isolates, 101(6.2%) were identified as *Acinetobacter baumannii*. Among the clinical specimens which yielded the growth of *Acinetobacter baumannii,* 66(65.3%) specimens were from male patients, while 35(34.6%) were from female patients. Patients' ages varied from as young as newborn babies to as old as 89 years.

Almost all types of specimens revealed the growth of *Acinetobacter baumannii*. Most of the isolates (34.6%) were recovered from respiratory specimens (including naso-bronchial lavage, sputum, and endo-bronchial washings), followed by pus and pus swab specimens (29.7%) (Figure).

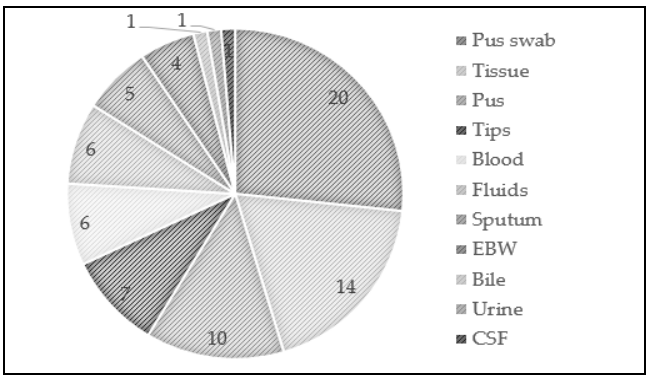

Figure: Frequency of Acinetobacter baumannii in Different Culture Specimens (n=101).

Antibiotic susceptibility testing of nine different antibiotics against *Acinetobacter baumannii* revealed that Doxycycline was the most sensitive antibiotic; 48.5% of the tested isolates were sensitive. Sensitivity to most of all other antimicrobials was 15% or less. The sensitivity of different drugs is shown in Table.

Table: Antimicrobial susceptibility profile of *Acinetobacter baumannii* (n=101).

Antibiotic	No. of Sensitive isolate	Percentage
Minocycline	60	59.4
Trimethoprim-sulphamethoxazole	16	15.8
Imipenem	12	11.8
Meropenem	6	5.9
Doxycycline	49	48.5
Amikacin	13	12.8
Gentamicin	24	23.7
Ciprofloxacin	4	3.9
Tazobactum-Piperacilin	3	2.9

DISCUSSION

Cases of multidrug-resistant *Acinetobacter baumannii* have been reported worldwide for quite some time now. Infections and especially outbreaks of MDR Acinetobacter are being regularly and constantly reported among patients admitted in the burn unit, intensive care unit (ICU) and those with central intravenous catheters and respiratory devices.[11] Many outbreaks have been reported and documented in ICUs, burns units and NICUs. [7-13] Such infections by this superbug have also been encountered in our setup for quite some time now. An outbreak was investigated in 2010 by our department, in which *Acinetobacter baumannii* was finally isolated as the cause of infection being spread by an infected ambu bag, which resulted in the death of two patients.[14] Similarly, a study was conducted in our department in which all the *Acinetobacter baumannii* isolated from pus and pus swab specimens were evaluated, and their antimicrobial susceptibility patterns were noted. 15 This superbug's Incidence of infection has risen for the last few decades now.[16]

The frequency of isolation of *Acinetobacter baumannii* from different clinical specimens varies in different studies. It will likely depend on geographical location, study groups, etc. In our study, around two-thirds of the isolates were from respiratory specimens (including sputum, nasotracheal lavage and endobronchial washings) and pus (including pus swab) specimens. In a similar study conducted in Lahore, Pakistan, in 2012, half of the Acinetobacter were isolated from pus specimens, followed by blood and urine.[17] In another study conducted in Katmandu, Nepal, 35% of the isolates were from sputum only, followed by urine, pus and pus swab specimens.[18] In a study conducted in Switzerland in 2018, most of the carbapenem-resistant Acinetobacter were isolated from blood cultures.[19] Similarly, many other studies have shown that the isolation of this organism from different clinical specimens varied among different studies and geographical locations.

Most of the *Acinetobacter baumannii* in our study were multi-drug resistant, which is the usual pattern of this organism worldwide. Similar results have been reported from other studies, like a study from India that reported 87% MDR isolates.[20] Minocycline is the most sensitive antibiotic in our study (59.4%), followed by Doxycycline (48.5%). Carbapenem resistance is a problem worldwide in these organisms. In our study, it too was quite high; rather, it has further reduced from 10-5% in the case of Meropenem. Similar Carbapenem resistance patterns have been reported in other studies conducted in Pakistan, India, and other countries. Even in Europe and America, high levels of carbapenem resistance are being reported. Resistance patterns of other antibiotics also remained very high in our study. Similar low susceptibility patterns are also documented worldwide for this pathogen.

In general, *Acinetobacter baumannii* is becoming more and more resistant daily. The antibiotic susceptibility profile of this organism should be regularly monitored, and laboratories worldwide should share their susceptibility patterns regularly so that appropriate measures can be taken on time. One major limitation of our study was that it included data over a short time. In addition, it was a purely laboratory-based study, and no correlation of the antibiotics used to treat patients with these infections was available.

CONCLUSION

Acinetobacter baumannii was isolated from 6.2% of specimens submitted for culture and susceptibility testing. Minocycline was the most sensitive antibiotic, followed by Doxycycline. Sensitivity to most of the other antimicrobials was 24% or less. An alarming resistant pattern demands strict infection control practices and judicious use of antibiotics.

Authors Contribution:

Following authors have made substantial contributions to the manuscript as under:

1,2: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

3,4: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

5,6: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of

any part of the work are appropriately investigated and resolved.

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