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Original Article

In Vitro Efficacy of Tetracyclines Against Multi Drug Resistant *Acinetobacter Baumannii* Amongst Intensive Care Patients

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ABSTRACT

Objective: To determine the anti-microbial susceptibility and frequency of *A.baumannii*, especially focusing the efficacy of Doxycycline and Minocycline against multi drug resistant (MDR) and extremely drug resist ant (XDR) *A.baumannii* in a tertiary care hospital.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Study conducted at the department of Pathology, Armed Forces Institute of Cardiology/ National Institute of Heart Diseases (AFIC/NIHD), Rawalpindi Pakistan, from Jan 2020 to Dec 2021.

Methodology: Data was collected by non-probability consecutive sampling. Antimicrobial susceptibility was assessed for *A.baumanni* isolated from hospital setting.

Results: A total of 6239 culture specimens were received from different wards of AFIC. Out of these 1439 (23%) yielded growth of different pathogens. Amongst 1439 positive cultures, 167 (11.6%) were *A.baumannii*. Multi drug resistant (MDR) *A.baumannii* were 9(5.4%), extremely drug resistant (XDR) 152(91%) and pan drug resistant (PDR) were 6(3.6%). Antibiotic susceptibility of *A. baumannii* revealed highest resistance for *cephalosporins* and *carbapenems* about 162(97%), ampicillin and beta lactamase inhibitor combinations about 157(94%), quinolones 152(91%), aminoglycosides 134(80%) and Trimethoprim+ sulfamethoxazole 131(78%). The least resistance observed from tetracyclines group of antibiotics which was 34(20%) from Minocycline and 54(32%) from Doxycycline.

Conclusion: A high resistant pattern was observed for *A.baumanni* against *cephalosporins, carbapenems*, ampicillin, beta lactamase inhibitor combinations, quinolones, aminoglycosides and Trimethoprim + sulfamethoxazole. Comparatively tetracycline antimicrobials i.e., minocycline and doxycycline were found to be less resistant than others to manage MDR and XDR cases.

Keywords: *Acinetobacter baumannii*, Antimicrobial susceptibility, Doxycycline, Minocycline, Tetracyclines, Multidrug resistant.

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INTRODUCTION

Antimicrobial resistance (AMR) has become obvious as a noteworthy phenomenon of increasing cost for healthcare facilities due to prolong hospital stay globally. It has contributed to significant morbidity & mortality in recent years.[1] Treatment options are vanishing in life threatening serious infections triggered by opportunistic multidrug resistant organisms.[2] Although the pharmaceutical industry is striving hard to bring new antimicrobials in the market for the last few years, but the resistance seems to be worsening with geometric progression.[3] Results from multicentre studies in the last decades have revealed that both community-acquired and hospital acquired AMR are on rise and posing serious threat to health care system to manage such infections.[1]

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Acinetobacter baumannii (A.baumannii) is an opportunistic nosocomial pathogen and is present in the hospital environment with its ability to survive for prolonged periods.[1] *A.baumannii* is notorious for causing all sort of infections including skin and soft tissue infection, respiratory tract infections, urinary tract infections, septicemia, bacteremia, & meningitis. The MDR and XDR, even the pan-drug resistant (PDR) *A.baumannii* has been isolated from almost all sort of specimens coming to the microbiological laboratories of the hospitals.[4] MDR is defined as "*non-susceptibility to at least one agent in three or more antimicrobial categories*", XDR is defined as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories)" and PDR is defined as "*non-susceptibility to all agents in all antimicrobial groups*".[5]

Mortality rate from septicemia caused by *A.baumannii* infections has been reported to be 34.0–

43.4% in the intensive care settings and 16.3% outside the intensive care settings. The therapeutic drug options for treating infections from highly resistant *A.baumannii* are coupled with significant toxicity of the limited number of antibacterial agents including carbapenems, aminoglycosides, tetracyclines, sulbactam and colistin. Although a lot of research has been going on, no new drug has been developed to treat MDR *A. baumannii* infections after marketing of tigecycline in 2005.[3] WHO has included A.baumannii in critical list of organisms for research and development of new antibiotics.[6]

Doxycycline and minocycline are long-acting broad-spectrum antibiotics and have excellent tissue penetration including bones. Both drugs are metabolized in liver and can be safely used in patients of renal failure for extra renal infections and dose adjustment is not required. These two drugs are active against many Gram positive and Gram negative bacteria. They are also affective against MDR & XDR *A. baumannii.*[7]

Substantial evidence about the emergence of resistant strains of *A.baumannii* around the world and in Pakistani hospitals especially in intensive care units have been reported in literature leading to poor outcome of patients.[7,8] According to the recommendations of Clinical Laboratory Standards Institute (CLSI) it is of utmost need to generate data regarding the resistance pattern/susceptibility of the organisms to combat this notorious pathogen infecting the patients in intensive care units and formulate antibiotic policy to combat this bug.[9]

In view of increase frequency of *A.baumannii,* challenges to manage this bug having high mortality rate, the current study was carried out. Study was aimed to find out frequency and anti microbial susceptibility of *A.baumannii*, especially focusing the efficacy of Doxycycline and Minocycline against MDR and XDR *A.baumannii* in a tertiary care hospital and to see the resistant pattern of different group of antimicrobials against *A.baumannii.*

METHODOLOGY

Study was conducted at the department of Pathology, Armed Forces Institute of Cardiology/ National Institute of Heart Disease (AFIC/NIHD), Rawalpindi Pakistan, from January 2020 to December 2021. This was a descriptive cross sectional study in which non probability consecutive sampling technique was adopted for sample collection.

Sample Size: By considering the 11.2% prevelence of *A.baumannii*[10] in clinical samples, the calculated sample size was n=153 but all the specimens were included in the study making n=6239 culture specimens.

Inclusion Criteria: All types of clinical specimens requested for culture and sensitivity from patients admitted in adult and pediatric intensive care units (ICUs) & high dependency units (HDUs) were incorporated in the study. All patient samples were dealt with standard microbiological laboratory practices and only samples yielding the growth of *A.baumannii* were included in the study.

Exclusion Criteria: Duplicate samples and contaminated specimens were excluded from the study.

Demographic details like hospital registration number, name, age, and gender of the patient were noted on a proforma.Permission from the Institutional Ethical Review Board was taken (IERB No. 3/22) and identity of patients were kept confidential. No discrimination based on age and gender was made. Identification of the pathogen was made by colony morphology, Gram staining, use of rapid tests like catalase, oxidase and biochemical reactions on analytical profile index (API) 20 NE (Biomerieux, France). Antimicrobial susceptibility testing (AST) was carried out on Mueller Hinton agar (Oxoid, UK) using modified Kirby-Bauer disk diffusion technique as per recommendations of Clinical and Laboratory Standards Institute (CLSI) 2020 & 2021.[11,12] Agar dilution method was used for colistin susceptibility testing which was confirmed by VITEK-2 system (Biomerieux, France). ATCC control strain of *A.baumannii* was used as control. Tigecycline (TGC) testing was done according to the breaking point by the European Committee for Antimicrobial Susceptibility Testing (EUCAST).[13] Disks of different drugs used for AST were Minocycline (MIN) 30µg, Doxycycline (DOX) 30µg, Amikacin (AK) 30µg, Gentamicin (CN) 10µg, Ciprofloxacin (CIP) 5µg, Levofloxacin (LEV) 5µg, Trimethoprim+ sulfamethoxazole (SXT) 25µg, Ceftazidime (CAZ) 30µg, Cefipime (FEP) 30µg, Ceftriaxone (CRO) 30µg, Imipenem (IMP) 10µg, Mero-penem (MEM) 10µg, Ampicillin+ sulbactam (SAM) 20µg, Piperacillin+ Tazobactam (TZP) 110µg. Statistical analysis was done by using IBM SPSS version 21.

RESULTS

A total of n=6239 culture specimens were received from different wards of this Cardiology

Institute, out of these 1439(23%) yielded the growth of different pathogens. Among 1439 positive cultures, 167(11.6%) were *A.baumannii*.

Out of these 167 *A.baumannii* 130(77.8%) specimens were from male patients while 37(22.2%) were from female patients. Age of patients range from 1 month to 80 years and mean age was 44.66 years. Among them 22% of patients were below the age of 3 years and majority (54.5%) of patients were between the age of 45 to 65 years. Distribution of specimens given in Table-I reflects 104 (62%) samples were from respiratory system and wound sites followed by blood culture and CVP line samples.

Table-I: Type of specimens received for culture & sensitivity

Specimen Source	Number of Specimens (n)	Percentage (%)
Respiratory samples	50	30
Blood	26	15.6
CVP Tip	24	14.4
Pus & Pus Swab	54	32.3
Others	13	7.7
Total	167	100

Antibiotic susceptibility of *A.baumannii* revealed highest resistance from cephalosporins and carbapenems followed by ampicillin/beta lactamase inhibitor combinations and quinolones, all range from 91 to 98%. Resistance to aminoglycosides were 134(80%), Trimethoprim+sulfamethoxazole 131(78%), & Tigecycline 124(74%). The reduced resistance was observed from tetracyclines group of antibiotics which was 34(20%) from Minocycline and 54(32%) from Doxycycline. The least resistance was noted from Colistin 6(3.6%). Detail of drugs used along with resistance pattern for AST is shown Figure-1 & 2 for number of isolates and their percentage respectively.

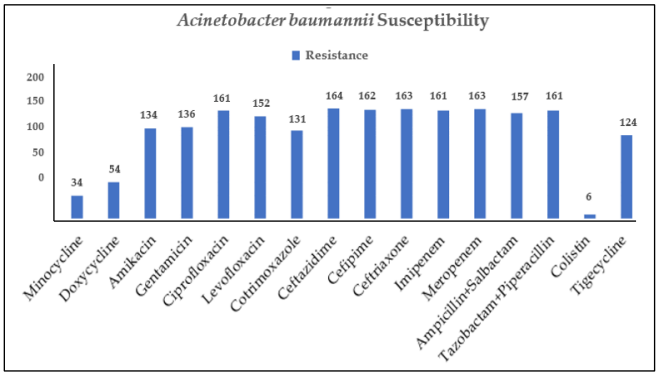

Figure-1: Antibiogram of *A. Baumannii* isolated from different clinical specimens

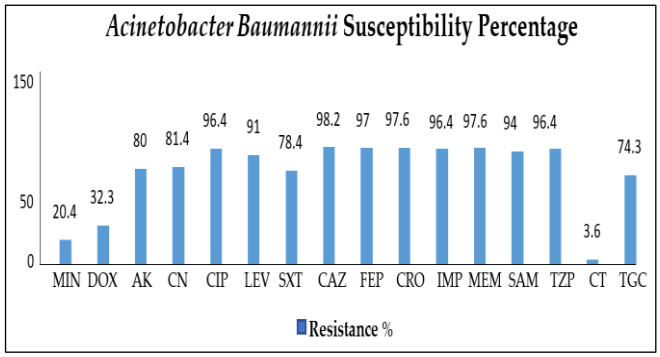

Figure-2: Resistance (%) of *A. baumannii* isolated from different clinical specimens Minocycline (MIN), Doxycycline (DOX), Amikacin (AK), Gentamicin (CN), Ciprofloxacin (CIP), Levofloxacin (LEV), Trimethoprim+sulfamethoxazole (SXT), Ceftazidime (CAZ), Cefipime (FEP), Ceftriaxone (CRO), Imipenem (IMP), Meropenem (MEM), Ampicillin+ sulbactam (SAM), Piperacillin+Tazobactam (TZP).

Distribution of *A.baumannii* based on resistance was such that 9(5.4%) were MDR, 152(91%) were XDR and 6(3.6%) were PDR. Minocycline and Doxycycline revealed much better efficacy against this superbug as compared to other 3rd or 4th generation cephalosporins, and carbapenems routinely used in ICUs for suspected infections.

DISCUSSION

Globally antibiotic resistance is a major threat to health sectors, it affects everyone irrespective of age, gender, or geographical condition. Although it is a natural phenomenon but injudicious use of antibiotics results in high resistance and increased fatality. Antibiotic resistance caused frequent hospitalization, prolong hospital stay, high medical cost, increased mortality and this over-burdened the healthcare system.[5]

Acinetobacter species is the major concern nowdays, *A.baumannii* is a gram negative bacteria that belongs to Moraxellaceae family which predominantly causes nosocomial infections. It is responsible for infections such as; urinary tract infections, bacteremia, meningitis, skin/wound and gastrointestinal infections, ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP).[14]

A.baumannii is one of the ESKAPE organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A.baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp.*), that is the reason of global threat to human health and a therapeutic challenge due to emerging and constantly increasing resistance. Carbapenem resistant *A.baumannii* (CRAB) was ranked by WHO as

number one priority for antibiotic research and development in 2018.[1]

According to Lashinsky *et al*. most of the *A.baumannii* strains were isolated from respiratory tract 16(73%), 4(18%) from soft tissues, and 2(9%) from the bone. There were no bloodstream infections reported by them.[15] While our study depicted more percentage of samples from respiratory system and wound sites followed by blood culture and CVP line samples. Most of the studies shows variation of *A.baumannii* prevalence from pus/wound samples i.e. 11.7–27.5%.[16] One of the study reported the isolates of *A.baumannii* from pus swabs were 2.46%, while our study reported 32.3% isolates from pus/wound samples. The disparity of *A.baumannii* prevalence is primarily due to the difference in identification method specifically when conventional procedures for identification were practiced. [16] The capability of *A.baumannii* to remain in healthcare setups for a longer period and its ability to persist for extensive times above the surfaces allows it to be a repeated and frequent reason of hospital acquired infections that results in multiple outbursts.[16,17]

Previous study reported the resistance of *A.baumannii* isolated strains to many drugs including; 100% by ampicillin & sulbactam, 83.82% by Piperacillin &tazobactam, 99.51% by ceftazidime, 85.78% by amikacin, 93.63% by gentamicin, 93.14% by cotrimoxazole and 97.55% by ciprofloxacin.[12] Same findings were reported by our study that *A.baumannii* revealed highest resistance of above 90% from cephalosporins, carbapenems, ampicillin & beta lactamase inhibitor combinations and quinolones.

The results of another study reported that the resistance rate of *A.baumannii* isolates is 96.1% by Piperacillin & tazobactam, 96.1% by ceftazidime, 88.8% by amikacin, 83% by gentamicin, 89.8% by cotrimoxazole and 96.6% by ciprofloxacin. It is found that most of the *A.baumannii* strains are susceptible to polymyxin-B, colistin & tigecycline.[18] This high resistance of Acinetobacter species against antimicrobials demands the other sources of treatment like homeopathy, herbal drugs, phage therapy and medicinal plants. Antimicrobial drugs like carbapenem, polymyxin & tetracycline were thought to be the most active choice of drugs against *A.baumannii* in the past decade.[19] Another study suggests that tigecycline and colistin are considered to be the last possible choice of drug so their findings necessitates the researchers and doctors to take cautionary measures to have favorable results.[19] The antimicrobial resistance of Acinetobacter species (490) isolated from 11 different European coun-tries during 1997–2000 showed the resistance against imipenem and meropenem was 16% and 18% respectively.[20] A previous study of 40 different hospitals of European region participated in the monitory program revealed a substantial increase of imipenem 42.5% and meropenem 43.4% resistance.[12] A different study publicized the resistance pattern of A.baumannii during the study period for imipenem from 0% to 42%.[16] All of these results showed an increasing trend toward carbapenem resistance but our findings revealed that it has reached to 96% because of injudicious use.

Over the past decade, the importance of minocycline has increased because of its invitro efficacy and susceptibility pattern against Acinetobacter species (e.g. MDR & XDR), along with its pharmacodynamics & pharmacokinetic properties like its capability to maintain optimum serum levels, tissue levels and to have bactericidal pattern. The resistance pattern of Acinetobacter species is the main area of concern which plays an important role in the field of antimicrobials. The Management of infections caused by MDR and XDR Acinetobacter species are a big concern for physicians as well as for medical microbiologists.[8]

RECOMMENDATIONS

1. Tetracyclines should be considered as first line treatment options in suspected cases amongst ICU settings.
2. Based upon the results extracted from current study, amongst even tetracycline group, minocycline should be preferred over doxycycline.
3. Effective implementation of infection control system is required to control resistant bugs in hospital settings.
4. Unnecessary use of antimicrobials must be prohibited to avoid multi drug resistance.

LIMITATIONS OF STUDY

Molecular confirmation of growth of A.baumannii isolates from clinical specimens and detection of resistant genes were not done for this study due to non-availability of funds. Molecular detection and genomic characterization of pathogens should be done in the succeeding study.

CONCLUSION

A high resistant pattern was observed for *A.baumannii* against cephalosporins, carbapenems, ampicillin, beta lactamase inhibitor combinations, quinolones, aminoglycosides, Trimethoprim+sulfamethoxazole and tigecycline. Comparatively tetracycline group of antimicrobials i.e. minocycline and doxycycline along with colistin were found to be less resistant than others to manage MDR and XDR cases.

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Conflcit of Interest: None.

Author's Contribution:

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

MF:Concept, Manuscript writing, final proof reading.

HK: Editing, article review, referencing

IAM: Editing, article review, proof reading

UM: Manuscript writing, referencing, editing

HZ: Data management, analysis and Result compilation.

WH: Article review, referencing, manuscript writing

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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