Cross-Sectional Study

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Prevalence and antimicrobial susceptibility of *Acinetobacter* spp. in a tertiary care hospital in Peshawar: a cross-sectional study

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Background: *Acinetobacter* spp. have been a primary cause of nosocomial infections worldwide, causing significant morbidity and mortality, especially in Pakistan. The purpose of this study was to investigate the trend of antimicrobial resistance over a 5-year period in a tertiary care hospital in Pakistan.

Methods: A retrospective cross-sectional study regarding the occurrence and antimicrobial resistance of *Acinetobacter* spp. recovered from clinical specimens that were referred to the Pathology Laboratory of Northwest General Hospital, Peshawar. The data from 2014 to 2019 was recorded and analyzed by the laboratory. Sociodemographic characteristics and laboratory record data was analyzed using SPSS, version 25. A chi-square test was applied to see the significance.

Results: Of 59 483 clinical samples, *Acinetobacter baumannii* strains were detected in 114 of them. The majority of the clinical samples were from blood (89.5%) followed by sputum (7.9%), wound swab (1.8%), and bone marrow (0.9%). *A. baumannii* has been found in 52 men (67.53%) and 28 women (75.67%), with an overall risk of 0.669 times. In 76 men (98.70%), sensitivity for ertapenem (99.1), colistin (96.49), and tigecycline (78.9%) were also observed which indicated the potential viability of these drugs to treat multidrug-resistant (MDR) *Acinetobacter* infections. The male-to-female risk ratio was 0.98 for colistin and 0.71 for amikacin.

Conclusion: Increased frequency of MDR supports the need for continuous surveillance to determine the prevalence and evolution of MDR *Acinetobacter* spp. in Pakistan. Colistin, tigecyclines, and ertapenem remain the possible line of drugs to treat MDR *Acinetobacter*.

Keywords: *A. baumannii* group, *Acinetobacter* spp., antimicrobial resistance, extended-spectrum β-lactamases, minimal inhibitory concentration, multidrug resistance

Introduction

Acinetobacter spp. are ubiquitous, nonfermenting, gram-negative bacteria that are classically known for causing opportunistic infections[1]. However, in recent times these bacteria are emerging as important nosocomial pathogens, mainly in patients with weak immune systems[2]. Patients on respiratory therapy equipment and indwelling catheters are quite susceptible to these pathogens and can have infections such as pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis, and meningitis[3].

Acinetobacter baumannii complex comprises *Acinetobacter nosocomialis*, *Acinetobacter pitti*, and *A. baumannii* which are the most clinically significant species out of the over 50 in the *Acinetobacter* genome, with *A. baumannii*, the deadliest among the species, being responsible for about 90% of the clinical infections caused by *Acinetobacter* spp. in humans[4].

HIGHLIGHTS

- *Acinetobacter* spp. are a big reason of nosocomial infections worldwide, causing significant morbidity and mortality, especially in Pakistan.
- Increased frequency of multidrug resistance (MDR) supports the need for continuous surveillance to determine the prevalence and evolution of MDR.
- Colistin, tigecyclines, and ertapenem remain the possible line of drugs to treat MDR *Acinetobacter*.

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A. baumannii can survive harsh conditions and is resistant to many disinfectants. Moreover, the emergence of strains resistant to a wide range of antimicrobials, including carbapenems, tetracyclines, colistin, and polymyxins, is very astonishing and has become an important global medical concern as it makes it difficult to treat infections caused by this pathogen[4,5]. The major mechanisms of *A. baumannii* responsible for resistance to antimicrobial agents are aminoglycoside-modifying enzymes, changes in penicillin-binding proteins production of broad-spectrum β-lactamases, and alterations in outer membrane proteins[2]. Furthermore, decreasing the permeability of the cell membrane to the drug and upregulation of the efflux pump led to an increasing prevalence of MDR *A. baumannii*[4].

This spread of antimicrobial resistance is facilitated by many factors including the presence of mobile genetic elements, overuse of antibiotics, poor infection control practices, and increased international travel[6]. According to WHO, carbapenem-nonsusceptible *Acinetobacter* spp. have emerged as the highest-weighted antimicrobial-resistant pathogens and have led to severe morbidities in ICU patients[4]. A study done in Brazil emphasized that extrahospital sources play an important role in the increased prevalence of MDR *Acinetobacter* spp. in the hospital setting[7].

Life-threatening infections caused by *A. baumannii* include meningitis, ventilator-associated pneumonia, bacteremia, wound, and urinary tract infections. *Acinetobacter* spp. can develop antibiotic resistance very quickly as a result of extensive evolutionary exposure to soil microbes that generate antibiotics. The adaptable creature uses a range of energy and carbon sources. These characteristics explain why *Acinetobacter* spp. may survive in the hospital environment in both damp and dry circumstances, aiding in transmission. *A. baumannii* treatment has been shown to be unsuccessful with a number of antimicrobial drugs, including penicillins, cephalosporins, tetracyclines, aminoglycosides, and quinolones due to the development of resistance determinants.

Many studies have shown that the rates of prevalence of MDR strains of *A. baumannii* are increasing in South Asia, the Arabian Peninsula, and many other parts of the world[6]. In Pakistan, the prevalence of infections caused by drug-resistant *Acinetobacter* spp. is increasing which is not only limiting the treatment options but also causing an increased economic burden on both the patient and the state. Currently, limited studies have been done in Pakistan to determine the incidence and drug resistance of the *Acinetobacter* spp. More research needs to be done to develop innovative strategies for the prevention and appropriate treatment of infections caused by the *Acinetobacter* spp. We have, therefore, conducted our study to check the prevalence and antimicrobial susceptibility of *Acinetobacter* spp. in the hospital setting of Pakistan.

Methodology

Study design

A retrospective cross-sectional study was conducted to determine the prevalence and antimicrobial susceptibility of *Acinetobacter* spp. among clinical specimens that were referred to the Pathology Laboratory of Northwest General Hospital, Peshawar.

Study period and area

The data from 2014 to 2019 was recorded and analyzed by the laboratory. The clinical specimens were collected from Northwest General Hospital, Peshawar.

Identification and sampling

On blood agar and McConkey agar, all of the acquired samples were cultivated aerobically in the laboratory. Blood samples were grown in trypticase soy broth and then transferred to blood agar and chocolate agar for further development. Gram staining and colony morphology, two common microbiological techniques, were used to identify and describe the isolates.

In addition, the API 20 E kit (Biomeriuex) was employed for identification. By using the traditional Kirby–Bauer disk diffusion method, the antimicrobial susceptibility of all the isolates was evaluated. Samples were prepared for culture using traditional, accepted techniques, and susceptibility testing was carried out using Kirby–Bauer's disk diffusion method. Following the recommendations of the Clinical and Laboratory Standards Institute (CLSI), antibiotics of the appropriate strength were utilized.

Inclusion and exclusion criteria

Acinetobacter spp. and other microbiological specimens isolated from all ages were referred to the hospital laboratory during the study interval. The samples which had incomplete information about patients or the antimicrobial susceptibility report that did not meet the criteria of laboratory guidelines were excluded from the study.

Data extraction method

A standardized questionnaire was used to collect clinical information about patients and the antibiotics used for treatment. For the *Acinetobacter* spp., antimicrobial susceptibility was also recorded. The complete data was, thereafter, entered into SPSS, version 25.

Statistical analysis

The data was clinically analyzed using SPSS, version 25. Chi-square test and cross-tabulation were used to compare the trends of antimicrobial resistance and prevalence of *Acinetobacter* spp. among patients admitted to Northwest General Hospital.

Ethical consideration

The study was conducted after approval from the Ethical Review Committee of Northwest General Hospital, Peshawar Ref No: NwGH/EC/06, dated 11/04/2020.

Data quality assurance

Standards of media preparations were maintained throughout the study. Quality assurance of media, reagents, and antibiotics was ascertained and Standard Operative Procedures given by the laboratory were closely followed.

Our study is fully compliant with the STROBE 2016 guidelines[8]. A complete STROBE 2016 checklist has been provided as a Supplementary File (Supplemental Digital Content 1, http://links.lww.com/MS9/A56). Our study has been registered on

Table 1

Test conducted for sensitivity check of *Acinetobacter*

Test name	*n* (%)
Blood for C/S	102 (89.5)
Sputum for C/S	9 (7.9)
Wound swab for C/S	2 (1.8)
Bone marrow C/S	1 (0.9)
Total	114 (100.0)

C/S, culture and sensitivity.

Research Registry with the following UIN: researchregistry8033[9]. Our study is in accordance with the Declaration of Helsinki.

Results

The goal of this study was to determine the prevalence of MDR *Acinetobacter* spp. in a tertiary care hospital of Peshawar including their antimicrobial susceptibility (Table 1). For this study, 59 483 samples were collected from 2014 to 2019, 33 689 of which showed no growth and 25 794 of which contained microorganisms, 114 of which tested positive for *Acinetobacter*. There were 77 males and 37 females in our study. In our sample size, the age of the patients was from 6 to 90 years with a mean age of 48.03 ($n = 114$, SD = 19.246), as shown in Figure 1.

Overall, 52 (67.53%) males and 28 (75.67%) females have been diagnosed with *A. baumannii* having a risk of 0.669 times with a 95% CI of 0.275–1.628, as shown in Table 2.

Meropenem was shown to be sensitive in 19 men (17.75%), but only in seven women (18.91%), with a male-to-female risk ratio of 0.71 [odds ratio (OR) = 0.71, 95% CI = 0.269–1.883].

Ciprofloxacin was shown to be sensitive in 16 men (20.77%), but only in eight women (21.62%), with a male-to-female risk ratio of 1.05 (OR = 1.05, 95% CI = 0.404–2.738). Amikacin was shown to be sensitive in 44 men (57.14%), but only in 18 women (48.64%), with a male-to-female risk ratio of 0.71 (OR = 0.71, 95% CI = 0.323–1.561). Cefpodoxime was shown to be sensitive in 76 men (91.56%), but only in 28 women (90.32%), with a male-to-female risk ratio of 0.98 (OR = 0.71, 95% CI = 0.962–1.013). Colistin was shown to be sensitive in 74 men (96.10%), but only in 36 women (97.29%), with a male-to-female risk ratio of 0.98 (OR = 1.45, 95% CI = 0.147–14.527). Ertapenem was shown to be sensitive in 76 men (98.70%), but only in 37 women (100%), with a male-to-female risk ratio of 0.98 (OR = 0.98, 95% CI = 0.962–1.013), as shown in Table 3.

The sensitivity of co-amauxiclav was determined to be 8.8%, with a significant *P*-value. Geneticin was found to be sensitive in 39 (34.2%) of the patients, while ceftazidime, cotrimaxazole, and trimethoprim were all shown to be sensitive in one (8.8%) of the patients (9.6%). For piperacillin/tazobactam, and cefotaxim, there was a sensitivity of 10, whereas for imipenem it was 26. (22.8%). There were 112 (98.2%) positive results for cefoperazone, whereas cefpodoxime had 104 (91.23%) positive results, and nalidixic acid had 98 (85.96%) positive results. There were 88.56% of patients with a substantial *P*-value who were responsive to ceftriaxone, as shown in Table 4.

Discussion

In our samples, the prevalence of *A. baumanni* was found to be 70.2%, while 29.8% of patients were infected with other *Acinetobacter* spp. According to our study, 67.5% of males reported *Acinetobacter* infection, compared with 32.5% of females. However, this could be due to the increased number of males reporting in hospitals. Joshi *et al.*[10] reported 50.20% infection in males. In our study, among 114 samples 96.5% were resistant to one or more drugs (Fig. 2).

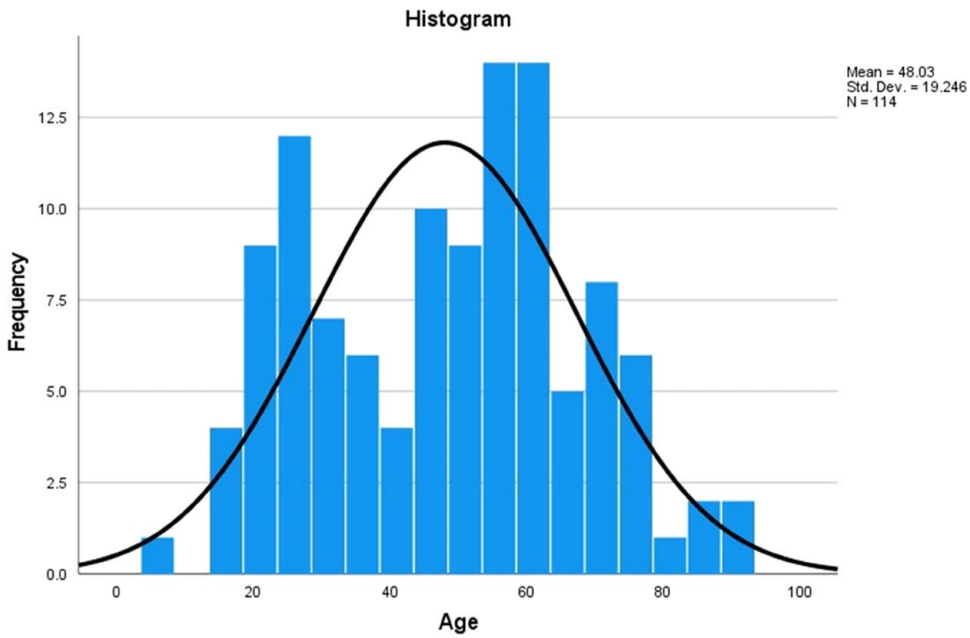

Figure 1. Age demographics of patients. Frequency tabulations and histogram were used to determine year-wise trend of antimicrobial sensitivity which revealed year 2016 had highest frequency of antimicrobial sensitivity (21.9%), followed by year 2017 (18.4%), 2019 (18.4%), 2018 (16.7%), as shown in Figure 2.

Table 2

Odds ratio of sex in comparison to *Acinetobacter baumannii* and other species

Sex	*Acinetobacter baumannii*	*Acinetobacter* spp.	*P*	Odds ratio	95% CI
Male	52	25	0.374	0.669	0.275–1.628
Female	28	9			

One of the mechanisms by which *A. baumanni* develops resistance is the presence of efflux pumps. These pumps cause the leakage of antibiotics and a wide range of substances out of the bacteria, creating MDR. Three systems, AdeFGH: RND, AdeIJK, and AdeABC, have been observed in the *A. baumanii*, among these AdeABC is most involved in the MDR *A. baumanii*[11].

Antimicrobial resistance among *Acinetobacter* spp. has expanded considerably in the previous 10 years and has made a significant predicament for public health. The most powerful drug class against *Acinetobacter* right now accessible are the carbapenems, however, resistant strains have arisen[12]. Despite the fact that carbapenem-resistant strains are expanding, carbapenems (imipenem, meropenem) stay one of the main helpful choices against these infections[12]. Our review uncovered 77.2% of strains were resistant to meropenem, and 77.2% were resistant to tinum (imipenem and cilastatin sodium). An Iranian review reports a resistance percentage of 73.3% to imipenem[13], which is tantamount to our study.

Our study revealed that 91.2% of strains were resistant to fortum (ceftazidime) and claforan (cefotaxime), while 50.9% of stains were resistant to megapime (cefepime). These values indicate a high prevalence of resistance to cephalosporins. In Abdar *et al.*'s[14] study, the resistance to ceftazidime was reported to be 93%, which is near to the present study.

Resistance of *A. baumannii* to fluoroquinolones has been ascribed to changes in the design of DNA gyrase or topoisomerase IV which are brought about by genetic transformations in the gyrA or parC qualities, separately, this leads to the lowered affinity of the drug in enzyme-DNA complex[15,16]. In our study, 78.9% of strains were resistant to ciproxin (ciprofloxacin) which is higher than the study conducted by Spence *et al.*[13] which reports 49.1% were resistant to ciprofloxacin.

We found that 91.2% of strains were resistant to amoxicillin (augmentin, penicillinase sensitive penicillin) which is comparable to a study conducted in Riyadh, Saudi Arabia, whereas 96% of *A. baumannii* were resistant to amoxicillin[17].

Our study revealed 65.8% strains were resistant to gentacin (aminoglycoside), while 45.6% were resistant to amikin (amikacin) which is in line with the study conducted by Ayenew *et al.*[18] that reports below 50% amikacin resistance. Tigecycline (a derivative of tetracyclines) showed 21.1% resistance which is lower than the study conducted by Navon-Venezia *et al.*[19] which reports 66% resistance but higher than the study conducted by Jo *et al.*[20] which reports 8% resistance.

Our study also revealed the sensitivity of *Acinetobacter* spp. to different drugs. The highest sensitivity was observed in ertapenem (99.1%). Ertapenem has potent in vitro activity against a broad spectrum of bacterial pathogens including gram-negative enteric-producing extended-spectrum β-lactamases and/or AmpC-type β-lactamases[21]. Further research and investigations are needed to verify the efficiency of ertapenem against *Acinetobacter* spp.

Our study revealed 96.49% of strains were sensitive to colistin. Colistin has become one of the major therapeutic options in the management of carbapenem-resistant *A. baumannii* infections. However, colistin resistance has rapidly emerged in *A. baumannii* after the reintroduction of this drug into clinical practice[22].

Table 3

Odds ratio of sex in comparison to various antibiotics

Sex	Resistant	Sensitive	*P*	Odds ratio	CI
Meropenem					
Male	88	19	0.49	0.71	0.269–1.883
Female	30	7			
Ciproxin					
Male	61	16	0.91	1.05	0.404–2.738
Female	29	8			
Amikacin					
Male	33	44	0.39	0.71	0.323–1.561
Female	19	18			
Cefpodoxime					
Male	7	76	0.48	0.98	0.962–1.013
Female	3	28			
Colistin					
Male	3	74	0.74	1.45	0.147–14.527
Female	1	36			
Ertapenem					
Male	1	76	0.48	0.98	0.962–1.013
Female	0	37			

Table 4

Resistance and sensitivity of *Acinetobacter* with various antibiotics

	n (%)		
Antibiotics	Resistant	Sensitive	*P* in association with age
Meropenem	88 (77.2)	26 (22.8)	0.156
Co-amoxiclav	104 (91.2)	10 (8.8)	0.00
Ciprofloxacin	90 (78.9)	24 (21.1)	0.32
Geneticin	75 (65.8)	39 (34.2)	0.00
Ceftazidime	104 (91.2)	1 (8.8)	0.00
Cotrimaxazole	104 (91.2)	10 (8.8)	0.00
Trimethoprim	103 (90.4)	11 (9.6)	0.30
Piperacillin/tazobactam	104 (91.2)	10 (8.8)	0.00
Cefotaxime	104 (91.2)	10 (8.8)	0.00
Imipenem	88 (77.2)	26 (22.8)	0.00
Amikacin	52 (45.6)	62(54.4)	0.44
Tigecycline	24 (21.1)	90 (78.9)	0.37
Cefepime and tazobactum	58 (50.9)	56 (49.1)	0.49
Cefoperazone/sulbactam	2 (1.8)	112 (98.2)	0.00
Cefpodoxime	10 (8.77)	104 (91.23)	0.00
Nalidixic acid	16 (14.04)	98 (85.96)	0.00
Ceftriaxone	26 (25.44)	88 (74.56)	0.00
Colistin	4 (3.51)	110 (96.49)	0.30
Ertapenem	1(0.9)	113 (99.1)	0.99

Limitations

The lack of molecular studies (genotyping) and the calculation of MIC values is a major limitation of this work. It was not possible to conduct a genetic investigation of the resistant phenotype or the mechanism of drug resistance. As a result, a prospective

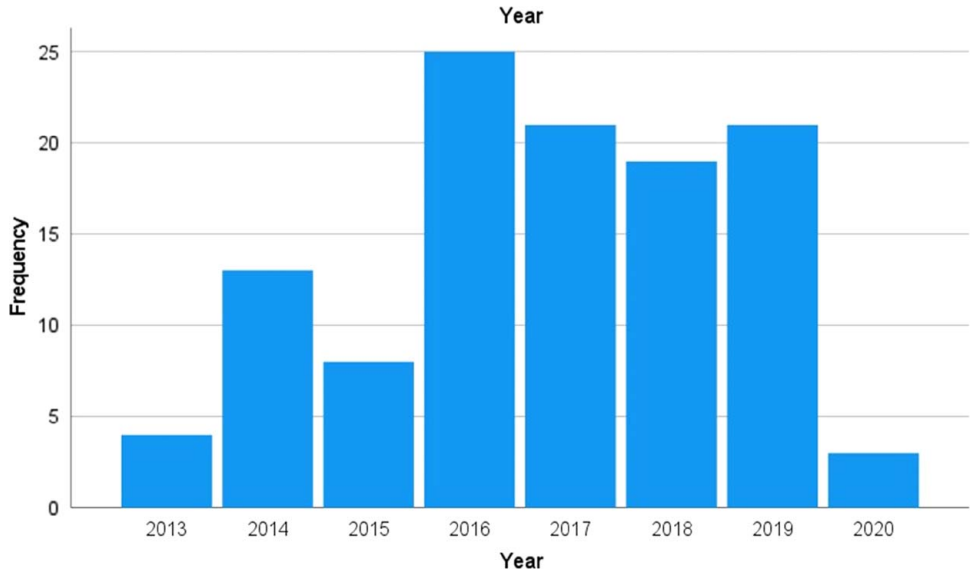

Figure 2. Year-wise trend of *Acinetobacter* sensitivity. Relevant clinical specimens were used to isolate *Acinetobacter* among which 98.5% species were isolated from blood, 7.9% from sputum, 1.8% from wound swab, and 0.9% from marrow cultures, as shown in Table 1.

investigation to assess the minimal inhibitory concentration values and genotyping of *A. baumannii* strains is advised to discover widespread drug-resistant clones in Pakistan. Detection of resistant strains of *Acinetobacter* spp. limited the study's scope. Further investigation into the relevant patient characteristics, such as whether the infection was contracted in the hospital, was also not possible due to the lack of complete clinical information.

Conclusions

This study showed that the frequency and rate of MDR *Acinetobacter* infections is high in our hospitals which could lead to its limited therapeutic options. Furthermore, *Acinetobacter* infections will continue to be a therapeutic challenge in our hospitals and healthcare facilities due to the increasing proportion of *Acinetobacter* spp. with MDR characteristics and resistance to potent antibiotics. Continuous monitoring and appropriate infection prevention and control programs need to be enhanced to avoid the spread of these pathogens in healthcare facilities. An extensive surveillance program is required to understand the origin and extent of *baumannii*. Research efforts should focus on the molecular basis and the discovery of new therapies for resistant strains. Our study showed that ertapenem, colistin, and tigecyclines were the most effective drugs against *A. baumannii*. Furthermore, to reduce the spread of MDR *Acinetobacter*, strict control of the hospital environment, hand hygiene, and optimization of antibiotic use are recommended.

Ethical approval

The study was conducted after approval from the Ethical Review Committee of Northwest General Hospital, Peshawar Ref No: NwGH/EC/06, Dated 11-042020

Consent

The informed consent from the patients was obtained considering Helsinki's Declaration.

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Author contribution

F.K., N.S., and W.K.: substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. A.N., N.N., W.A., and I.A.: drafting the work or revising it critically for important intellectual content. H.M.: Final approval of the version to be published. S.A., M.S.A.K., and M.H.S.: agreement 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.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

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