ORIGINAL RESEARCH

Epidemiology and Multidrug Resistance of *Pseudomonas aeruginosa* and *Acinetobacter baumanni* Isolated from Clinical Samples in Ethiopia

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Background: *A. baumannii* and *P. aeruginosa* are important nosocomial pathogens in health-care settings. Both are intrinsically resistant to many drugs and are able to become resistant to the virtually most antimicrobial agents. An increasing prevalence of infections caused by multidrug-resistant isolates has been reported in many countries.

Methods: An institutional-based cross-sectional five-year retrospective study was conducted to assess the antimicrobial resistance trend of *P. aeruginosa* and *A. baumani*. 893 *A. baumani* and 729 *P. aeruginosa* isolates were included in the study. Conventional method was used for identification and antimicrobial susceptibility was determined by Kirby-Bauer disc-diffusion method. The isolates were from suspected bloodstream infections, wound infections, urinary tract, or surgical site nosocomial infections. Socio-demographic and other variables of interest were collected using a structured check list from a patient record data. Data were analyzed using SPSS version 26 software. P value <0.05 was considered statistically significant.

Results: A total of 1622 *A. baumanii* and *P. aeruginosa* were isolated from various clinical specimens recorded from the year 2017–2021. Out of which *A. baumanni* was 893 (60.6%) and *P. aeruginosa* was 729 (39.4%). Blood was the major source of the isolates (18.3%), followed by urine (16%), and tracheal aspirate (10.6%). Antimicrobial resistance among *A. baumanni* over the five years were; ampicillin (86% to 92%), ceftriaxone (66.7% to 82.2%), and ciprofloxacin (58.5% to 66.7%). In *P. aeruginosa* a significant increase in resistance was seen from 2017 to 2021 to Amoxicillin-clavulanate (74.1% to 84.2%), chloramphenicol (62% to 81.9%), and gentamicin (40% to 44.8%).

Conclusion: A five-year antimicrobial resistance trend analysis of *A. baumanni* and *P. aeruginosa* showed increasing multi drug resistance and resistance to highly potent antimicrobial agents in Ethiopia. It should be addressed with infection control measures, surveillance, and alternative new therapeutic strategies to circumvent the spread of multi-drug resistance.

Keywords: *A. baumanii*, antibiotic resistance, Ethiopia, MDR, *P. aeruginosa*

Introduction

Antimicrobial resistance is quickly gaining global attention, particularly as the number of microbes resistant to currently available antimicrobials rises.[1] It includes both gram-positive and gram-negative bacteria and has a global prevalence rate of 60% or higher.[2,3]

Infections caused by drug-resistant *Acinetobacter baumanii* and *Pseudomonas aeruginosa* species are a growing source of Hospital acquired infection and a major public health concern.[4] Pneumonia, bacteremia, meningitis, urinary tract infection, and wound infection are only a few of the illnesses caused by these bacteria.[5,6] Infections caused by drug-resistant *Acinetobacter spp*. and *Pseudomonas s*pp are linked to prolonged hospital stays and mortality.[7,8]

Due to its extraordinary ability to withstand medicines, eradication of *P. aeruginosa* has become increasingly difficult. Most antibiotics are known to be resistant to *P. aeruginosa* strains due to their high levels of inherent and acquired resistance mechanisms, biofilm formation.[9–11] Alternative therapeutic techniques that present unique pathways against *P. aeruginosa* infections are becoming increasingly desired and receiving.[11]

Pseudomonas aeruginosa and *Acinetobacter baumanni* have been a leading cause of nosocomial infections, causing significant morbidity and mortality over the entire world including Ethiopia.[12] Multidrug-resistant *A. baumannii* and *P. aeruginosa* thrive in hospitals, where they can easily spread from patient to patient via health-care personnel's hands.[12] The most important features of these bacteria species are their ability to persist in the hospital environment and rapidly develop resistance to a wide variety of antibiotics due to different mechanisms such as, reduced outer membrane permeability, efflux pump systems, enzymatic inactivation, and biofilm formation.[13–15] As a result, practically all -lactams, aminoglycosides, and quinolones are often resistant to them. Antimicrobial drug usage, drug prescriptions without susceptibility testing, self-medication, and prolonged hospitalization have all been linked to the development of MDR.[11,13] However, data on the prevalence of nosocomial multi drug resistance (MDR) *A. baumanni* and *P. aeruginosa* infections in Ethiopia, in general, and the study area, in particular, is scarce. Therefore, the purpose of this study was to assess the magnitude and AMR pattern of *A. baumanni and P. aeruginosa* from clinical samples in retrospective approach.

Methods

A cross-sectional retrospective analysis was conducted to assess the magnitude and AMR pattern of *A. baumanni* and P. *aeruginosa* from clinical samples that were tested for bacterial presence and subsequent susceptibility testing dated from January 2017 to December 2021, from two large teaching hospital in Ethiopia (Black lion specialized hospital and Yekatit 12 hospital medical college). All hospitals are situated in the city of Addis Ababa, Ethiopia. The clinical information extracted from the microbiology laboratories included type of sample analyzed, name of pathogens isolated and the names of antibiotics used for susceptibility testing and the susceptibility results as recorded in the laboratory report. All information were recorded by the assigned laboratory personnel/data collector and reviewed by senior microbiologist.

Laboratory Analysis, Clinical Samples and Collection of Bacterial Isolates

Several types of clinical specimens, including urine, blood, sputum, pus, tracheal aspirate, wound swab, ear discharge, pleural fluid, and cerebrospinal fluid (CSF) were cultured. Both laboratories sampled for the current study employed similar standard microbiological culturing techniques. All the laboratories performed similar microscopic identification and biochemical identification using the Clinical Laboratory Standard Institute guidelines (CLSI guideline).[16] Antimicrobial susceptibility test was performed by both laboratories using the Kirby-Bauer disk diffusion method in Muller Hinton agar and interpreted using CLSI.[16]

Data Analysis

Collected data was entered into Microsoft Excel and loaded into SPSS, version 26 for analysis. Proportions of predominant isolates, sociodemographic characteristics, and antibiotic resistance profiles were presented using tables and figures. $P < 0.05$ was considered significantly associated among variables.

Results

A total of 1622 patient's data that were isolated from various clinical specimens was recorded from the year 2017–2021 for antimicrobial susceptibility test of *A. baumanni* and p. *aeruginosa*. Out of which 881 (54.3%) were males and 741 (45.7%) were females, whereas the age ranged from 1 to 90 with the mean and median ages of 39.3 years and 37 years, respectively. *A.baumanni* was the predominant isolate 893 (55.05%) than *P. aeruginosa* which was 729 (45%) (Table 1).

In our study, 893 *A. baumanni* and 729 *P. aeruginosa* were analyzed to investigate the trends of antimicrobial resistance. In the analysis, we found that *A. baumanni* had the most prevalence in all the five years ranging from 60.6% to 62.7%, while *P. aeruginosa* ranges was 38.2%, in 2020 *A. baumanni* was 56.4%, and *Pseudomonas aeruginosa* was 43.6% and in 2021 *Acinetobacter* was 62.7%, while *Pseudomonas* was 37.3% (Figure 1).

Table 1 Socio-Demographic and Clinical Characteristics of Study Participants in TASH and Yekatit 12 Hospital Medical College, 2017–2021
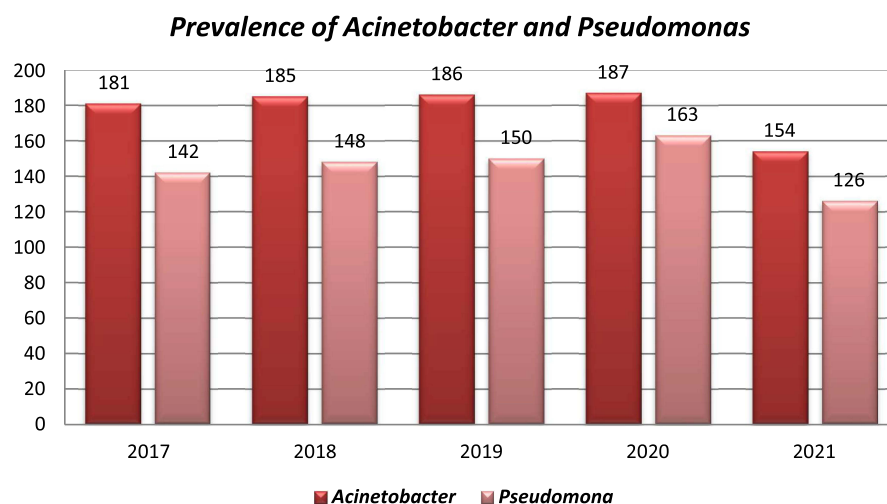
Variables		Frequency	Percent
Sex N=1622	Male	881	54.3
	Female	741	45.7
Age N=1622	<1	236	14.5
	1–14	290	17.8
	15–24	254	15.65
	25–44	343	21.1
	45–65	304	18.7
	>65	195	12
Type of specimen N=1622	Blood	297	18.3
	Urine	261	16
	CSF	161	9.9
	Tracheal aspirate	173	10.6
	Pus	168	10.3
	Sputum	157	9.6
	Wound	154	9.5
	Pleural fluid	125	7.7
	Ear discharge	122	7.5
Isolated Bacteria N=1622	*A. baumanii*	893	55
	P. aeruginosa	729	45

Among the two labs, 9 different types of clinical specimen were processed, but the highest level of the isolates was found in the blood 203 (26.3%), followed by urine 167 (21.6%), tracheal aspirate 79 (10.2%), and pus 74 (9.6%) specimen. *P. aeruginosa* was the predominant isolate in urinary tract infection (25%), sepsis (16.4%), and respiratory infections (13.5%) (Figure 2).
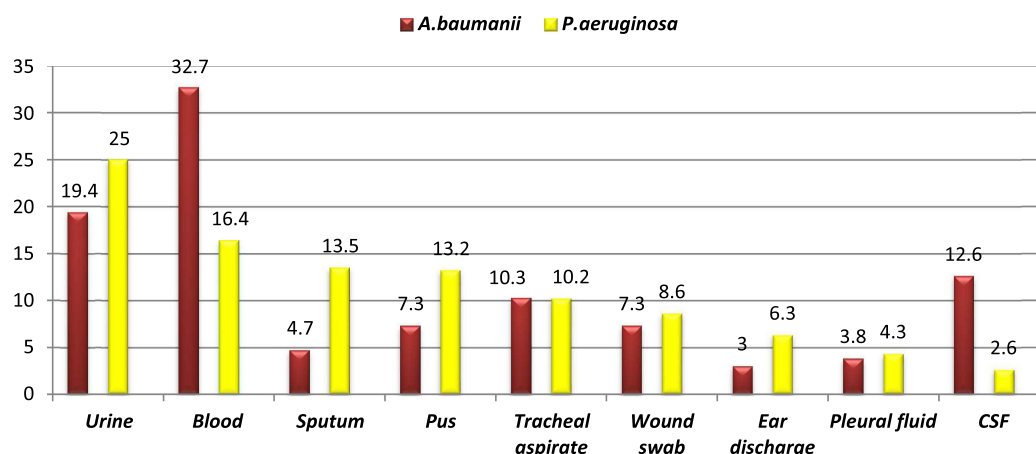
P. aeruginosa resistance to antibiotics over the five years showed that higher resistance to Ampicillin (98%), followed by Amoxicillin-clavulanate (91.3%), and Nitrofurantoin (86%), while it was most sensitive to Tobramycin 68 (39.1%), Cefoxitin, Azithromycin 33.3%, and Amikacin 135 (24.5%).

Most *P. aeruginosa* in these study sites were recovered from urine, followed by blood, sputum, pus and tracheal aspirate. A significant increase in resistance was seen in Amoxicillin-clavulanic acid (94.1% to 100%), Chloramphenicol (88.9% to 100%), Gentamicin (40% to 44.8%), Cefepime (31% to 90.9%), P. tazobactam (22.2% to 26.7%), Meropenem (20.7% to 38.9%), Cefotaxime (71.4% to 100%), Ciprofloxacin (18.5% to 25.5%), Tobramycin (42.9% to 33.3%), Ceftazidime (30.2% to 16.7%), Ceftriaxone (76.5% to 66.7%), Amikacin (9.4% to 9.2%) showed decrease in resistance pattern (Figure 3).

An increasing trend of antimicrobial resistance for *A. baumanni* was observed in the five years ampicillin (86% to 92%), followed by Amoxicillin-clavulanate (81% to 84.7%), chloramphenicol (76% to 82.3%), and Cefotaxime (66.7% to 82.2%). However, a decreasing trend of resistance was observed for tobramycin (61.1% to 25.2%), followed by Tazobactam (84.6% to 63.2%), and Amikacin (31.6% to 26.2%) (Figure 4).

Prevalence of Acinetobacter and Pseudomonas

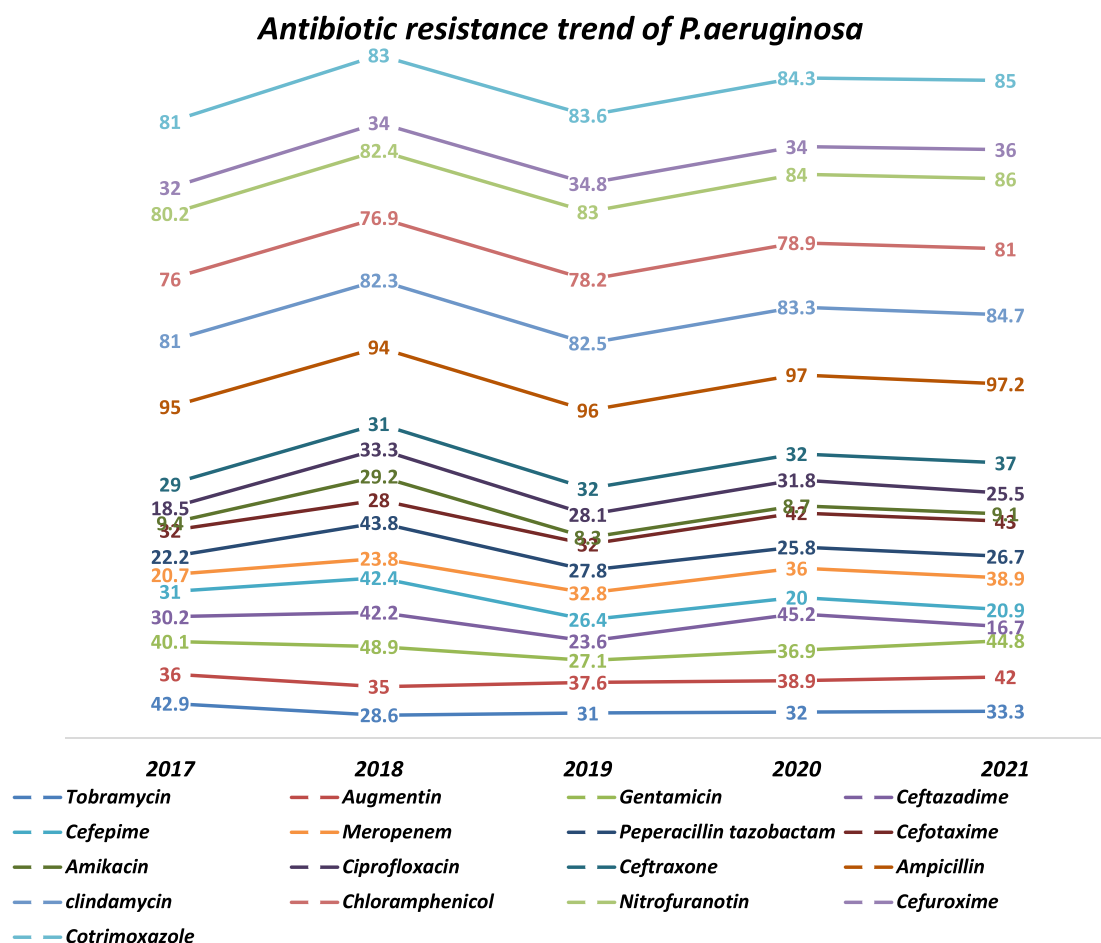Figure 1 Distribution of *A. bauumanni* and *P. aeruginosa* from 2017 to 2021.

Figure 2 Distribution of *P. aeruginosa* and *A. baumanii* across clinical specimens from 2017 to 2021.

Overall, 73.7% of *A. baumanni* and 58.9% of *P. aeruginosa* were multidrug resistant. 9.7% of *A. baumanni* isolates showed resistance to greater than nine antibiotics while 6.7% *P. aeruginosa* were resistant to more than nine antibiotics (Table 2).

Discussion

The nightmare of the rising numbers of multidrug-resistant organisms (MDROs) requires the implementation of effective stewardship programs.[11,17] However, this should be preceded by making available evidence-based knowledge regarding the local antimicrobial resistance pattern, which is fundamental. The excessive use of antibiotics has led to a vast widespread prevalence of antimicrobial resistance.[2] As time passes, bacterial pathogens will defy every antibacterial option, thus, becoming extremely hard to control. Hence, the WHO identified it as an international health prime concern.[13,18]

In our study, 893 *A. baumanni* and 729 *P. aeruginosa* were analyzed to investigate the trends of antimicrobial resistance. In the analysis we found that *A. baumanni* had the most prevalence in all the five years. The prevalence of *A. baumanni* in 2017 was 60.6%, while *P. aeruginosa* was 39.4%, and in 2021 *A. baumanni* was 62.7%, while *P. aeruginosa* was 37.3%. The difference regarding type and frequency of pathogens could be linked to several factors

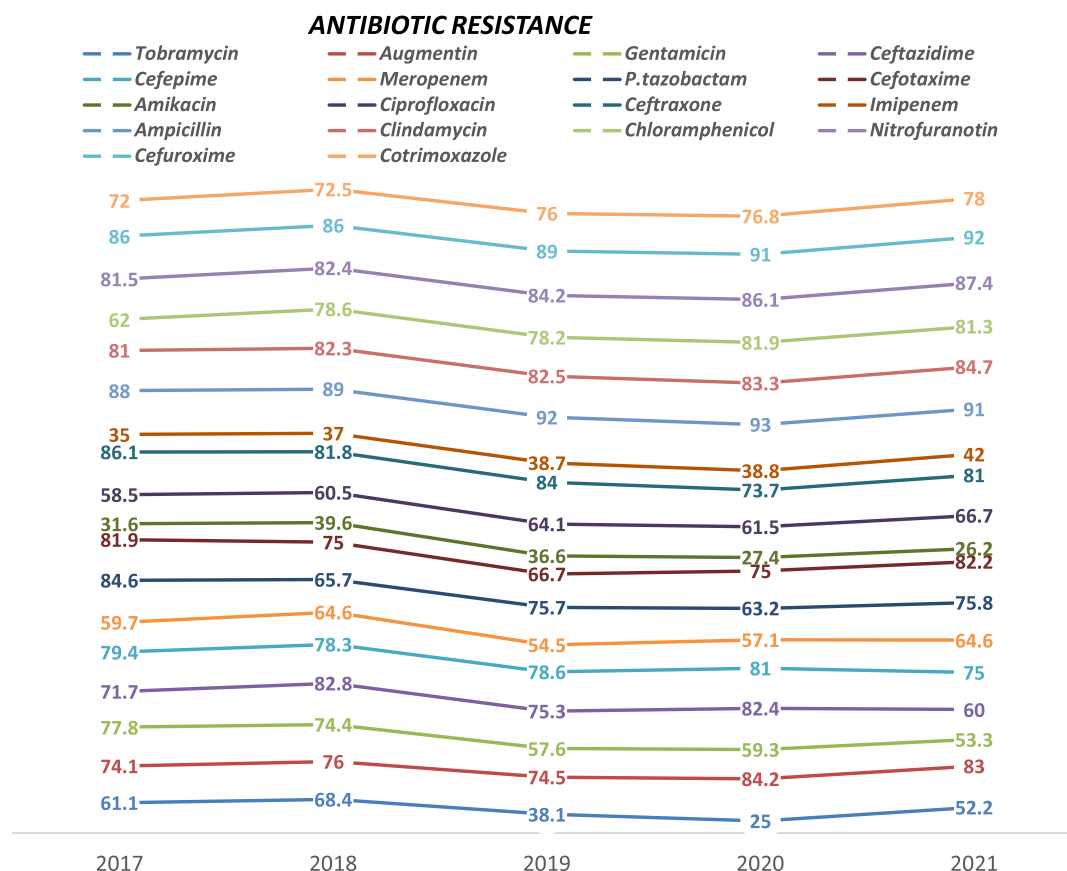## Antibiotic resistance trend of P.aeruginosa

Figure 3 Trend of antimicrobial resistance pattern for *Pseudomonas aeruginosa*.

like environmental conditions, health practices, patient conditions, personal hygiene, number of patients involved in each study, and laboratory procedures.[19]

In our study, the highest prevalence of infections due to *P. aeruginosa* was observed in the Urinary tract (25%) specimens followed by blood (16.4%), and sputum (13.5%). De Francesco et al, and Yayn et al reported that most of the isolates of *P. aeruginosa* were obtained from the respiratory tract followed by urinary tract, wound and blood[3,20] While the highest prevalence infection due to *A. baumanni* was observed from blood (32.7%), urinary tract (19.4%) followed by CSF (12.6%) and different studies also reported the isolation of *A. baumanni* from these clinical specimens. However, the current finding was lower than the study conducted in Dessie which isolated *A. baumanni* from wound specimen at a rate of 51.4% and in Iran at the rate of 56.7% which is higher than this study.[10,12]

In this study, the multidrug resistance prevalence showed that 73.7% of *A. baumanni* and 58.9% of *P. aeruginosa* were multidrug resistant. The highest percentage of resistance among *A. baumanni* were exhibited towards Ampicillin that showed the most resistant 147 (98%) followed by Amoxicillin-clavulanic acid 146 (91.3%), Nitrofurantoin 33 (86%), and Ceftriaxone 160 (82.5%), while Amikacin being the most sensitive 135 (24.5%). Resistance in *P. aeruginosa* and *A. baumannii* to broad-spectrum antimicrobial agents in hospital settings is now an emerging issue worldwide.[21] Surveillance studies indicate that the rates of resistance to antimicrobial agents among *A. baumannii* are increasing in Mexico,[22] the Arabian Peninsula and in many parts of the globe.[23,24] While in *P. aeruginosa*, out of the drugs tested for the resistance pattern Ampicillin, Amoxicillin-clavulanate, chloramphenicol, Cefotaxime and Ceftriaxone had shown higher resistance pattern. However, these isolates showed the maximum sensitivity pattern for tobramycin (61.1% to 25.2%), followed by tazobactam (84.6% to 63.2%), and amikacin (31.6% to 26.2%). Compared to a cross-sectional study conducted at Ethiopian public health institute (EPHI) in 2021 the *Pseudomonas* species were highly resistant to amikacin

ANTIBIOTIC RESISTANCE

- Tobramycin
- Cefepime
- Amikacin
- Ampicillin
- Cefuroxime
- Augmentin
- Meropenem
- Ciprofloxacin
- Clindamycin
- Cotrimoxazole
- Gentamicin
- P.tazobactam
- Ceftraxone
- Chloramphenicol
- Ceftazidime
- Cefotaxime
- Imipenem
- Nitrofuranotin

Figure 4 Trend of antimicrobial resistance of *A. baumanni* from 2017 to 2021, Ethiopia.

in our study (9.1%), Ceftazidime was lower in our study (16.7%) compared to the study by Tesfa et al which was 35%. Most of the findings in different countries confirmed that *pseudomonas* was becoming increasingly resistant to different types of antimicrobial agents.[25] In Ethiopia, a high prevalence of carbapenem-resistant *Acinetobacter* spp. was reported from one previous phenotypic study by Ayenew et al,[26] which is comparable to the prevalence of carbapenem resistance in this study. However, a systematic review in Africa indicates *P. aeruginosa* and *A. baumannii* showed that the lowest prevalence of carbapenemase-producing *A. baumannii* (4.7%).[27] However, most available studies including a study for hospital environment[28] reported higher prevalence of the MDR *P. aeruginosa* and *A. baumannii* in Ethiopia, calling for the application of genotypic methods to studies on mechanisms of resistance and spread.

The trend analysis in antimicrobial resistant from 2017 to 2021 showed that an increasing pattern from year to year for both *A. baumanni* and *P. aeruginosa*. Antibiotic policies and infection control measures are considered of immense value in fighting the mounting trends of nosocomial incidents. In addition, genuine

Table 2 MDR Pattern of *P. Aeruginosa* and *A. Baumanni* Isolates from Clinical Isolates 2017–2021, Ethiopia

Bacteria Species	Antibiogram								
	R1	R2	R3	R4	R5	R6	R7	R8	>R9
A. baumanii	135	97	107	98	91	102	84	80	87
P. aeruginosa	208	89	91	66	63	59	53	49	49

Abbreviations: R1, resistance to one class of antibiotic; R1, resistance to two antibiotics; R3, resistance to three class of antibiotics; >R9, Resistance to more than nine class of antibiotics.

efforts are needed to develop new antimicrobial agents against these pathogens and to monitor the efficacy of the presently accessible drugs.[29,30] For instance, hand hygiene has been found to have impact in controlling infection. Infections due to drug-resistant gram-negative rods are an emerging risk factor for increased mortality in ICU. A number of studies have indicated the rising trends of pathogens from other body systems and hospital wards.[19,29–31]

Compared to the previous years, carbapenem resistance in both *P. aeruginosa* and *A.baumanni* has increased significantly in 2021 and this is comparable with previous studies conducted in Africa and Europe.[25,32–34] The trend analysis in antimicrobial resistance showed that in the year 2021 there was high resistant trait in Ampicillin, Chloramphenicol, Cefepime, Gentamycin, which is comparable with similar study in Saudi Arabia and this could be due to pan drug abuse, intrinsic resistance, acquired resistance and other environmental factors.[17,23,35]

A study conducted at *Kathmandu* university hospital in Nepal in 2020, *Pseudomonas spp* was resistant to ciprofloxacin and Ceftazidime while ours was 31.8% for Ciprofloxacin and 45.2% for Ceftazidime. In the same study 94.4% of *Acinetobacter* were resistant to Ciprofloxacin, 88.89% were resistant to Piperacillin-tazobactam while our result was 61.5% for Ciprofloxacin and 63.2% for Piperacillin-tazobactam.[36] The difference between our study and other studies could be linked to several factor-like environmental conditions, health practices, patient conditions, personal hygiene, drug abuse and number of patients involved.[31,37,38]

This study carries some limitations as the retrospective nature of it cannot eliminate the risk of selection bias and did not allow to make a better correlation between timing, type, and duration of empirical antimicrobial therapy as reported risk factors and the exact correlation between resistance and clinical outcomes. For the same reason, we could not verify the exact mechanism of resistance of *A. baumannii* and *P.aeruginosa*.

Conclusion

The majority of the pathogens in the studied hospital have evolved resistance to most of the antibiotics. In conclusion, there has been an increasing trend of antimicrobial resistance in *A. baumanni* and *P. aeruginosa*. Both species remain a therapeutic challenge in hospitals and health-care setting due to the increasing rate of with traits of MDR and resistance to high potent antimicrobial agents. Continuous surveillance and appropriate infection prevention and control program needs to be strengthened to circumvent the spread of these pathogens in health-care facilities.

Data Sharing Statement

The data sets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Declaration: Ethics Approval and Consent to Participate

Ethical clearance for this study was provided by Addis Ababa University, College of health science, department of medical Laboratory ethical review committee DRERC/479/21/MLS. Participants' study data were recorded in codes and were kept private and confidentially. As this was a retrospective study informed consent was not collected from study participants but a permission was obtained from the hospitals to collect and access the laboratory logbook and it was also in accordance to Helsinki II declaration.

Consent for Publication

Not applicable as details like; videos or images related to study subjects were not recorded for this study.

Disclosure

The authors listed above declare that there is no competing interest.

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