BMC Infectious Diseases

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Antimicrobial resistance profile, biofilm forming capacity and associated factors of multidrug resistance in *Pseudomonas aeruginosa* among patients admitted at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College in Addis Ababa, Ethiopia

Matifan Dereje Olana[1,2,3*], Daniel Asrat[2] and Göte Swedberg[3]

Abstract

Background *Pseudomonas aeruginosa* is one of the leading causes of nosocomial infections and the most common multidrug-resistant pathogen. This study aimed to determine antimicrobial resistance patterns, biofilm-forming capacity, and associated factors of multidrug resistance in *P. aeruginosa* isolates at two hospitals in Addis Ababa, Ethiopia.

Methods A cross-sectional study was conducted from August 2022 to August 2023 at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital Medical College. Culture and identification of *P. aeruginosa* were done using standard microbiological methods. An antimicrobial susceptibility test was done by Kirby-Bauer disk diffusion according to CLSI recommendations. The microtiter plate assay method was used to determine biofilm-forming capacity. SPSS version 25 was used for data analysis. Bivariate and multivariable logistic regression were used to assess factors associated with multidrug resistance in *P. aeruginosa*. The Spearman correlation coefficient (rs = 0.266)) was performed to evaluate the relationship between biofilm formation and drug resistance.

Results The overall prevalence of *P. aeruginosa* was 19.6%. High levels of resistance were observed for ciprofloxacin (51.8%), ceftazidime (50.6%), and cefepime (48.2%). The level of multidrug-resistance was 56.6%. The isolates showed better susceptibility to ceftazidime-avibactam (95.2%) and imipenem (79.5%). Overall, 95.2% of *P. aeruginosa* were biofilm-producing isolates, and 27.7% and 39.8% of isolates were strong and moderate biofilm producers, respectively. A positive correlation and statistically significant relationship was observed between resistance to multiple drugs and

*Correspondence:
Matifan Dereje Olana
maathy4@gmail.com

Full list of author information is available at the end of the article

the level of biofilm formation (rs = 0.266; p-value = 0.015). Previous history of exposure to ciprofloxacin (OR, 5.1; CI, 1.12–24.7, p-value, 0.032) was identified as an independent associated factor for multidrug resistance in *P. aeruginosa*.

Conclusion The present study indicates an association between multidrug resistance in *P. aeruginosa* and its biofilm formation capabilities. Additionally, over half of the isolates were resistant to multiple drugs, with prior use of ciprofloxacin linked to the development of multidrug-resistance. These findings suggest that antibiotic stewardship programs in hospital settings may be beneficial in addressing resistance.

Keywords *P. aeruginosa*, Biofilm, Multidrug-resistance, Associated factors, Ethiopia

Background

Pseudomonas aeruginosa (*P. aeruginosa*) is an aerobic, non-fermentative, Gram-Negative bacterium that causes acute and chronic infections, especially in immunocompromised patients [1]. It can cause a variety of hospital-acquired infections (HAIs), including bloodstream infections (BSI), wound infections (WI), urinary tract infections (UTI), and pneumonia [2]. *P. aeruginosa* is one of the main causes of HAIs globally and belong to the ESKAPE pathogens, which also include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *and Enterobacter* spp [3]. The World Health Organization (WHO) has indicated *P. aeruginosa* among priority pathogens, with significant intrinsic resistance and a broad potential to develop multi-drug resistance, and constitute a profound threat to global health that urgently requires new antibiotics [4]. *P. aeruginosa* is intrinsically resistant to many antibiotics, including ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, ertapenem, cefotaxime, ceftriaxone, tigecycline, trimethoprim, trimethoprim-sulfamethoxazole, and chloramphenicol [5, 6].

Drug resistance in *P. aeruginosa* involves several mechanisms, including intrinsic, acquired, and adaptive resistance [6]. Innate resistance is due to an overexpressed efflux pump, low outer membrane permeability, and the production of antibiotic-inactivating enzymes [7]. Acquired resistance is caused by mutational changes and the acquisition of resistance genes by horizontal gene transfer [6, 7]. The adaptive resistance in *P. aeruginosa* involves the formation of biofilm that serves as a diffusion barrier to limit antibiotic access to the bacterial cells [6].

According to a meta-analysis study of the antimicrobial resistance profile in Ethiopia, high multidrug-resistance (MDR) was found in clinical isolates of *P. aeruginosa* [8]. There have also been additional studies that demonstrate rising antibiotic resistance in *P. aeruginosa* [9]. *P. aeruginosa* is a common pathogen in hospital settings because of its innate resistance to several antibiotics, its capacity to develop further resistance mechanisms, and its ability to form biofilm as a survival strategy in harsh environments [10]. Biofilms are microbial communities that are enclosed by the self-secreted extracellular polysaccharide matrix and provide a barrier against the host immune system and impair antimicrobial drug penetration [11]. Although prior research has demonstrated a high MDR in Ethiopia, the biofilm-forming capacity and its association with drug resistance and related factors of MDR have not been thoroughly investigated. Therefore, this study aimed to determine the antimicrobial resistance pattern, biofilm-forming capacity, and its association with drug resistance and associated factors of MDR in *P. aeruginosa* isolates at Tikur Anbessa Specialized Hospital (TASH) and Yekatit-12 Hospital Medical College (Y12HMC), Addis Ababa, Ethiopia.

Materials and methods

Study design, setting and period

A cross-sectional study was conducted in TASH and Y12HMC from August 2022 to August 2023. TASH is the largest teaching hospital in Ethiopia. Y12HMC is one of the hospitals in the Addis Ababa City Administration and has been giving routine health services for Addis Ababa as well as referral cases from different regional states of the country. TASH was selected because it represents Ethiopia's healthcare landscape, receiving patients from various regions. Y12HMC was chosen due to its dedicated burn center, where *P. aeruginosa* is a key bacterial isolate associated with burn patients.

Study population and sample size

All patients admitted to TASH and Y12HMC during the study period were a source of population for sampling. The sample size was calculated by employing a single population proportion formula by taking prevalence (P=0.5) [12], using a 95% confidence interval with a 5% margin of error and a 10% non-response rate, the total sample size was 422.

$N = z\ 2\ p\ (1\text{-}p)/D^2 = (1.96)\ 2\ *0.5(1\text{-}0.5)/(0.05)^2 = 384 + 10\% = 422$

Following that, hospitals were given a proportionate share of the sample size based on average monthly admission. TASH has 282 admissions and Y12HMC has 214 admissions. The proportion of admissions at each hospital serves as the basis for the sample distribution.

TASH: (282/496)*422 = 240 and Y12HMC: (214/496)*422 = 182

Therefore, in order to maintain the proportionate representation of each hospital's admissions, 240 samples were assigned to TASH and 182 samples to Y12HMC.

Patients of all ages admitted for more than 48 h during the study period and diagnosed with suspected UTI, BSI, and WI, who volunteered to participate and were able to give clinical samples were included in the study.

Sample collection

A total of 90 blood samples, 10 ml from adult patients and 1–5 ml from children with suspected BSI, were collected aseptically and put into a blood culture bottle containing Tryptic Soy Broth (TSB) (Oxoid, England). Inoculated broth was incubated aerobically at 35–37 °C for seven days and inspected for turbidity daily [13, 14].

A total of 140 five-ml urine samples were collected aseptically from catheterized (n=98) and midstream urine from non-catheterized (n=42) patients suspected of UTI.

A total of 192 wound swab samples were collected from patients suspected of infected burn wounds and surgical site wound infections. A sterile cotton wool swab was used to collect swabs from the depths of infected sites. The swab was placed in a bottle with normal saline and then inoculated on appropriate culture media [15].

Culture and identification of *P. aeruginosa*

Culture and identification were done using conventional standard microbiological methods. Blood cultures that showed turbidity were subcultured on blood agar and MacConkey agar (BioMérieux, Craponne, France). Urine and wound swab samples were directly inoculated on blood agar and MacConkey agar. All plates were incubated at 35–37 °C for 16–48 h for bacterial growth. Further identification at the species level was done through biochemical tests including oxidase, catalase, triple sugar iron, and growth at 42 °C [13]. Urine cultures from non-catheterized patients that grew $\geq 10^5$ CFU per ml of urine and from catheterized patients that grew $\geq 10^2$ CFU/ml were taken as significant bacteriuria that indicates UTI [16]. All *P. aeruginosa* isolated were kept in STGG media (skim milk-tryptone-glucose-glycerin) at -80°C for further molecular characterizations.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method for eleven antibiotics, by the 2021 Clinical and Laboratory Standards Institute (CLSI) guidelines [5], with discs from Oxoid: Imipenem (IMI, 10 µg), Meropenem (MEM, 10 µg), Gentamicin (GM, 10 µg), Netilmicin (NET, 30 µg), Ciprofloxacin (CIP, 5 µg), Levofloxacin (LEV, 5 µg), Ceftazidime (CAZ, 30 µg), Cefepime (CPM, 30 µg), Piperacillin-Tazobactam (PTZ, 100/10µg), Ceftazidime-avibactam (CZA, 30/20µg), and aztreonam (ATM, 30 µg) [5]. *P. aeruginosa* was categorized as MDR, Drug resistant (DR), and susceptible isolates based on the criteria described by Magiorakos et al. [17]. DR *P. aeruginosa* is defined as non-susceptibility to more than one antimicrobial agent in less than three antimicrobial categories. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [17].

Biofilm formation assay

A microtiter plate assay was employed for the determination of biofilm formation as described previously [18]. The overnight cultures of tested isolates in Mueller-Hinton broth were adjusted to match a 0.5 McFarland standard. Then, 180 µl of TSB (Oxoid) and 20 µl of bacterial suspension were added into a flat bottom 96-well polystyrene microtiter plate to a final volume of 200 µl. The plate was incubated overnight at 37ºC for 24 h. Following overnight incubation, planktonic cells were washed off with water; the plate was dried and stained with a 1% crystal violet for 15 min. Afterwards, 250 µl of 33% acetic acid was added to each well, and the biofilm formation was quantified by measuring optical density (OD) at 595 nm. Biofilm assays were performed in triplicates, the mean absorbance was determined, and the OD cut-off value was calculated as described previously [19].

Quality control

Several QC procedures were followed to ensure the quality control of experiments used in this study. The media were prepared in accordance with the manufacturer's instructions, and non-inoculated plates were incubated at 37 °C for 24 to 48 h to verify sterility. The pH of media was checked to make sure it was within the suggested range. *Staphylococcus aureus* (ATCC 25923) was used to evaluate the performance of Blood Agar and catalase test. *Escherichia coli* (ATCC 25922) was used to confirm the appropriate differentiation of fermentation on MacConkey Agar. *P. aeruginosa* (ATCC 27853) was used to evaluate performance of oxidase test and triple Sugar Iron (TSI) Agar test. *P. aeruginosa* (ATCC 27853) was used as the positive control in the biofilm formation assay, and sterile media without bacteria was added as a negative control to confirm that there was no contamination. To guarantee consistency of results, all tests were conducted in triplicate, the inoculum density was standardized to a 0.5 McFarland standard, and plates were incubated at 37 °C for 16–18 h to allow for optimal bacterial growth.

Statistical analysis

The data was prepared using a Microsoft Office Excel sheet and imported to SPSS version 25 for analysis. Descriptive statistics were used to describe relevant variables. Categorical variables were expressed in terms of

Table 1 Socio-demographic characteristics of patients at TASH and Y12HMC, Addis Ababa, Ethiopia

Variables	Categories	Frequency (no. %)
Age in years	0–15	98(23)
	16–40	188(44.1)
	41–60	74(17.4)
	> 60	62(14.6)
Sex	Male	252(59.7)
	Female	170(40.3)
Residence	Urban	268(63.5)
	Rural	154(36.5)
Hospitals	TASH	240(56.9)
	Y12HMC	182(43.1)
Hospital stay time	3–10 days	182(43.1)
	11–20 days	113(26.7)
	21–30 days	52(12.3)
	> 30 days	75(17.8)
Clinical samples	Blood	90(21.3)
	Urine	140(33.2)
	Wound	192(45.5)

Table 2 Characteristics of patients with positive culture for *P. aeruginosa* at TASH and Y12HMC, Addis Ababa, Ethiopia

Variables	Categories	Frequency (no. %)
Age in years	0–15	21(25.3)
	16–40	36(43.4)
	41–60	13(15.7)
	> 60	13(15.7)
Sex	Male	52(62.7)
	Female	31(37.3)
Residence	Urban	56(67.5)
	Rural	27(32.5)
Hospitals	TASH	47(56.6)
	Y12HMC	36(43.4)
History of Hospitalization	Yes	39(47.0)
	No	44(53%)
History Surgery	Yes	11(13.3
	No	72(86.7)
History of invasive medical procedure usages	Tracheostomy	11(13.3)
	Urinary Catheter	19(22.9)
	Indwelling gastric tube	4(4.8)
	None	49(59.0)
Hospital stay time	3–10 days	36(43.4)
	11–20 days	22(26.5)
	21–30 days	10(12)
	> 30 days	15(18.1)

frequency and percentage. The bivariate and multivariable logistic regression analyses were used to identify the associated factors of multidrug resistance. Variables with $p<0.25$ were taken as a candidate for multivariable logistic regression on bivariate. Then, those with $p<0.05$ on multivariable logistic regression were considered statistically significant risk factors of multidrug resistance. Spearman correlation (rs) was performed to evaluate the relationship between biofilm formations and drug resistance.

Results

Socio-demographic data

During the study period, 422 study participants were included, with 252 (59.7%) male and 170 (40.3%) female. Out of 422 study participants, 268 (63.5%) were from urban areas, 188 (44.1%) were from the age group 16–40 years, 240 (56.9%) were from TASH, and the majority of patients, 182 (43.1%), had hospital stay time between 3 and 10 days (Table 1).

Pseudomonas aeruginosa isolates

Out of 422 clinical samples processed, 83 (19.6%) *P. aeruginosa* isolates were identified. Among the 83 *P. aeruginosa* isolates, 47 (56.6%) were from TASH, 52 (62.7%) were from male and 36 (43.4%), were from age group 16 to 40 years old (Table 2).

All BSI were from TASH, and all burn wound infections were from Y12HMC. The distribution of infections among hospitals is shown in Fig. 1.

Antibiotic susceptibility patterns of *P. aeruginosa*

Out of 83 *P. aeruginosa*, 69 (83.1%) were resistant to at least one of the tested drugs. The highest level of resistance of *P. aeruginosa* was observed against ciprofloxacin (51.8%), followed by ceftazidime (50.6%), and cefepime (48.2%). A low level of resistance was observed to ceftazidime-avibactam (4.8%). The resistance levels of *P. aeruginosa* to various antibiotics are shown in Table 3.

Among the 83 isolates tested, 14 (16.9%) were sensitive to all antibiotics, 22 (26.5%) isolates were DR isolates and 47(56.6%) were MDR isolates.

Biofilm profiles and association with drug resistance

Out of 83 *P. aeruginosa* isolates tested for biofilm formation, 79 (95.2%) were biofilm producers (strong, moderate or weak) and four (4.8%) were non-biofilm-producing isolates (Fig. 2).

The Spearman correlation coefficient was calculated to evaluate the relationship between biofilm formation and drug resistance in *P. aeruginosa*. There was a weak positive correlation between biofilm-forming capacity and antibiotic resistance in *P. aeruginosa*, and the association was statistically significant ($n=83$; rs=0.266; p-value=0.015). The average optical densities of isolates increased from susceptible isolates (0.45) to drug resistance isolates (0.52) and MDR isolates (0.61). The OD value increased with the level of resistance. This indicates that most of the isolates with high OD values were MDR isolates. Sixteen (69.6%) of strong biofilm-producing

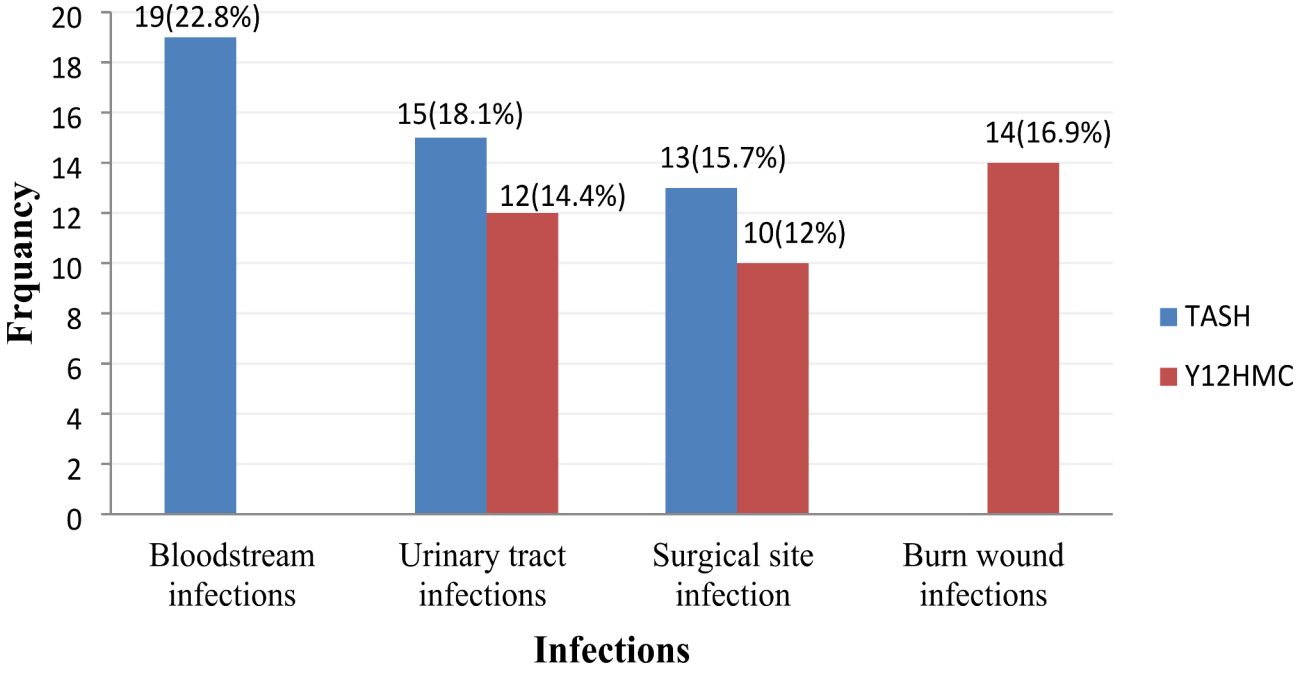

Fig. 1 The distribution of infections among TASH and Y12HMC, Addis Ababa, Ethiopia

Table 3 Antibiotic susceptibility patterns of *P. aeruginosa* isolated from patients at TASH and Y12HMC, Addis Ababa, Ethiopia

Antibiotics	*P. aeruginosa* resistance rate (*n* = 83)		
	R	I	S
MEM	19(22.9)	10(12)	54(65.1)
IMP	14(16.9)	3(3.6)	66(79.5)
CN	36(43.4)	0(0.0)	47(56.6)
NET	22(26.5)	2(2.4)	59(71.1)
CIP	43(51.8)	10(12)	30(36.1)
LEV	31(37.3)	3(3.6)	49(59)
CAZ	42(50.6)	13(15.7)	28(34.9)
FEP	40(48.2)	14(16.9)	28(34.9)
PTZ	19(22.9)	1(1.2)	63(75.9)
CZA	4 (4.8)	0(0.0)	79(95.2)
ATM	35(42.2)	0(0.0)	48(57.8)

MEM: meropenem; IMP: imipenem; CN: gentamicin; NET: netilmicin; CIP: ciprofloxacin; LEV: levofloxacin; CAZ: ceftazidime; FEP: cefepime: PTZ: piperacillin-tazobactam; CZA: ceftazidime-avibactam; ATM: aztreonam; S, susceptible; I, intermediate; R, resistant

isolates and 19 (57.6%) of moderate biofilm-producing isolates were MDR *P. aeruginosa* isolates (Table 4; Figs. 2 and 3).

Analysis of factors associated with MDR in *P. aeruginosa*

Variables in different categories were entered into bivariate and multivariateble logistic regression for the risk analysis of MDR in *P. aeruginosa*. Among the variables, including the 41–60 age group, urban residence, types of antibiotics used in the last three months (cephalosporin, ciprofloxacin, gentamicin, and meropenem), history of invasive medical procedure, and isolates from blood and urine showed significance ($p<0.25$) in bivariate logistic regression and were analyzed together for multivariable logistic regression. In multivariable logistic regression, only one variable, previous usage of ciprofloxacin, was statistically associated with MDR in *P. aeruginosa* (OR: 5.1; 95%CI: 1.12–24.7; p-value: 0.032). Multivariable logistic regression showed that *P. aeruginosa* isolated from those with previous exposure to ciprofloxacin were about five times more likely to develop MDR as compared to those without previous exposure to ciprofloxacin (Table 5).

Discussion

P. aeruginosa is one of the most common gram-negative pathogens associated with nosocomial infections and is one of the major worldwide public health concerns [20]. It is an opportunistic pathogen and causes both acute and chronic infections, especially in individuals with compromised immune systems [21]. It is estimated that *P. aeruginosa* has a prevalence of 7.3–11% of all healthcare-associated infections and is associated with high morbidity and mortality rates [22, 23]. Its mortality rate was reported in the range of 17.9–44.6% in healthcare-associated infections [24–26].

In the present study, the prevalence rate of *P. aeruginosa* isolates was 19.6%, which was relatively consistent with previous studies conducted in Ethiopia (19.3%) [27], Iran (21.3%) [18], China (19.8%) [28], and Pakistan (21.2%) [29]. However, it is slightly higher than other studies from Ethiopia (12.86%) [30], and Italy (13.4%)

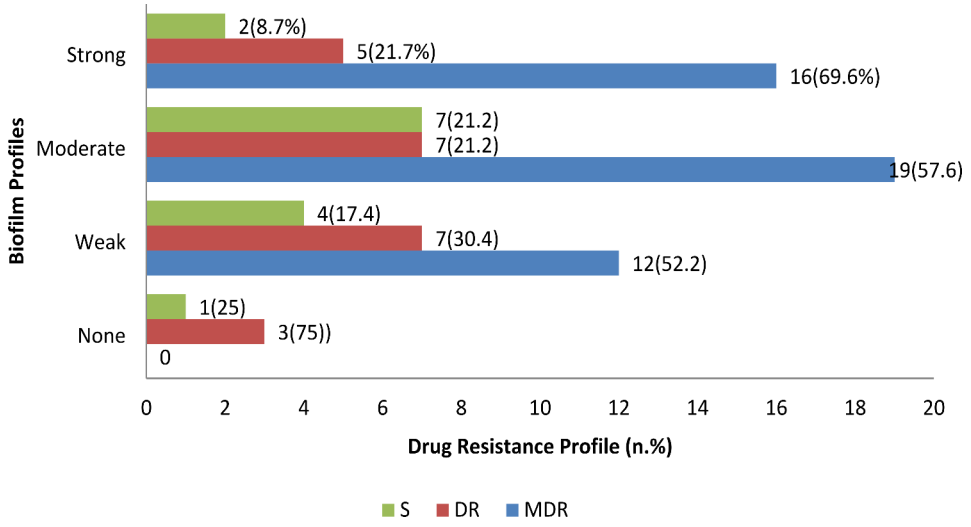

Fig. 2 Distribution of resistance among isolates with different biofilm formation capacities. S, sensitive to all; DR, drug resistant; MDR, multidrug resistant

Table 4 Correlation between biofilm formation capacities and drug resistance profiles of *P. aeruginosa* isolates

Resistance profile	Susceptible isolates	DR-isolates	MDR- isolates	Correlation coefficient	*P*-value
(n. %)	14(16.9)	22(26.5)	47(56.6)	0.266	0.015
OD average	0.45	0.52	0.61		

Optical density (OD) was determined at 595 nm; DR, drug resistant; MDR, multidrug-resistant

[31], but lower than several other reports from Ethiopia (49.3%) [32], Tanzania (24.2%) [33], Egypt (28.3) [34], China (34.5%) [35], Sweden and Norway (25.8–48.9%) [36]. While our methodology aligns with some studies, differences in the prevalence may arise from variations in population characteristics, local healthcare practices, and infection prevention strategies across countries.

P. aeruginosa possesses a high level of intrinsic resistance to multiple antibiotics, and the emergence of MDR strains has become a significant public health problem globally [37]. In the present study, we found that the MDR rate in *P. aeruginosa was* 56.6%, which was within a range of the previous studies conducted in Ethiopia *(*4.6–84%) [9, 38, 39], and relatively lower than the findings reported in Brazil (76.2%) [40], Iraq (76.06%) [41], and Egypt (70%) [42]. Pérez et al. [43] reported a lower MDR rate (30.2%) of *P. aeruginosa* isolates collected from three European countries (Greece, Italy, and Spain). Other studies demonstrated low rates of MDR from Iran (16.5%) [44], Ghana (43.6%) [45], Kenya (31%) [46], Nepal (42%) [47], Iraq (50%) [48], Germany (34%) [49], and China (22.3%) [50]. The variation in MDR between countries can be attributed to differences in antimicrobial stewardship strategies and varying definitions of MDR, along with factors such as healthcare access, the use of antibiotics in agriculture, and local epidemiological trends.

The present study found a high level of resistance to ciprofloxacin (51.8%) in *P. aeruginosa*, which was relatively compatible with studies reported from Ethiopia (61.1%) [38], India (50%) [51] and Uganda (50-64%) [52, 53]. Lower levels of resistance against ciprofloxacin were reported in previous studies conducted in Ethiopia (18–36%) [27, 39], Iran (29%) [54] and Spain (38%) [55]. Higher levels of resistance to ciprofloxacin have been reported in China (80.4%) [19], Brazil (94.3%) [40], and Egypt (70%) [42]. The rate of resistance to levofloxacin in present study was 37.3%, which was lower than the study conducted in India (67%) [56], and higher than the study conducted in Ethiopia (24%) [9]. Resistance towards the aminoglycoside drugs gentamicin and netilmicin were 43.4% and 26.5%, respectively. A wide range of resistance against gentamicin was reported from previous studies conducted in Ethiopia (7-62.97%) [9, 30]. Higher levels of resistance against both gentamicin and netilmicin were reported in India (81.03% vs. 58.6%) [57]. The level of resistance against aztreonam in the present study was 42.2%. Higher rates of resistance against aztreonam were reported from studies in Egypt (69%) [42], and India (81.8%) [58]. The discrepancies in resistance rates among countries may stem from multiple factors, including local healthcare practices, which influence prescribing behaviors and antibiotic stewardship. Additionally, variations in diagnostic practices, socioeconomic factors, and public awareness can all contribute to these differences.

Carbapenems are well-known β-lactam antibiotics with broad-spectrum activity and are used for treating severe infections caused by gram-negative bacteria [59]. However, the use of carbapenems is threatened by the global

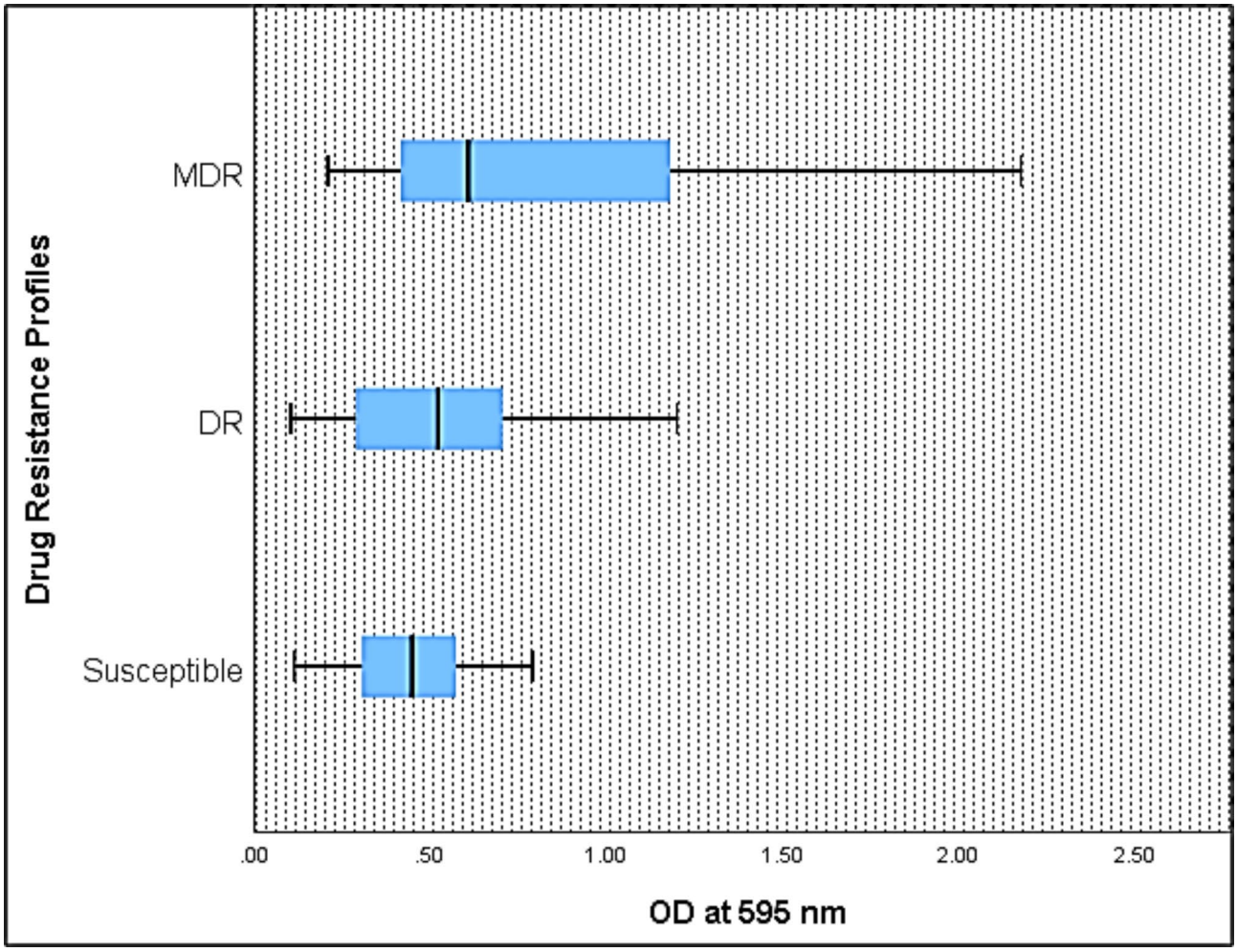

Fig. 3 Boxplot of optical density for isolates with different resistance profiles

emergence of carbapenem-resistant strains [60]. In the present study, 22.9% of *P. aeruginosa* isolates were resistant to meropenem, comparable with previous studies in Ethiopia (19.4%) [61] and India (18.2%) [58]. The resistance rate to imipenem in this study was 16.9%, which was in agreement with the studies conducted in Ethiopia (18%) [9], and India (18.2%) [58]. However, higher rates of resistance to meropenem and imipenem were reported in Poland (61.6% vs. 41.1%) [62]; India (80% vs. 78%) [56]; Saudi Arabia (42.4% vs. 36.4%) [63], and Iran (30% vs. 70%) [64]. The lower frequency of carbapenem resistance in *P. aeruginosa* observed in the present study, compared to other studies, may be attributed to variations in carbapenem prescribing rates. These differences could limit *P. aeruginosa* exposure to these antibiotics, thereby reducing the selective pressure that drives the development of resistance.

Various studies reported that the combination drug ceftazidime-avibactam has a better effect against MDR *P. aeruginosa* isolates [65, 66]. The present study revealed better sensitivity to ceftazidime-avibactam (95.2%) and piperacillin-tazobactam (75.9%) in *P. aeruginosa* isolates, which was relatively higher than previous findings conducted in three European countries (Greece, Italy, and Spain) [43]. This difference may be due to the nature and source of tested isolates.

The present study revealed that 95.2% of *P. aeruginosa* were biofilm producers, which was relatively consistent with findings published in previous studies conducted in Iran (70%) [64], and South Korea (92.7%) [67]. In this study, 34.04% and 40.42% of MDR isolates were strong and moderate biofilm-forming isolates, respectively, in agreement with study conducted in Saudi Arabia [63]. The present study discovered a significant weak positive correlation (rs=0.266; p-value=0.015) between biofilm production and drug resistance,, comparable to studies carried out by Iran [18], and Ethiopia [68]. Additionally, a previous study conducted in South Korea found a statistically significant genetic linkage between biofilm formation and drug resistance in *P. aeruginosa* [67]. Nevertheless, in the present study, further molecular analysis

Table 5 Bivariate and multivariable analysis of risk factors associated with multidrug-resistant *P. Aeruginosa* isolates

Variables	MDR (%)	Bivariate logistic regression		Multivariable logistic regression	
		COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Age in Years					
0–15	52.4	2(0.48–8.77)	0.335	1.9(0.31–10.4)	0.444
16–40	61.1	1.4(0.37–5.55)	0.604	0.8(0.17–3.74)	0.763
41–60	38.5	3.6(0.71–18.25)	**0.122**	2.62(0.41–15.9)	0.296
>60*	-				Ref
Residence					
Urban	63.6	2.3(0.92–5.90)	**0.074**	0.28(0.68–1.15)	0.077
Rural*	-				Ref
Types of antibiotics used in last three months (Yes/No*)					
Cephalosporin	66.04	2.92(1.15–7.4)	**0.023**	2.6(0.81 – 8.1)	0.111
Gentamicin	88.9	7.17(0.85- 60)	**0.069**	3.9(0.369 – 41.4)	0.258
Ciprofloxacin	68	1.9(0.74 – 5.3)	**0.173**	5.1(1.12–24.7)	**0.032**
Meropenem	83.3	4.16(0.47-37.9)	**0.202**	3.1(0.23- 39.9)	0.392
History of invasive medical procedures usage (Yes/No*)					
Yes	47.1	0.52(0.212–1.26)	**0.145**	0.37(0.09–1.5)	0.272
Source of isolates(Clinical samples)					
Blood	42.1	2.9(0.92–8.97)	**0.071**	1.5(0.356 – 6.4)	0.355
Urine	51.8	1.9(00.69–5.37)	**0.206**	0.9(0.21–3.53)	0.858
Wound*	-				Ref

"*" reference categories; "CI" confidence interval; "OR" odds ratio; "**bold**," significant; Cephalosporin", replaced for at least one from ceftriaxone, cefepime or ceftazidime). Significant variables in the binary logistic analysis ($p < 0.25$) were entered into a multiple-variable analysis. Significant variables in multivariable analysis ($p < 0.05$) were identified as independent risk factors in this study

is required to prove the genetic linkage between biofilm and drug resistance.

In this study, multivariable logistic regression showed that isolates from patients with previous exposure to ciprofloxacin were about five times more likely to develop MDR compared to isolates from those without previous exposure to ciprofloxacin (OR: 5.1; 95%CI: 1.12–24.7; p-value: 0.032). Similarly, we found the highest resistance rate to ciprofloxacin. Widespread use and higher prescribing rate of ciprofloxacin may be a cause of increasing resistance against this drug, and is driving the selection of resistance genes that lead to multiple drug resistance in *P. aeruginosa.* This observation corresponds with findings from a previous study that identified recent antibiotic therapy as an independent factor for MDR in *Pseudomonas aeruginosa*, although our study specifically emphasized previous ciprofloxacin exposure as the only significant factor [69].

Study limitations

The present study has several limitations. Only conventional microbiological methods were used for laboratory investigation, and a simple biofilm assay was performed, excluding information provided by molecular approaches. Furthermore, the cross-sectional study design limits the capacity to prove causation. In this study, only previous use of ciprofloxacin was statistically associated with MDR in *P. aeruginosa*, and therefore further study with a strong study design may be beneficial to better understand the risk of MDR development. Additionally, further molecular analysis is necessary to identify the resistance gene determinants responsible for drug resistance in *P. aeruginosa.*

Conclusion

In this study, the highest resistance rate was observed for ciprofloxacin, followed by ceftazidime and cefepime. Over half of the isolates were resistant to multiple drugs, with prior use of ciprofloxacin associated with the development of MDR. The study also showed a high prevalence of biofilm-producing isolates and an association between resistance to multiple drugs and the level of biofilm-producing capacity. These findings suggest that optimizing antibiotic use, monitoring resistance patterns and implementing infection control measures in hospital settings may be beneficial in addressing resistance. It can also be used as baseline data for further studies to inform public health policy and the current national initiatives to fight antibiotic resistance.

Abbreviations

BSI	Blood stream infections
HAIs	Hospital-acquired infections
MDR	Multidrug-resistant
TASH	Tikur Anbessa Specialized Hospital
TSB	Tryptic Soya broth
UTI	Urinary tract infections
WHO	World Health Organization
WI	Wound infections
Y12HMC	Yekatit-12 Hospital Medical College

Acknowledgements
We would like to thank Addis Ababa University and Uppsala University for supporting this study. We express our appreciation to Yekatit 12 Hospital Medical College Hospital and Tikur Anbessa Specialized Hospital for enabling us to carry out the study. We appreciate all of the medical professionals, nurses, and microbiologists who assisted us in conducting this study. Finally, we would like to express our sincere appreciation to all study participants.

Author contributions
Conceptualization and Investigation, M.D.O.: Methodology, M.D.O., D.A. and G.S.; Validation, M.D.O, D.A. and G.S.; Analysis, M.D.O., D.A .and G.S.; Data curation, M.D.O.; Supervision and Funding acquisition, D.A .and G.S. Writing—Original Draft, M.D.O. All authors read and approved the final manuscript.

Funding
This study was supported by Addis Ababa University and Addis Ababa University Swedish International Development Cooperation Agency project.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The research project was ethically approved by the Department Research Ethics Review Committee (DRERC) of Microbiology, Immunology and Parasitology and Institutional Review Board (IRB) (Protocol number: 054/22DMIP) of College of Health Sciences of Addis Ababa University and then by National Ethics Review Committee (NERC) (ref no: 17/152/845/23). Before being recruited for the study, the purposes of study were explained to participants or participants' parents or guardians. The children whose parents or guardians provided informed consent as well as the study participants who provided written informed consent were included to this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Medical Laboratory Sciences, Collage of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia
[2]Department of Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia
[3]Department of Medical Biochemistry and Microbiology, Biomedical Centre, Uppsala University, Uppsala, Sweden

Received: 11 July 2024 / Accepted: 16 December 2024
Published online: 28 December 2024

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