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Prevalence and associated factors of multi-drug resistant bacteria among different clinical specimens at wad Medani, Sudan: a four-year, cross-sectional study

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Multi-drug resistant organisms (MDROs) are a global health concern. Therefore, the aim of the study is to evaluate the prevalence of MDROs and associated factors among different clinical specimens. This was a retrospective cross-sectional study, conducted between January 2020 to October 2023 using recorded laboratory data of culture and drug sensitivity from the Pathology Center for Diagnosis and Research, University of Gezira, Wad Medani City, Sudan. Among the 1766 investigated clinical samples, 1255 (71.1%) were from female. The overall MDROs prevalence was 694 (39.3%). Blood samples 45(52.3%) and wound swabs 191(41.2%) had the most MDROs. *Staphylococcus aureus (S. aureus*) and *Proteus* spp. 42(75%) had the highest MDR among the gram-positive bacteria (GPB*)* and the gram-negative bacteria (GNB) respectively. A significant association was observed between cerebrospinal fluid (CSF) and urine samples with the presence of MDROs. This study revealed a moderate prevalence of MDROs. Which is more prevalent in blood samples, wound swabs and in GNB. *S. aureus* and *Proteus* spp. had the greatest MDR among the GPB, and GNB, respectively. CSF and urine samples were associated with the presence of MDROs. Implementing these findings enables the development of strategies to prevent AMR in Sudan and other comparable low-resource environments.

Keywords Prevalence, Associated factors, Multi-drug resistant, Bacteria, Wad Medani, Sudan

Abbreviations

AMR	Antimicrobial resistance
MDROs	Multi-drug resistant organisms
WHO	World Health Organization
P. aeruginosa	Pseudomonas aeruginosa
E. coli	Escherichia coli
PCDR	Pathology Center for Diagnosis and Research
CLSI	Clinical and Laboratory Standards Institute
GNB	Gram-negative bacteria
GPB	Gram-positive bacteria
CI	Confidence intervals
COR	Crude odd ratio
AOR	Adjusted odd ratio
CSF	Cerebrospinal fluid
ICU	Intensive Care Unit

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Antimicrobial resistance (AMR) is a global health concern. It has been reported that AMR bacteria directly led to 1.27 million deaths worldwide in 2019[1]. The AMR also affects the global economy, and the economic cost of AMR might reach $100 trillion by 2050 if it is not addressed[2].

AMR has worsened over the past few decades due to the increasing frequency of multi-drug resistant organisms (MDROs) from both hospital- and community-acquired illnesses[1,3]. With an estimated five years prevalence of 622,390 MDROs infections among hospitalized patients in the USA[4]. With numerous other organizations and researchers, the World Health Organization (WHO) agrees that the development and spread of MDROs is an urgent issue that must be addressed with a global coordinated action plan[1,3,5].

MDROs are defined as microorganisms that are nonsusceptible to at least one antibiotic from three or more classes of antimicrobial agents[6]. MDROs can affect the healthcare, veterinary, and agricultural sectors as well as individuals at any stage of life[7]. Moreover, MDROs have negative detrimental effects, such as prolonged hospital stays, increased morbidity and mortality risk, and increased medical and social expenses[7]. However, these effects are far more pronounced when MDROs infect vulnerable individuals[8,9].

Globally, various studies investigating the epidemiology and antimicrobial profiles of MDROs have reported different prevalence rates and risk factors among different countries, such as Australia 36%[10], Nigeria 59.3%[11], Saudi Arabia 68.2%[12], and Ethiopia 70.5%[13]. However, most studies conducted in Sudan estimate the prevalence of MDROs among specific microorganisms, such as *Pseudomonas aeruginosa* (*P. aeruginosa*), is 13.6% [14], and that of *Escherichia coli (E. coli*) is 92.2%[15].

The Global World Health Organization stated that Africa is one of the areas that lacks functioning AMR surveillance measures[16]. To address this problem of AMR, the WHO urges healthcare professionals to adopt antimicrobial stewardship programs to lessen the burden of antibiotic resistance. However, before any stewardship program is implemented, details about the common microorganisms and their antimicrobial resistance profile are needed[5]. Hence, it is crucial to understand the prevalence of MDROs and associated risk factors, particularly in areas with minimal surveillance data. Given that the pattern of MDROs appears to change temporally and geographically, certain MDROs could be rare in some places but endemic in others[1,5]. On top of that considering local microbial profiles and antimicrobial resistance is essential for guiding both empirical and specific therapeutic recommendations[17]. In Wad Medani city, Sudan there was no published data on the prevalence of MDROs and associated factors. Therefore, this study aimed to evaluate the prevalence of MDROs and associated factors among different clinical specimens in Wad Medani, Sudan.

Materials and methods

Study design

This was a retrospective cross-sectional study.

Study setting and period

The study was conducted between January 2020 to October 2023 using recorded laboratory data that was routinely obtained for diagnostic purposes at the Pathology Center for Diagnosis and Research (PCDR), Faculty of Medicine, University of Gezira, Wad Medani City, Sudan. The PCDR serves as the reference laboratory and encompasses all hospital settings at Wad Medani. Which include six university teaching hospitals, two Military Hospitals, and one Police Hospital. Wad Medani city is the capital of Gezira State. It covers a total area of 65 km2 and population of the city were 515.230 Based on 2008 census.

Data source

Data was collected manually from the center's registries (a paper-based record) of the PCDR. The registries contain descriptions of the microbiological procedures and anonymous patient information, which include: the patient's code number, date, age, sex, type of clinical sample, type of bacterial strain, and results of tested antibiotics. Data quality and completeness checks were conducted at the end of data collection by the investigators.

Inclusion and exclusion criteria

The data for all samples examined at the PCDR for culture and drug sensitivity were included in this study.

Exclusion criteria

All samples exhibited no growth or fungal growth.

Antibiotics that were tested but are not included in the study reference for the definition of MDROs[6].

Antibiotics that were examined but are not listed in the Clinical and Laboratory Standards Institute (CLSI) guidelines for the particular bacteria[18] .

Laboratory procedures at the PCDR

Sample collection

The PCDR applies standard microbiological approaches for sample collection; for example, after the patients were instructed to clean their genitalia, urine samples were obtained, and a freshly voided midstream urine specimen (10–20 mL) was collected in a dry, wide-mouthed sterile, and leak-proof container[19]. For patients with catheters, urine was aspirated promptly from the catheter using a sterile needle with a syringe and then placed in a sterile container[20]. All specimens were delivered to the microbiology laboratory of the PCDR aseptically for culture and drug sensitivity tests.

Identification of the isolated organism

The obtained samples were inoculated onto blood agar and MacConkey agar plates for urine samples using a calibrated loop (0.001 mL)[19]. Cultures were incubated in an aerobic atmosphere at 37 °C for 24 h. A positive

urine culture was defined as a colony count of ≥ 10^5CFU/mL for midstream urine[20]. Similarly, all plates were incubated accordingly based on their specimen type and the organism expected[19].

For all positive cultures, morphological characteristics, Gram staining, and confirmatory biochemical tests were used to identify the bacterial isolates. Gram-negative bacteria (GNB) were identified by inoculation on MacConkey agar plates, followed by biochemical tests such as H2S production, indole production, utilization of citrate/carbohydrates, urease tests, and oxidase tests. Additionally, gram-positive bacteria (GPB) were identified by the catalase reaction, coagulase test, optochin test, bacitracin test, and hemolytic activity test on blood agar[19].

Antimicrobial susceptibility

The antimicrobial susceptibilities of the bacterial isolates were ascertained by Mueller–Hinton agar plates (Oxoid, England) using the Kirby–Bauer disk diffusion method according to CLSI 2020 guidelines[18].

GPB isolates were tested against the following antimicrobials: ampicillin (10 µg), cotrimoxazole (1.25/23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg), vancomycin (30 µg), gentamycin (10 µg), piperacillin/tazobactam (100/10 µg), clindamycin (2 µg), linezolid (30 µg), and erythromycin (15 µg)[17].

GNB isolates were tested against ceftriaxone (30 µg), ampicillin/sulbactam (10/10 µg), cefuroxime (30 µg), ampicillin (10 µg), cotrimoxazole (1.25/23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg), gentamycin (10 µg), amoxicillin/clavulanic (20/10 µg), piperacillin/tazobactam (100/10 µg), chloramphenicol (30 µg), and cefotaxime (30 µg)[17]. The CLSI 2020 guideline breakpoints were used to interpret zone diameters[18].

Quality control

To guarantee the authenticity of the results, quality control techniques were employed as standard practices throughout the entire laboratory work process. Before use, the staining reagents, antibiotic discs, and culture media were examined for normal shelf life[21]. Following preparation and autoclaving at 121 °C for 15 min, all culture plates and antibiotic discs were stored at the recommended refrigeration temperature. The standard reference bacterial strains were investigated as a positive control on agar plates with biochemical assays and antibiotic discs[21]. Which includes: *E. coli* strain ATCC 25,922, *P. aeruginosa* ATCC 27,853, *S. aureus* ATCC 29,123, and *streptococcus pneumoniae* ATCC 49,619[18]. The samples were processed carefully by highly qualified microbiologists.

Variable definition

MDROs: microorganisms not susceptible to at least one antimicrobial in three or more antimicrobial categories[6].

Coliform: included bacterial species of genera other than *E. coli* and *Klebsiella* (*Citrobacter*, *Enterobacter*, *Serratia*, etc.).

Study variables

Age, sex, year, type of bacterial strain, and type of clinical sample were considered independent variables, while the MDROs were considered the dependent variable in this study.

Statistical analysis

The Statistical Package for the Social Science (SPSS) version 27.0 was used to analyze the data. Qualitative data are presented as frequencies (percentages). The Pearson chi-squared test and Fisher's exact test were used after checking the applicability conditions to compare categorical data. Univariable and multivariable binary logistic regression analyses were used to determine the factors associated with MDROs bacteria. All variables associated with MDROs at a p value < 0.200 in the univariable model were included in the multivariable model using the enter method. The initial multivariable model was then subjected to the "stepAIC" function to generate the final multivariable logistic regression model through a stepwise process that combines forward and backward selection. The results are displayed as odds ratios with 95% confidence intervals (CIs), and a statistically significant difference was considered at a p value ≤ 0.05.

Ethical consideration

This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was evaluated and approved by the Ethical Committee, Ministry of Health, Gezira State, Sudan (12/6/2023). Due to the retrospective nature of the study and the data were provided to us anonymously, the Ethical Committee, Ministry of Health, Gezira State, Sudan (12/6/2023) waived the need of obtaining informed consent.

Ethical approval and informed consent

Ethical permission for this study was approved by the Ethical Committee, Ministry of Health, Gezira State, Sudan (12/6/2023). Due to the retrospective nature of the study, the Ethical Committee, Ministry of Health, Gezira State, Sudan (12/6/2023) waived the need of obtaining informed consent.

Results

Socio-demographic characteristics

Out of the 1766 investigated clinical samples, 1255 (71.1%) were from females, and 511 (28.9%) were from males. Regarding the age groups of the investigated clinical samples, most of the samples were in 30–44 years 584(33.1%) followed by ≥ 60 years 432(24.5%), and 5–14 years 53(3%) was the least one (Table 1). Most of the isolated sample were 790(44.73%) at 2022 (Table 1). Overall, growth for GNB was observed in 993(56.2%), while GPB in 773(43.8%) of studied samples.

Characteristic	2020 *N*(%)	2021 *N*(%)	2022 *N*(%)	2023 *N*(%)	Overall *N*(%)	*p*-value[a]
Sex						< 0.001
Male	78(45.6)	92(32.9)	175(22.2)	166(31.6)	511(28.9)	
Female	93(54.4)	188(67.1)	615(77.8)	359(68.4)	1255 (71.1)	
Age group[b]						< 0.001
Under 5	8(4.7)	14(5)	15(1.9)	35(6.7)	72(4.1)	
5–14	9(5.3)	5(1.8)	13(2.4)	20(3.8)	53(3)	
15–29	29(17)	25(8.9)	117(14.8)	77(14.7)	248(14)	
30–44	61(35.7)	103(36.8)	281(35.6)	139(26.5)	584(33.1)	
45–59	26(15.2)	69(24.6)	183(23.2)	99(18.9)	377(21.3)	
≥ 60	38(22.2)	64(22.9)	175(22.2)	155(29.5)	432(24.5)	

Table 1. Sociodemographic characteristics of study participants at PCDR from January 2020 to October 2023. [a]Pearson's Chi-squared and fisher exact test; [b]Age in year.

Characteristic	MDR *N* = 694 (39.3%)	No MDR *N* = 1072 (60.7%)	Overall *N* = 1766	*p*-value[a]
Sex				< 0.001
Male	241(47.2)	270(52.8)	511(28.9)	
Female	453(36.1)	802(63.9)	1255(71.1)	
Age group				< 0.001
Under 5	20(27.8)	52(72.2)	72(4.1)	
5–14	26(49.1)	27(50.9)	53(3)	
15–29	92(37.1)	156(62.9)	248(14)	
30–44	200(34.2)	384(65.8)	584(33.1)	
45–59	148(39.3)	229(60.7)	377(21.3)	
≥ 60	208(48.1)	224(51.9)	432(24.5)	
Years				0.214
2020	80(46.8)	91(53.2)	171(9.7)	
2021	107((38.2)	173(61.8)	280(15.9)	
2022	303(38.4)	487(61.6)	790(44.7)	
2023	204(28.9)	321(61.1)	525(29.7)	
Type of sample				0.040
Blood	45(52.3)	41(47.7)	86(4.9)	
Urine	424(38.3)	684(61.7)	1108(62.7)	
CSF	4(23.5)	13(76.5)	17(1)	
Ear swab	3(21.4)	11(78.6)	14(0.8)	
Wound swab	191(41.2)	273(58.8)	464(26.3)	
Vaginal swab	27(35.1)	50(64.9)	77(4.4)	

Table 2. Distribution of patient characteristics according to their multidrug resistance status at PCDR from January 2020 to October 2023. [a]Pearson's Chi-squared and fisher exact test. N (%), Number (percentage); MDR, Multi drug resistance.

Prevalence and distribution of multidrug resistance bacteria

Overall, 694 (39.3%) of the isolated bacteria exhibited MDROs patterns. Among the females, 453 (36.1%) of the isolated bacteria were MDROs, while in males, 241 (47.2%) (95% CI; *p* value < 0.001) were MDROs, as summarized in (Table 2). The 5–14 years age group had a high MDROs detection rate of 26 (49.1%), followed by the ≥ 60 years 208 (48.1%) and 45–59 years 148 (39.3%) age groups, while the < 5 years age group 20 (27.8%) had the lowest MDROs detection rate (95% CI; *p* value < 0.001) (Table 2).

Regarding years of isolation, 2020 had the highest MDROs detection rate of 80 (46.8%), while 2023 had the lowest MDROs detection rate of 204 (28.9%) (95% CI; *p* value 0.214) (Table 2). However, blood samples had the highest MDROs detection rate 45(52.3%), followed by wound swabs at 191(41.2%), while ear swabs had the lowest MDROs detection rate 3(21.4%) (95% CI; *p* value 0.040) (Table 2).

The prevalence of MDROs among the GPB isolates was 112 (14.5%), while that among the GNB isolates was 582 (58.6%) (95% CI; *p* value < 0.001). In particular, MDROs were detected in 101 (14.6%) of the isolated S. *aureus* strains, while MDROs were detected in 11 (13.8%) *Streptococcus* spp. (Table 3). Furthermore, *Proteus* spp. 42(75%) had the highest percentage of MDROs isolated from GNB, followed by *E. coli* 462(71.2%), while

	Level of antibiotic resistance *n* (%)										
	N	R0	R1	R2	R3	R4	R5	R6	R7	R8	Total MDR *R* ≥ 3
Gram negative Isolates											
E.coli	649	7(1.1)	65(10)	115(17.7)	230(35.4)	187(28.8)	29(4.5)	12(1.8)	4(0.6)	0	462(71.2)
Klebsiella spp	79	0	11(13.9)	14(17.7)	28(35.4)	23(29.1)	0	2(2.5)	1(1.3)	0	54(68.4)
Proteus spp	56	2 (3.6)	4(7.1)	8(14.3)	19(33.9)	10(17.9)	7(12.5)	4(7.1)	1(1.8)	1(1.8)	42(75)
Coliform[a]	90	30(33.3)	25(27.8)	15(16.7)	12(13.3)	8(8.9)	0	0	0	0	20(22.2)
P.aeruginosa	119	71(59.7)	26(21.8)	18(15.1)	4(3.4)	0	0	0	0	0	4(3.4)
Total	993	110(11.1)	131(13.2)	170(17.1)	293(29.5)	228(23)	36(3.6)	18(1.8)	6((0.6)	1(0.1)	582(58.6)
Gram positive Isolates											
S. aureus	693	307(44.3)	183(26.4)	102(14.7)	80(11.5)	21(3)	0	0	0	0	101 (14.6)
Streptococcus spp	80	29(36.3)	24(30)	16(20)	7(8.8)	2(2.5)	1(1.3)	1(1.3)	0	0	11 (13.8)
Total	773	336(43.5)	207(26.8)	118(15.3)	87(11.2)	23(3)	1(0.1)	1(0.1)	0	0	112 (14.5)

Table 3. Multi-Drug resistance patterns of gram negative and gram positive isolates at PCDR from January 2020 to October 2023. [a]Included bacteria species of genera other than *E. coli* and *Klebsiella* (*Citrobacter*, *Enterobacter*, *Serratia*, etc.). R0, resistance to no antibiotics; R1-8, resistance to 1, 2, 3, 4, 5, 6,7, and 8 antibiotics; MDR, multi drug resistance; ≥R3, resistance to 3 or more antibiotics from different classes (resistant to one or more antibiotics in three or more classes).

	Isolated gram negative bacteria									
	E. coli		*Proteus spp*		*Coliform*		*Klebsiella spp*		*P. aeruginosa*	
Antibiotic	S; N (%)	R; N (%)	S; N (%)	R; N (%)	S; N (%)	R; N (%)	S; N (%)	R; N (%)	S; N (%)	R; N (%)
AMC	7 (1.3)	533 (98.7)	6 (17.6)	28 (82.4)	0	72 (100)	1 (1.4)	73 (98.6)	–	–
CTX	155 (35.2)	285 (64.8)	20 (40)	30 (60)	27 (39.1)	42 (60.9)	37 (66.1)	19 (33.9)	–	–
AMP	20 (7.6)	243 (92.4)	1 (5.6)	17 (94.4)	3 (9.1)	30 (90.9)	2 (7.4)	45 (92.6)	–	–
GM	58 (69.9)	25 (30.1)	24 (68.6)	11 (41.4)	7 (70)	3 (30)	–	–	32(74.4)	11 (25.6)
CIP	164 (49.1)	170 (50.9)	26 (74.3)	9 (25.7)	28 (73.7)	10 (26.3)	14 (30.4)	32 (69.6)	41 (64.1)	23 (35.9)
AK	268 (72.8)	100 (27.2)	38 (77.6)	11 (22.4)	32 (53.3)	28 (46.7)	23 (62.2)	14 (37.8)	67 (79.8)	17 (20.2)
TZP	58 (54.7)	48 (45.3)	22 (75.9)	7 (24.1)	14 (100)	0	10 (71.4)	4 (28.6)	25 (61)	16 (39)
TE	110 (31.3)	241 (68.7)	10 (18.5)	44 (81.5)	22 (36.7)	38 (63.3)	10 (33.3)	20 (66.7)	–	–
COT	50 (27.6)	131 (72.4)	16 (30.8)	36 (69.2	6 (24)	19 (76)	–	–	–	–
LE	315 (70)	135 (30)	28 (82.4)	6 (17.6)	47 (71.2)	19 (28.8)	37 (74)	13 (26)	52 (74.3)	18 (25.7)
CH	67 (68.4)	31 (31.6)	19 (65.5)	10 (34.5)	12 (75)	4 (25)	–	–	–	–
AMC	213 (59.5)	145 (40.5)	0	2 (100)	29 (53.8)	24 (46.2)	27 (62.8)	16 (37.2)	–	–
CTR	15 (19.2)	63 (80.8)	14 (43.8)	18 (56.3)	3 (25)	9 (75)	–	–	–	–
CXM	19 (6.1)	290(93.9)	1 (5.6)	17 (94.4)	3 (8.3)	33 (91.7)	0	38 (100)	–	–

Table 4. Antimicrobial susceptibility pattern of GNB from different clinical specimen at PCDR from January 2020 to October 2023. S, susceptible; R, resistance; AMC, ampicillin/sulbactam; CTX, cefotaxime; AMP, ampicillin; GM, Gentamycin; CIP, Ciprofloxacin; AK, Amikacin; TZP, Piperacillin/tazobactam; TE, Tetracycline; COT, Cotrimoxazole; LE, Levofloxacin; CH, Chloramphenicol; AMC, amoxicillin/clavulanic acid; CTR, ceftriaxone; CXM, Cefuroxime.

P. aeruginosa 4(3.4%) had the lowest percentage of MDROs detected (Table 3). The antibiotic susceptibility patterns of GNB and GPB are summarized in (Table 4) and (Table 5), respectively.

Factors associated with multi-drug resistance

The univariate and the multivariable logistic regression analyses of factors associated with MDROs were summarized in (Table 6). Univariate analysis revealed that the odds of MDROs detection were 1.58 times greater in males than in females (COR (crude odd ratio) 1.58; 95% CI 1.283–1.946; p value < 0.001). In addition, patients in the 30–44 years of age group (COR 1.849; 95% CI 1.051–3.253; p value 0.033) and those < 5 years of age group (COR 2.504; 95% CI 1.188–5.277; p value 0.016) had 1.849 times and 2.504 times greater odds of MDROs detection, respectively, than did those aged 5–14 years. GNB reduced MDROs detection rate by 0.120 times compared to that of GPB (COR 0.120; 95% CI 0.094-0.152; p value < 0.001). Furthermore, *compared with E. coli*, *P. aeruginosa*, *Streptococcus* spp., *S. aureus*, and *coliform bacteria* were found to increase MDROs detection rate. Moreover, compared with blood samples, ear, vaginal, and wound swabs and cerebrospinal fluid (CSF) and urine samples were found to increase the MDROs detection rate.

Gram positive bacteria	AST	Antimicrobial agents level N (%)											
		AMP	GM	CIP	AK	TZP	TE	COT	E	LE	VA	CD	LZ
S. aureus	S	–	111 (72.5)	293 (73.3)	87 (54.7)	34 (75.6)	161 (44.6)	113 (37.9)	134 (48.9)	–	122 (50.8)	38 (56.7)	237 (80.1)
	R	–	42 (27.5)	107 (26.8)	72 (45.3)	11 (24.4)	200 (55.4)	185 (62.1)	140 (51.1)	–	118 (49.2)	29 (43.3)	59 (19.9)
Streptococcus species	S	9 (40.9)	17 (77.3)	35 (81.4)	–	–	–	–		35 (53.8)	22 (61.1)	–	31 (72.1)
	R	13 (59.1)	5(22.7)	8 (18.6)	–	–	–	–		30 (46.2)	14 (38.9)	–	12 (27.9)

Table 5. Antimicrobial susceptibility pattern of gram positive isolates from different clinical specimen at PCDR from January 2020 to October 2023. AST, antimicrobial susceptibility test; AMP, ampicillin; GM, Gentamycin; CIP, Ciprofloxacin; AK, Amikacin; TZP, Piperacillin/tazobactam; TE, Tetracycline; COT, Cotrimoxazole; E, erythromycin; LE, Levofloxacin; VA, Vancomycin; CD, clindamycin; LZ, linezolid; S, susceptible; R, resistance.

Characteristic	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Gender				
Female	Reference	–	–	–
Male	1.580 (1.283–1.946)	< 0.001	1.377(1.036–1.831)	0.027
Age group[a]		< 0.001		0.001
5–14	Reference	–	–	–
15–29	1.633(0.899-2.966)	0.107	1.844(0.866-3.924)	0.112
30–44	1.849(1.051–3.253)	0.033	2.033(0.9934.159)	0.052
45–59	1.490(0.837-2.653)	0.175	1.590(0.767-3.299)	0.213
≥ 60	1.037(0.586-1.835)	0.901	1.582(0.767-3.264)	0.214
< 5	2.504(1.188–5.277)	0.016	6.587(2.504–17.328)	< 0.001
Year		0.216		
2020	Reference	–		
2021	1.421(0.967-2.089)	0.074		
2022	1.423(1.013–1.971)	0.042		
2023	1.383(0.977-1.959)	0.068		
Isolates		< 0.001		< 0.001
E. coli	Reference	–	–	–
P. aeruginosa	71.029 (25.838–195.262)	< 0.001	78.66(28.322-218.471)	< 0.001
Klebsiella species	1.144(0.691-1.893)	0.601	1.213(0.724 – 2.030)	0.463
Streptococcus species	15.489(8.021–29.942)	< 0.001	15.168(7.799–29.500)	< 0.001
Proteus species	0.824(0.439-1.544)	0.545	0.886(0.450-1.745)	0.727
S. aureus	14.481(11.045–18.987)	< 0.001	14.854(11.234–19.640)	< 0.001
Coliform[b]	8.647(5.114–14.620)	< 0.001	8.459(4.926–14.527)	< 0.001
Type of bacteria stain				
Gram positive	Reference			
Gram negative	0.120(0.094-0.152)	< 0.001		
Samples		0.048		0.014
Blood	Reference	–	–	–
Urine	0.1.771(1.149–2.75)	0.011	2.715(1.503–4.903)	< 0.001
CSF	3.567(1.077–11.818)	0.037	7.563(1.760-32.504)	0.007
Ear swab	4.034(1.049–15.445)	0.042	3.699(0.720-18.990)	0.117
Wound swab	1.569(0.989-2.489)	0.056	2.482(1.328–4.637)	0.004
Vaginal swab	2.033(1.081–3.820)	0.028	2.116(0.929-4.823)	0.074

Table 6. Univariate and multivariable logistic regression of factors associated with multidrug resistance at PCDR from January 2020 to October 2023. [a]Age in year; [b]Included bacteria species of genera other than *Escherichia coli* and *Klebsiella* (*Citrobacter*, *Enterobacter*, *Serratia*, etc.). *COR*, crude odds ratio, *CI*, confidence interval, *AOR*, adjusted odds ratio.

The multivariable logistic regression revealed that the odds of MDROs detection were 2.504 times greater in the < 5 years age group (AOR (adjusted odd ratio) 2.504; 95% CI 1.188–5.277; *p* value 0.016) than in the 5–14 years age group. In addition, the odds of MDROs detection were 1.377 times greater in males than in females (AOR 1.377; 95% CI 1.036–1.831; *p* value 0.027). Generally, *compared with E. coli*, *P. aeruginosa*, *Streptococcus* spp., *S. aureus*, and *coliform bacteria* were found to increase the MDROs detection rate. Other factors, such as CSF, were also associated with MDROs in univariate (COR 3.567; 95% CI 1.077-11.8181; *p* value 0.037) and multivariable analyses (AOR 7.563; 95% CI 1.760-32.504; *p* value 0.007) and urine in univariate (COR 1.771; 95% CI 1.149–2.75; *p* value 0.011) and multivariable analyses (AOR 2.715; 95% CI 1.503–4.903; *p* value < 0.001).

Discussion

The epidemiological study of bacterial infection and susceptibility to antibiotics is crucial for infectious disease management, which is significant in developing countries, especially in Sudan, where 74% of healthcare workers do not have access to infection prevention and control strategies[22,23]. In Sudan, information about MDROs was limited, this was supported by a recent WHO report on antibiotic resistance in the African Region[24]. Consequently, we discussed the results of this study alongside the existing information from Sudan and other neighboring and global countries.

This study revealed that the overall prevalence of MDROs among different clinical specimens was 39.3%. This finding was in line with a study conducted in adult Moroccoian Intensive Care Unit (ICU) 41%[25] and in long-term care facility residents, Australia 36%[10]. However, the result of this study was less than the study conducted on Libyan war casualties admitted to a Tertiary Care Hospital, Germany 60%[26], in the ICU, Ibadan, Nigeria 59.3%[11], in the ICU of King Fahad Hofuf Hospital 68.2%[12], and in the adult ICU, China 51.55%[27]. However, the highest MDROs rate was reported by a systematic review and meta-analysis carried out in Ethiopia 70.5% [13]. On top of that, the findings of this study were greater than those of studies conducted in Oman 10.8%[28], Poland (23.2%)[29], and Norway 0.3%[30]. The potential rationale for the higher and lower prevalence of MDROs compared with other studies might be due to variations in the studied population, study setting, type of sample, type of isolated bacteria, and variations in the implementation of strategies for infection control and prevention between countries, particularly between developed and developing centuries. This outcome highlights the significance of ongoing surveillance of antimicrobial resistance. Moreover, comprehensive investigations need to be carried out to assess the MDROs across various hospital departments and diverse patient populations.

In this study, blood samples had the highest MDROs detection rate, followed by wound swabs, while ear swabs had the lowest MDROs detection rate. This result is different from that of a study carried out in Eastern Saudi Arabia among GNB, in which the highest number of MDROs were isolated from urine samples, followed by blood, respiratory, and swabs[31]. Moreover, another study carried out at a tertiary hospital in China reported that sputum had the most MDROs, followed by secretions, urine, blood, and pus[7]. This discrepancy could be explained by differences in the studied samples and studied microorganisms. The significant occurrence of MDROs in blood samples requires additional research to identify the specific source of MDROs, along with wound swabs to assess the prevalence of MDROs across various wound types.

Concerning the overall prevalence of MDROs among GPB isolates, in this study, the isolation rate was 14.5%. The result of this study was significantly less than most of the published studies. Among these studies, a study conducted in upper Egypt among samples isolated from wound infections 50%[32], a hospital-based study from surgical site infections 60.6%[33], in addition to the study conducted in Addis Ababa, Ethiopia, to evaluate gram-positive cocci isolates from different clinical specimens 44%[34]. Moreover, a study conducted in Dhaka, Bangladesh on isolates from infected wound samples 68.8%[35]. This significant variation might be related to the fact that these studies were conducted in only wound samples in addition to one study conducted only on GPB, while this study was conducted among different clinical samples and both GNP and GPB. Further study on a molecular basis should be implemented to verify this finding.

In this study, the overall prevalence of MDROs among GNB isolates was 58.6%. The findings of this study were comparable to those of studies conducted in Tanzania 61.4%[33] and in upper Egypt 54.9%[32]. However, this finding was less than that of a study conducted in Eastern Saudi Arabia among GNB 64.3%[31], in Addis Ababa, Ethiopia to evaluate Gram-negative bacilli isolates from different clinical specimens 94.5%[36], and in Tikur Anbessa Specialized Hospital, Ethiopia among bacterial bloodstream infections isolates 95.3%[37]. On the other hand, the results of this study were greater than those of a study conducted in the ICU of King Fahad Hofuf Hospital 51%[12] and in Bouali, Iran 49.61%[38]. The higher and lower prevalence compared with other studies could be justified by the variation in the studied population, study setting, type of studied samples, and type of studied bacteria.

In particular, *S. aureus* had the highest percentage of MDROs among GPB, at 14.6%. The MDROs prevalence in this study was comparable to that in a large hospital in Khartoum state, Sudan, at 10.4%[14]. This finding contradicts many studies conducted worldwide, of these conducted in five different states in Sudan 93.7%[39], in Addis Ababa, Ethiopia to evaluate Gram-Positive Cocci 24.1%[34], and in the adult ICU, China 55.56%[27]. These variations might be related to differences in study settings, in addition to one study being conducted only on GPB, while this study was conducted among different clinical samples and both GNP and GPB.

In this study, *Proteus* spp. had the highest percentage of MDROs among GNB, at 75%. The results of this study were in line with those of studies carried out in Eastern Saudi Arabia 72.4%[31] and Dhaka, Bangladesh 75.9%[35]. On the other hand, the results of this study were greater than those of a hospital-based study carried out among various clinical samples in India 40.74%[40]. In addition, to the laboratory-based study conducted in the northern region of Ghana 43%[41]. Furthermore, higher than the study conducted in upper Egypt from samples isolated from wound infections at 41.7%[32]. However, the finding of this study was less than in a study conducted in Bouali, Iran 84.2%[38]. The higher and lower prevalence of MDROs compared with other studies could be related to variations in the study design, study setting, and type of studied sample.

The second most common MDRO among GNB was *E. coli* 71.2%. The present results were less than those of studies conducted at different hospitals in Khartoum State, Sudan 92.2%[15], and in Addis Ababa, Ethiopia, which evaluated gram-negative bacilli isolates from different clinical sources 99.3%[36].

On the other hand, this result was in agreement with those of studies conducted in Ibadan, Nigeria 71.4%[11], India 70.04%[40], and Eastern Saudi Arabia 68.1%[31]. On top of that, this percentage was higher than that in studies conducted among different clinical samples in large hospital, Khartoum State, Sudan 24.7%[14], and in adult ICU, China 32.23%[27]. The higher and lower prevalence of MDROs compared with other studies could be explained by the fact that these studies were hospital-based, while our study was laboratory-based and included both inpatients and outpatients. Given the high MDR rate of *E. coli* in this study, hospitals should promptly and substantially change their antibiotic prescription guidelines to provide physicians with an integrated strategy for the rational, safe, and effective use of antimicrobial drugs, and a comprehensive educating campaign should be put in place to reduce the resistance rate of MDROs.

P. aeruginosa in this study had the lowest prevalence of MDROs 3.4%. This finding, in concordance with different studies, revealed that *P. aeruginosa* had the lowest percentage of MDROs among GNBs. Of these studies conducted among different clinical samples in a large hospital in Khartoum state, Sudan 13.6% were the least MDROs[14], in Eastern Saudi Arabia 37.9% were the least MDROs[31], and in Tanzania 4% were the least MDROs[33]. However, this result contradicts the findings of a study conducted in upper Egypt from samples isolated from wound infections identified the *P.aeruginosa* MDROs isolation rate was the highest at 54.2%[32]. The low prevalence of MDROs among *P. aeruginosa* could be related to that only five antibiotics were tested for *P. aeruginosa* susceptibility, while the difference from other studies could be linked to the difference in the studied samples.

In this study, various factors were shown to predispose patients to MDROs according to univariable and multivariable binary logistic regression analyses. Male sex, age < 5 years, *P. aeruginosa*, *Streptococcus* spp., *S. aureus*, and *coliform*, CSF and urine samples were shown to increase the prevalence of MDROs, while GNB was shown to reduce the MDROs detection rate. Different studies have reported different risk factors[27,31,37,41]. These differences might be linked to three factors. The first is related to the sample sizes used to identify the MDROs isolation rates, which were respectively 483, 1508, 1486, and 1222 for ICU China, Saudia Arabia, Ethiopia, and Gana, while our study included a total of 1766. The second is that the first three studies exclusively considered GNB, while this study examined both GNB and GBP. Third, differences among laboratory-, hospital-, and ICU-based studies. Additional research should be conducted to assess various factors not examined in this study, such as prior antibiotic use, length of hospital stay, patient's comorbidities, etc.

Strengths and limitations

In the strength of this study, we evaluated different types of clinical samples over four years. Furthermore, the data were collected from the PCDR, Faculty of Medicine, University of Gezira. This facility works as the reference laboratory and encompasses all hospital settings at Wad Medani. These findings provided an accurate depiction of the MDROs detection rate and associated risk factors.

The limitations of our study include the failure to record additional potential risk variables, such as clinical history, comorbidities, length of hospital stay, and prior antibiotic use. Given that the clinical samples were obtained for diagnostic purposes independently of this study. Additionally, due to a lack of resources and access to molecular techniques, we did not assess the existence of MDROs on a molecular basis.

Conclusion

This study revealed a moderate prevalence of MDROs among different clinical specimens. This phenomenon is more prevalent in males and GNB. *S. aureus* had the highest MDROs prevalence among GPB, and *Proteus* spp. had the highest MDROs prevalence among GNB, followed by *E. coli*. Concerning the factors associated with MDROs, male sex, *P. aeruginosa*, *Streptococcus* spp., *S. aureus*, and *coliform*, CSF, and urine samples were the most important risk factors for the prevalence of MDROs. These findings will be helpful in determining the factors associated with the emergence of MDROs in bacteria. Therefore, a regular assessment of these factors, bacterial profile, and antibiotic resistance should be carried out to develop an effective strategy for managing the emergence of MDROs. Implementing these findings enables the development of both short- and long-term strategies to prevent AMR, as well as development of antimicrobial stewardship and infection control measures in Sudan and other comparable low-resource environments. Additional prospective research is needed to assess the influence of comorbidities, duration of hospitalization, and previous antibiotic usage on the prevalence of MDROs, and to investigate the presence of MDROs at a molecular level.

Data availability

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

Received: 3 October 2024; Accepted: 30 April 2025
Published online: 04 May 2025

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Author contributions

Y.B.H. and S.F.A. designed the project. Y.B.H., S.F.A. and M.F.A. performed the statistical analysis. Y.B.H., S.F.A.., Z.M.A., W.S.M., F.O.A., Y.M.Y. and A.A.A. discussed the results and drafted the paper. Y.B.H., S.F.A. and M.F.A. drafted the final editing of paper. Y.B.H., S.F.A. and M.F.A. critically revised the paper. All authors have read and agreed to the published version of the manuscript.

Funding

This study did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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