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ORIGINAL ARTICLE

Multidrug-Resistant Bacteria Among Culture Isolates at University of Gondar, Specialized Referral Hospital, Northwest Ethiopia: a Five-Year Retrospective Study

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SUMMARY

Background: **Updated information on multidrug-resistant bacteria is essential because multidrug-resistant bacteria are one of the most important current threats to public health and prevalence of multidrug-resistant bacteria varies from time to time. A large number of people die annually due to infections caused by multidrug-resistant pathogens, with millions more suffering from serious infectious complications. The aim of this study was to determine the rate of multidrug-resistant bacteria isolates among culture requested patients at University of Gondar Specialized Referral Hospital, Northwest Ethiopia.**
Methods: **A hospital-based retrospective cross-sectional study was conducted on all culture-positive patients' results at University of Gondar Specialized Referral Hospital, Northwest Ethiopia.**
Results: **Of the 2,161 bacterial isolates, the overall prevalence of multidrug-resistant bacteria was 40.5% (876/2,161). The multidrug-resistance rate of Gram-negative bacteria was 52.4% (493/942) and Gram-positive bacteria was 31.4% (383/1,219). From all isolates, 22.3% (481/2,161) were from wound discharge followed by 19.57% (428/2,161) from blood and 18.14% (224/2,161) from urine. The predominant bacteria isolated were *S. aureus* (44.1% (952/2,161)) followed by *E. coli* (16.6% (359/2,161)), *Klebsiella* species (13.2% (179/2,161)), and *Citrobacter* (4% (86/2,161)). The isolates showed high levels of resistance to ampicillin (81.99% (692/844)), cotrimoxazole (66.62% (531/797)), and penicillin (62.66% (381/608)) and lower resistance to vancomycin (6.79% (46/677)), amikacin (20% (18/95)), and nitrofurantoin (20.90% (21/110)). Primary MDR isolates were *Serratia* species (83.3% (5/6)), *Enterobacter* species (66.7% (20/30)), *Klebsiella pneumonia* (65.3% (89/176)), and *Enterococcus* species (57.1% (8/14)). The majority of MDR isolates were from inpatients (58.6% (p-value < 0.0001)), males (53.77%), age group < 2 years (30.7%), and blood sample (25.22%).**
Conclusions: **A higher level of resistance to ampicillin, cotrimoxazole, penicillin and lower level of resistance to vancomycin, amikacin, and tobramycin were detected. Slightly lower overall rates of multidrug-resistant bacteria are isolated and a higher resistance rate is observed on *Serratia* species, *Enterobacter* species, *K. pneumonia*, *Enterococcus* species, and *E. coli*. Hospital admission is highly associated with the isolation of multidrug-resistant bacteria. Hence patient hospital stay should be considered in the prevention of multi-drug resistant bacterial infections.**
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KEY WORDS

multidrug resistance, antimicrobial, bacterial isolate

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LIST OF ABBREVIATIONS

AMR - Antimicrobial Resistant
CDC - Disease Control and Prevention
CLSI - Clinical Laboratory Standards Institute
CoNS - Coagulase Negative Staphylococcus
CSF - Cerebrospinal Fluid
ECDC - European Centre for Disease Prevention and Control
ESBL - Extended Spectrum Beta Lactamase
FDA - Food and Drug Agencies
ICU - Intensive Care Unit
IDSA - Infectious Diseases Society of America
MDR-MRSA - Multidrug Resistant-Methicillin Resistant *Staphylococcus aureus*
MDRO - Multidrug Resistant Organism
MRSA - Methicillin Resistant strain of *Staphylococcus aureus*
NMDR - Non-multidrug resistance
PBP - Penicillin Binding Protein
SD - Standard Division
SPSS - Statistical Package for the Social Sciences
UoGCSTH - University of Gondar Comprehensive Specialized Teaching Hospital
UTI - Urinary Tract Infection
WHO - World Health Organization

INTRODUCTION

The discovery of anti-microbial treatments has been one of the greatest achievements in the history of modern medicine. These drugs played a significant role in the prevention and treatment of various forms of infectious diseases across the globe. As a result, many potentially life-threatening diseases, deaths and disabilities were lessened. However, the subsequent use of anti-microbial agents was challenged by the occurrence of drug resistant pathogens [1,2]. Antibiotic resistance refers to the mechanism of bacteria to resist the effects of antibiotics, so the bacteria are not destroyed and their growth continues to occur [3]. It arises through various and complex molecular mechanisms [4]. A wide range of morbidities and mortalities are attributable to the resistance of important bacterial pathogens to common antimicrobial therapies. According to the WHO, approximately 700,000 people die each year due to the outcomes of antibiotic resistance. Annual deaths are expected to reach 10 million by 2050 [5]. A published report of the WHO provided a record of twelve bacteria and their families which become resistant to a large number of antibiotics. The antibiotic-resistant priority pathogens listed were: *Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacteriaceae, Enterococcus, Staphylococcus aureus, Helicobacter pylori, Campylobacter* species, *Salmonellae, Neisseria gonorrhoeae, Streptococcus pneumoniae, Haemophilus influenzae*, and *Shigella* species [6].
Multidrug resistance (MDR), a major threat worldwide, is defined as non-susceptibility to at least one agent in three or more antimicrobial drug classes [7]. Its emergence and spread in Gram-positive and -negative bacteria become a significant burden. Globally, infections caused by multidrug-resistant pathogens are responsible for large numbers of deaths and millions more cases of sufferings and complications. Infections due to MDR strains are accompanied by adverse outcomes at the individual, health care, and society levels. Consequences include increased infections as a result of failure or delay of antibiotic treatment, prolonged hospitalization, increased economic burden, loss of productivity, and impacts on the health care system as a whole [8-10]. Indiscriminate and improper use of antibiotics associated with rising income and affordability, lack of stewardship in hospital and poor control of over-the-counter sales are highly contributing to the rapid spread of MDR pathogens [11]. Developing countries are the leading victims of the condition where the irrational use of antimicrobials is of great concern [12,13].
In Ethiopia, the emergence of antimicrobial resistance is related to the challenges faced in identifying the specific etiologic agents and their antimicrobial susceptibility pattern due to the absence of standard laboratories and diagnostic tests. In addition, self-prescription of antibiotics, poor awareness of providers, and lack of access to local antibiogram are among the contributors. Limited capacity for microbiology testing combined with a high burden of life-threatening bacterial infections raises empirical antibiotic prescription, where AMR is noticed only after a therapeutic failure occurs [14].
Antimicrobial resistance profiles of bacteria vary across populations because of differences in geography, local antimicrobial prescribing practices, and prevalence of resistant bacterial strains [15]. These differences are not stable and may change rapidly especially in places where misuses of antibiotics are common particularly in developing countries. So, updated information on multidrug resistance bacteria could improve clinical practice by guiding empirical antibiotic choice. It is also essential for devising future research interventions and management strategies that address antibiotic resistance. The main aim of this research was to identify multidrug-resistant bacteria from different clinical specimens at the University of Gondar and to give updated information. This may guide the rational use of the existing antimicrobial agents and clinical decision making.

MATERIALS AND METHODS

This study was conducted at the University of Gondar Comprehensive specialized hospital located in Gondar. The hospital provides healthcare services for over five million people in Gondar town and the surrounding districts and it has an accredited referral level laboratory with seven sections including a separate reception room. The microbiology section is one of the principal areas, and it is estimated that 9,600 samples are delivered an-

nually. In this section, culturing is one of the main activities, mainly applicable to bacterial isolation and identification. A five-year hospital based retrospective study was conducted from January 2014 to December 2018. Data were collected and reviewed from the Medical Microbiology Laboratory's registration book by using a checklist. Patient's information such as socio demographic characteristics, clinical data and antimicrobial sensitivity test results were obtained from the record book. We examined results that registered culture confirmed bacteria and antimicrobial susceptibility tests that were done according to the standard operational procedure and also resistance patterns interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (2018) [16]. In the Microbiology Laboratory the bacteria were identified based on morphological characteristics, Gram stain, and biochemical tests. Identification of Gram-positive bacteria was done using Gram stain, hemolytic activity on blood agar plates, catalase reaction, and coagulase test. Gram-negative bacteria were identified based on colony morphology on blood agar and MacConkey agar, followed by biochemical reactions namely oxidase, triple sugar iron, Sulphur-Indole-Motility, citrate, lysine decarboxylase and urease tests. After bacterial identification, antimicrobial susceptibility tests were done on Mueller-Hinton agar using the Kirby-Bauer disk diffusion method for ampicillin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, norfloxacin, gentamicin, oxacillin and sulfamethoxazole, penicillin, erythromycin, clindamycin, cefoxitin, vancomycin, tetracycline, and amoxicillin. Data were entered to EPI-Info version-7 to check data completeness and data clearance then transferred to SPSS version 20 for analysis. Summary statistics were performed using frequencies, mean, standard deviation, and proportions for categorical data. Chi-square test was employed to compare the proportion of bacterial isolates between variables. p-value less than 0.05 at 95% confidence interval was considered statistically significant.

RESULTS

Socio-demographic characteristics

A total of 2,161 bacterial isolates, each with a complete record, were included in this study at the University of Gondar Referral Teaching Hospital from January 2014 to December 2018. The majority of the isolates were from males (52.9% (1,144/2,161)). The age range of the study participants were from 1 to 93 years, with a mean ± SD age of 20.29 ± 20.315 years and the highest patient age category was less than 5 years (34.6% (747/2,161)), followed by 19 - 29 years of age (15.5% (336/2,161)). The majority (43.1% (428/993)) of the inpatients are under five years of age (p-value < 0.001) and 56.2% (558/993) of the inpatients are males (p-value 0.005) (Table 1).

Prevalence of bacterial isolates

Bacterial isolates were collected from different sources of clinical specimens, including blood, wound discharge, secretions, urine, pus, or any other site that was clinically suspected for bacterial infection and *S. aureus* was the predominant isolate (44% (952/2,161)) followed by *E. coli* (16.6% (359/2,161)), *Klebsiella pneumonia* (9.2% (179/2,161)) and *Citrobacter* (4% (86/2,161)). The majority of bacteria were isolated from wound discharge (22.3% (481/2,161)) followed by blood (19.57% (428/2,161)) and urine (18.14% (396/2,161)). The predominant isolates obtained from wound discharge was *S. aureus* (13.5% (291/2,161)) followed by *E. coli* (2.25% (53/2,161)) and *Citrobacter* (1.11% (24/2,161)), while *S. aureus* (8.8% (190/2,161)) and *E. coli* (8.6% (185/2,161)) were the most common isolate from blood and urine samples, respectively. *Salmonella* (1.2% (16/2,161)), *Shigella* species (0.05%) and *S. aureus* (0.1%) were the only pathogen isolated from stool. *N. gonorrhoeae* (0.14% (4/2,161)), *E. coli* (0.1% (3/2,161)) and *S. aureus* (0.1% (3/2,161)) were the only organisms isolated from vaginal discharge (Table 2).

Antimicrobial susceptibility patterns of isolates

In this study, bacterial isolates showed a higher level of resistance to ampicillin (692/844 (81.99%)) cotrimoxazole (531/797 (66.62%)), and penicillin (381/608 (62.66%)). In contrast, vancomycin (623/677 (92.31%)), tobramycine (4/5 (80%)), and amikacin (72/95, (75.75%)) were found to have a high level of susceptibility rate (Table 3).

Prevalence of MDR isolates

In this study, the MDR status of bacteria was tested against 13 classes of antimicrobials. The overall prevalence of MDR bacterial infections in this study was 40.5% (876/2,161). Of all MDR isolates, the majority were from male patients (471/876 (53.77%)) and children < 2 years (269/876 (30.7%)) represented the highest proportion of patients with MDR bacterial infections. This was followed by age group 20 - 29 years (129/876 (14.73%)) and adolescents 10 - 19 years (116/876 (13.24%)). Statistically significant association was seen in age group 10 - 19 (Table 4).

The overall MDR rate of Gram-negative bacteria was 493/942 (52.3%). Higher rates of MDR isolates were seen among *Serratia* spp. (5/6 (83.3%)), *Enterobacter* spp. (20/30 (66.7%)), *Klebsiella pneumonia* (89/176 (65.4%)), and *E. coli* (119/359 (54%)). Additionally, 66.6% of *Serratia* spp. was resistant to five classes of antibiotics. On the other hand, the MDR rate of Gram-positive isolates was 383/1,219 (31.4%). Higher rates of MDR were obtained among *Enterococcus species* (8/14 (57.14%)), coagulase negative *Staphylococci* (CoNS) (40/102 (39.2%)), and *S. aureus* (303/952 (31.8%)). No MDR rate for Providencia referigeri and Nisseria meningitides were detected (Table 5).

Table 1. Frequency of study participants according to age group, gender and patients' visits at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

Characteristics		Inpatient Frequency (%)	Outpatient Frequency (%)	p-value	OR (95% CI)
Age group (years)	Less than 5	428 (43.1)	319 (27.3)	0.000	2.22 (1.65, 2.97)
	5 - 9	72 (7.3)	67 (5.7)	0.007	1.78 (1.17, 2.70)
	10 - 19	132 (13.3)	192 (16.4)	0.459	1.14 (0.81, 1.60)
	20 - 29	124 (12.5)	212 (18.2)	0.844	0.97 (0.70, 1.35)
	30 - 39	86 (8.7)	139 (11.9)	0.906	1.02 (0.71, 1.48)
	40 - 49	56 (5.6)	82 (7.0)	0.577	1.13 (0.74, 1.73)
	50 & above	95 (9.6)	157 (13.4)		
Gender	Male	558 (56.2)	586 (50.2)	0.005	1.27 (1.08, 1.51)
	Female	435 (43.8)	582 (49.8)		
	Total	993 (100.0)	1,168 (100.0)		

In this study, the predominant MDR isolates were obtained from urine (25.57% (224/876)), blood (25.22% (221/876)), and wound discharge (19.3% (169/876)). However, no MDR isolates were detected from cervical discharge, biopsy, and tongue patch (Table 6).

Among MDR isolates, 58.60% (516/876) were from inpatients while the rest were from outpatient visits (41.40% (360/876)) (p-value < 0.0001; (OR (95% CI), 0.412 (0.345 ± 0.491)). In this study, a decreasing trend of MDR was observed over the last 5 years. This decrease was statistically significant (Table 7).

The prevalence of MDR was higher in 2014 than in other years. Moreover, data comparison of 2017 and 2018 showed an increased prevalence of MDR from 34.96% to 40.50% (Figure 1).

DISCUSSION

Multidrug-resistant bacterial infection has become a real threat in developing countries, including Ethiopia. In this study, pathogenic bacteria isolated from various clinical specimens showed drug resistance. The predominant bacteria isolated included *S. aureus* (44%) followed by *E. coli* (16.6%) and *Klebsiella* species (13.74%). This finding agrees with previous studies done in Ethiopia [17,18]. The possible reason for the high frequency is that the majority of these isolates are normal flora on the skin and gut of healthy individuals. Once they breach the skin and soft tissues and are displaced from their resident to other sterile sites, they can easily disseminate [19].

In this study, a higher proportion of bacterial isolates were reported in the age group of 0 - 5 years. This finding is in agreement with another study in Ethiopia [17]. The possible reason for the high prevalence in this age group might be the lack of resistance, previous exposure to infections, poor personal hygiene, and higher exposure to contaminated environments. Concerning the antimicrobial resistance, overall high levels of resistance were demonstrated against ampicillin (81.99%) followed by cotrimoxazole (66.62%), penicillin (62.66%), and tetracycline (61.08%). This finding is supported by previous studies in Ethiopia [17,20]. It might be because of easy availability and over and indiscriminate use of these drugs outside the hospitals. On the contrary, our results showed inconsistencies when compared with those reported by others studies [21,22]. The potential differences in the rate might be due to differences in the study population, drug usage, and medical setup. On the other hand, vancomycin, tobramycin, amikacin, and ciprofloxacin were the most effective antibiotics having sensitivity rates of greater than 70%. This finding aligned with another study in Ethiopia [22].

The overall MDR rate of the isolates in this study was 40.5% which was consistent with studies conducted in other parts of Ethiopia and world [22-27] where 35.5 - 55.7% MDR rate was reported. Our study was lower than reports from other studies in Ethiopia and other countries [17,23]; they reported MDR rates of 70.4% - 90.4%. It may be due to global interventions made by WHO, CLSI, and other organizations on the control measures of MDR. Multi-drug resistant Gram-negative bacteria species have become a big threat to the world, especially for developing countries [17,19,28]. This study also showed similar trends of MDR by Gram negative bacteria. This might be that in developing countries there is an irrational use of antimicrobials, improper dosage regimens, misuse of antimicrobials, and extended duration of therapy. As a result, Gram-negative bacteria easily resist antibiotics and result in increased antimicrobial resistance [29]. Among Gram-negative bacteria, *Serratia* spp., *Enterobacter* spp., and *K. pneumonia* were reported as the most predominant

Table 2. Frequency of bacterial isolates from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 – 2018 (Part 1).

Types of bacteria isolated (n)	Type of specimen (%)																Total (%)
	AF	TP	AD	TD	Urine	ABD	ND	VD	Sputum	UD	WD	ED	EARD	UMBD	Pus	PLUF	
S. aureus (952)	0.10	0.05	0.05	0.10	2.73	0.05	0.14	0.14	0.14	0.04	13.5	0.83	3.37	0.2	9.6	0.9	44
Proteus species (81)	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0	1.03	0.28	1.62	0.0	0.23	0.05	3.54
Shigella species (35)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.09	0.05	0.0	0.0	0.05	0.0	1.62
Salmonella spp. (16)	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.05	0.0	0.05	0.0	0.76
Citrobacter spp. (86)	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	1.11	0.14	0.42	0.0	0.3	0.1	4
Enterobacter spp. (30)	0.0	0.0	0.0	0.0	0.56	0.05	0.0	0.0	0.0	0.0	0.14	0.05	0.05	0.0	0.3	0.0	1.41
Pseudomonas spp. (11)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.19	0.0	0.0	0.0	0.52
Streptococcus Viridians (55)	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.42	0.05	0.14	0.0	0.7	0.0	2.56
Providencia spp. (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.05	0.05	0.0	0.0	0.0	0.15
S. agalactia (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.09	0.0	0.05	0.0	0.1	0.1	0.37
Escherichia coli (359)	0.05	0.0	0.0	0.05	8.6	0.0	0.14	0.14	0.0	0.0	2.65	0.09	0.1	0.0	1.8	0.1	16.6
P. aeroginosa (23)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.18	0.09	0.5	0.0	0.1	0.05	1.07

Table 2. Frequency of bacterial isolates from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018 (Part 1 - continued).

Types of bacteria isolated (n)	Type of specimen (%)								
	Blood	PF	SF	Stool	CSF	BD	Biopsy
S. aureus (952)	8.8	0.6	0.79	0.10	0.6	1.0	0.05
Proteus species (81)	0.18	0.0	0.05	0.0	0.0	0.0	0.05
Shigella species (35)	0.2	0.0	0.0	1.2	0.09	0.0	0.0
Salmonella spp. (16)	0.3	0.0	0.0	0.05	0.05	0.0	0.0
Citrobacter spp. (86)	0.6	0.05	0.05	0.0	0.1	0.05	0.0
Enterobacter spp. (30)	0.2	0.05	0.0	0.0	0.0	0.0	0.0
Pseudomonas spp. (11)	0.14	0.05	0.0	0.0	0.0	0.0	0.0
Streptococcus Viridians (55)	0.93	0.09	0.05	0	0.09	0.0	0.0
Providencia spp. (3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. agalactia (8)	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Escherichia coli (359)	1.5	0.7	0.05	0.0	0.1	0.0	0.0
P. aeroginosa (23)	0.05	0.0	0.0	0.0	0.5	0.0	0.0

Table 2. Frequency of bacterial isolates from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018 (Part 2).

Types of bacteria isolated (n)	Type of specimen (%)							Total (%)
	WD	ED	EARD	UMBD	Pus	PLUF		
H. influenzae (8)	0.05	0.0	0.14	0.0	.05	0.0		0.39
N. gonorrhoeae (9)	0.0	0.0	0.0	0.0	0.0	0.0		0.42
N. meningitides (3)	0.0	0.0	0.0	0.0	0.0	0.0		0.14
Serratia spp. (6)	0.05	0.0	0.0	0.0	0.0	0.05		0.29
S. pyogenes (61)	0.74	0.19	0.32	0.0	0.7	0.1		2.82
Enterococcus spp (14)	0.14	0.05	0.0	0.0	0.0	0.0		0.65
S. pnemonea (27)	0.12	0.05	0.09	0.0	0.1	0.0		1.65
Morganella spp. (1)	0.0	0.0	0.0	0.0	0.0	0.0		0.5
CoNS (92)	0.37	0.0	0.19	0.0	0.2	0.2		4.54
Other Klebsiella spp. (95)	0.32	0.14	0.32	0.0	0.8	0.1		4.54
K. pnemonea (176)	0.97	0.19	0.5	0.0	0.6	0.3		9.2
Total (2,161)	22.3	2.25	8.1	0.2	16.2	1.99		100

Table 2. Frequency of bacterial isolates from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018 (Part 2 - contined).

Types of bacteria isolated (n)	Type of specimen (%)																	
	Blood	PF	SF	Stool	CSF	BD	Biopsy	AF	TP	AD	TD	Urine	ABD	ND	VD	Sputum	UD
H. influenzae (8)	0.0	0.05	0.0	0.0	.05	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0
N. gonorrhoeae (9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2
N. meningitides (3)	0.0	0.0	0.0	0.0	0.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0
Serratia spp. (6)	0.14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0
S. pyogenes (61)	0.3	0.14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.14	0.0	0.05	0.0	0.05	0.0
Enterococcus spp (14)	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.19	0.0	0.0	0.0	0.0	0.0
S. pnemonea (27)	0.2	0.14	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	.05	0.0	0.0	0.0	0.0	0.04	0.0
Morganella spp. (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0
CoNS (92)	2.13	0.09	0.09	0.0	.05	.05	0.0	0.0	0.0	0.0	0.0	1.15	0.0	0.0	0.0	0.0	0.0
Other Klebsiella spp. (95)	1.4	0.19	0.09	0.0	0.05	0.0	0.0	0.0	0.0	0.05	0.0	1.2	0.0	0.0	0.05	0.0	0.0
K. pnemonea (176)	2.6	1.14	0.05	0.0	0.32	0.05	0.0	0.0	0.0	0.0	0.0	2.22	0.09	0.0	0.05	0.0	0.0
Total (2,161)	19.57	3.29	1.21	1.25	2.08	1.21	0.1	0.19	0.05	0.05	0.29	18.14	0.19	0.43	0.60	0.32	0.28

PF - peritoneal, SV - synovial fluid, BD - breast discharge, AS - ascetic fluid, TP - tongue patch, AD - anal discharge, TD - tracheal discharge, AD - a95bdominal discharge, UR - urethral discharge, CV - cervical discharge, WD - wound discharge, ED - eye discharge, EARD - ear discharge, UMBD - umbilical discharge, PLUF - pleural fluid.

Table 3. Overall antimicrobial resistance pattern of bacteria isolated from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

Antimicrobial agent	Frequency	Susceptibility pattern		
	n	Resistant (%)	Sensitive (%)	Intermediate (%)
Ampicillin	844	81.99	15.52	2.49
Amoxicillin/clavulanate	692	53.60	40.5	5.89
Ceftriaxone	870	32.5	64.1	3.4
Chloramphenicol	1,008	31.8	64.93	3.27
Ciprofloxacin	1,147	27.28	70.88	1.84
Clindamycin	534	23.97	54.3	21.73
Erythromycin	878	45.44	52.84	1.72
Gentamicin	841	37	58.97	4.03
Methicillin	68	44.11	51.47	4.42
Naldic acid	272	44.11	50.73	5.16
Norfloxacin	557	33.03	64.45	2.52
Vancomycin	677	6.79	92.31	0.9
Penicillin	608	62.66	33.38	3.96
Cotrimoxazole	797	66.62	31.86	1.52
Tetracycline	997	61.08	29.78	9.14
Cefuroxime	252	37.69	58.73	3.58
Cefoxitin	270	36.66	62.59	0.75
Augementin	205	50.73	40	9.27
Oxacillin	367	44.95	44.14	10.91
Doxycycline	60	45	43.33	11.67
Nitrofurantoin	110	20.90	73.63	5.47
Tobramycine	5	20	80	0.00
Ceftazidime	28	39.28	57.14	3.58
Amikacin	95	18.94	75.78	5.28
Meropinime	40	50	50	0.00

n - total no. of antibacterial agents.

Table 4. Age and gender specific distribution of MDR bacteria at University of Gondar Specialized Referral Hospital, North-west Ethiopia, 2014 - 2018.

Age (years)	Gender		Total (n/%)	p-value
	Male (n, %)	Female (n, %)	Male + female	
< 2	160/269 (18.26)	109/269 (12.44)	269 (30.7)	-
2 - 9	64/112 (7.30)	48/112 (5.47)	112 (12.78)	0.086
10 - 19	68/116 (7.76)	48/116 (5.48)	116 (13.24)	0.007
20 - 29	48/129 (5.48)	81/129 (9.24)	129 (14.73)	0.051
30 - 39	43/91 (4.90)	48/91 (5.48)	91 (10.40)	0.242
40 - 49	24/56 (2.74)	32/56 (3.65)	56 (6.40)	0.348
50 - 59	21/38 (2.40)	17/38 (1.94)	38 (4.33)	0.147
60 - 69	19/32 (2.16)	13/32 (1.48)	32 (3.65)	0.309
≥ 70	24/33 (2.74)	9/33 (1.02)	33 (3.76)	0.578
Total	471/876 (53.77)	405/876 (46.23)	876 (100)	0.524

n - represents total frequency of MDR isolates in specific age group and gender.

Table 5. Frequency and resistant pattern of MDR isolates from different clinical specimens at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

Bacterial isolates (n)	MDR		Class of antimicrobial n (%)				
Gram positive bacteria	n	%	R3	R4	R5	R6	≥ R7
Staphylococcus aureus (952)	303	31.8	154 (16.17)	94 (9.87)	43 (4.5)	9 (0.95)	3 (0.32)
Streptococcus species (151)	32	21.2	20 (13.24)	7 (4.6)	2 (1.32)	0 (0.00)	0 (0.00)
Enterococcus species (14)	8	57.14	4 (28.6)	3 (21.4)	1 (7.14)	0 (0.00)	0 (0.00)
CoNS (102)	40	39.2	12 (11.7)	9 (8.8)	9 (8.8)	0 (0.00)	0 (0.00)
Total (1,219)	383	31.4	190 (15.5)	113 (9.3)	55 (4.5)	9 (0.7)	3 (0.2)
Gram negative bacteria							
Proteus species (81)	41	50.6	6 (7.4)	12 (14.8)	4 (4.9)	1 (1.2)	1 (1.2)
Salmonella species (16)	9	56.2	3 (18.7)	3 (18.7)	1 (6.2)	2 (12.5)	0 (0.00)
Shigella species (35)	7	20	2 (5.7)	3 (8.5)	2 (5.7)	0 (0.00)	0 (0.00)
Citrobacter spp. (86)	47	54.6	21 (24.4)	12 (13.9)	9 (10.5)	3 (3.5)	2 (23.3)
Enterobacter spp. (30)	20	66.7	10 (33.3)	5 (16.6)	4 (13.3)	1 (3.3)	0 (0.00)
Pseudomonas species (11)	6	54.5	2 (18.2)	3 (27.3)	0 (0.00)	1 (9)	0 (0.00)
Providencia species (3)	0	0.00	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Escherichia coli (359)	194	54	85 (23.7)	69 (19.2)	32 (9.5)	6 (1.7)	2 (0.6)
Pseudomonas aeroginosa (23)	11	47.8	6 (26.1)	5 (21.7)	0 (0.00)	0 (0.00)	0 (0.00)
Haemophilus influenzae (8)	1	12.5	1 (12.5)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Nisseria gonorrhoeae (9)	3	33.3	2 (22.2)	1 (11.1)	0 (0.00)	0 (0.00)	0 (0.00)
Nisseria meningitides (3)	0	0.00	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Serratia spp. (6)	5	83.3	1 (16.6)	0 (0.00)	4 (66.7)	0 (0.00)	0 (0.00)
Marginalia morganii (1)	0	0.00	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Others Klebsiella species (95)	60	6.74	24 (0.2.70)	22 (2.44)	8 (8.4)	2 (2.1)	3 (3.2)
Klebsiella pneumonia (176)	89	65.4	37 (27.2)	30 (22.1)	16 (12)	6 (4.4)	0 (0.00)
Total (942)	493	52.3	226 (23.9)	147 (16)	78 (8.3)	29 (3.1)	7 (0.7)
Over all Total (2,161)	876	100	416 (47.48)	280 (32)	133 (15)	38 (4.33)	9 (1.05)

n - represent frequency bacteria isolated, n - represent frequency MDR isolates, R3 - Resistance for three classes of antimicrobials, R4 - Resistance for four classes of antimicrobials, R5 - Resistance for five classes of antimicrobials, R6 - Resistance for six classes of antimicrobials, ≥ R7 - Resistance for seven or more classes of antimicrobials.

Table 6. Percentage of MDR isolates from clinical specimens at University of Gondar, Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

Types of specimens	MDR in n (%)	Types of specimens	MDR in n (%)
Blood	221 (25.22)	Urine	224 (25.57)
Peritoneal fluid	20 (2.3)	Wound discharge	169 (19.3)
Synovial fluid	5 (0.6)	Abscess	46 (5.25)
Stool	6 (0.68)	Eye discharge	20 (2.30)
CSF	10 (1.14)	Ear discharge	72 (8.22)
Breast discharge	9 (1.02)	Pus	40 (4.56)
Biopsy	0 (0)	Pleural fluid	13 (1.48)
Ascetic fluid	1 (0.14)	Tongue patch	0 (0)
Anal discharge	1 (0.14)	Tracheal discharge	1 (0.14)
Abdominal discharge	2 (0.30)	Nasal discharge	3 (0.34)
Vaginal discharge	5 (0.57)	Sputum	3 (0.34)
Urethral discharge	4 (0.46)	Cervical discharge	0 (0)
Umbilical discharge	1 (0.14)	Total	876 (100)

Table 7. Five-year trends of MDR bacteria at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

Years	No. of bacterial isolated		p-value	OR (95% CI)
	NMDR	MDR		
2014	287/541	254/541	-	-
2015	209/362	153/362	0.166	0.827 (0.633 ± 1.082)
2016	232/362	130/362	0.001	0.633 (0.482 ± 0.832)
2017	279/429	150/429	0.000	0.607 (0.468 ± 0.788)
2018	278/467	189/467	0.039	0.768 (0.598 ± 0.987)

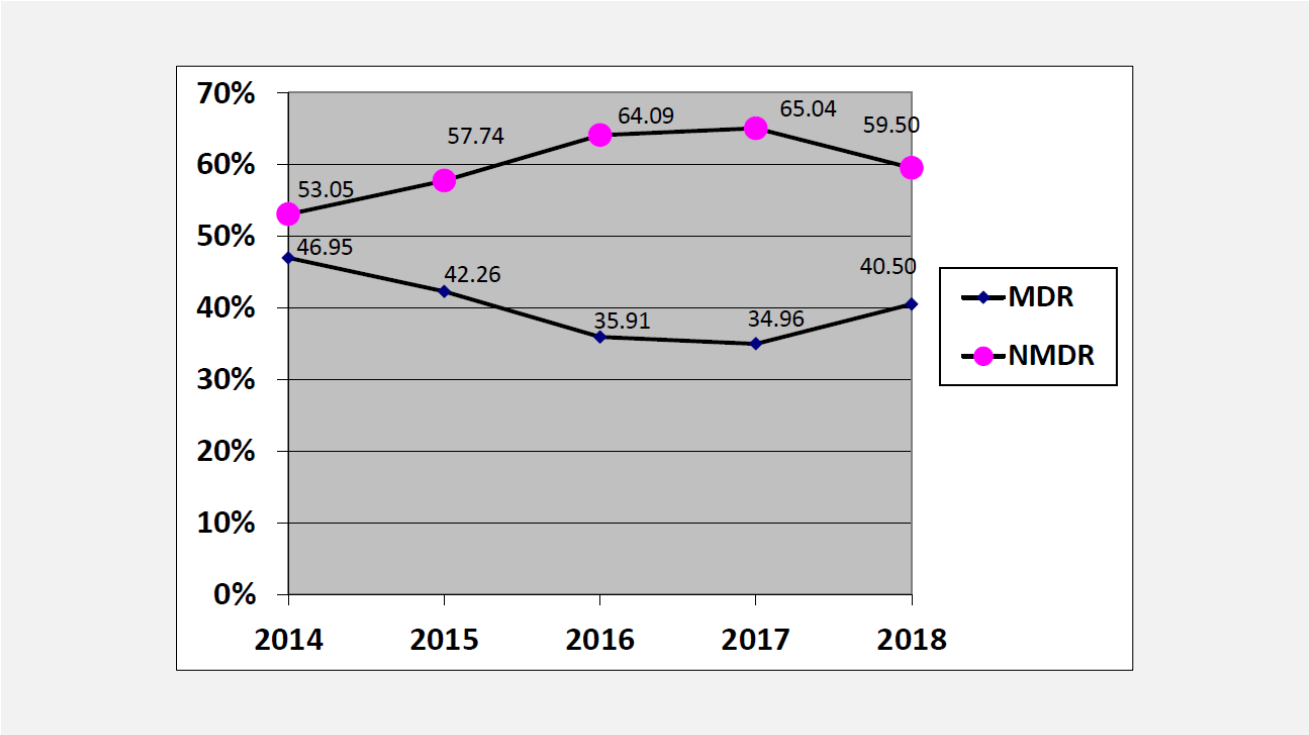

Figure 1. Five-year trends of multidrug resistant bacteria isolated at University of Gondar Specialized Referral Hospital, Northwest Ethiopia, 2014 - 2018.

MDR isolates in this study. This result is inconsistent with other studies and the possible explanation for this disparity is the difference in the types of bacteria isolated and diagnostic techniques utilized among hospitals. In this study, 31.4% of Gram-positive bacteria were demonstrated as MDR. This was similar with the 34.5% MDR rate documented for these bacteria in Ethiopia [23].

In contrast to our findings, 72.2 - 84.4% MDR was reported in others studies of Ethiopia [17,21,30]. Such disparity might be the difference in the type of organism isolated, study population, antimicrobial prescription pattern, study area in terms of laboratory infrastructure, infection prevention practices, poor up-to-date knowledge of clinicians on AMR, and operational definition of MDR. Of the Gram-positive isolates, about 57.14% of *Enterococcus* species, 31.8% of *S. aureus*, and 29% CoNS were MDR. In the case of *S. aureus*, our result lined up with studies conducted in Ethiopia and other parts of the world [19,23,25,30]. However, it was inconsistent with others with respect to *Enterococcus* species and CoNS. This disparity might be attributed to the different type of organism isolated and also diagnostic techniques employed.

In our study, the number of MDR isolates was higher in males (53.77%) and in the age group less than two years

(30.7%). Similarly, a statistically significant higher association was observed among inpatients (58.6%) than outpatients (41.4%) with MDR. This variation might be because inpatients are exposed to contaminated hospital environments, surgical equipment, and ill patients during the stay in hospital, this may lead to hospital acquired MDR infection. The other possible reason is that inpatients are more likely to be immune suppressed compared to outpatients. Hence, they are more exposed to a more infection possibilities than outpatients [31]. In this study, a decreased trend of MDR was observed in the past 5 years. This might be due to intervention and efforts made by WHO, CLSI, and others organizations on serious public health effects of MDR for the last five years and encouraging increased knowledge for clinicians and laboratory professionals on AMR in the study area.

CONCLUSION

The most significant finding revealed by the present study was the decrease in the rate of multidrug resistant bacteria over three years (2016 to 2018). The isolation rate of *S. aureus*, *E. coli*, and *Klebsiella* species were high. The isolates showed higher levels of resistance to ampicillin, cotrimoxazole, and penicillin and lower levels of resistance were seen for vancomycin, amikacin, and tobramycine. A higher resistance rate is observed on *Serratia* species, *Enterobacter* species, *K. pneumonia*, *Enterococcus* species, and *E. coli*. Hospital admission was highly associated with MDR. Hence patient hospital stay should be considered in the prevention of multi-drug resistant bacterial infections. Continuous and periodic evaluation on the rate of MDR is needed to provide updated information for clinicians in choosing the appropriate antibiotic for the optimum outcome.

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Ethics approval and consent to participate: Ethical approval was obtained from the research and ethical review committee of the School of Biomedical and Laboratory Science at the University of Gondar, College of Medicine and Health Sciences. Patient records or information were kept confidential after collection. Permission to collect the data from the registration book was obtained from the Hospital administration and Medical microbiology laboratory staffs.

Consent for Publication:
Not applicable.

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

Authors' Contributions:
All authors contributed equally to the proposal development, analysis of data, and interpretation of the result and write up of the manuscript.

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The authors declare that they have no competing interests.

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