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Multidrug resistant (MDR) and extensively drug-resistant (XDR) gram negative bacteria at a tertiary care hospital, in Lalitpur, Nepal

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Abstract

Introduction: Prevalence of multi and extensively drug resistant (MDR and XDR) gram negative bacteria (GNB) are increasing trend and becoming a threat to public health. There is no single effective antibiotic to treat these pathogens. These superbugs need continuous surveillance to develop relevant treatment protocols and early therapeutic approaches. In this study, we evaluated the trends in prevalence, etiology and antibiogram of MDR and XDR GNB isolated from various samples in a tertiary care hospital.

Method: It was a hospital based descriptive cross-sectional study conducted at Patan Hospital, Patan Academy of Health Sciences. All the records of the patient whose culture and sensitivity report yielded gram negative bacteria from 1st January 2021 to 31st December of 2023 was extracted from the hospital electronic database and analyzed. Ethical approval was taken from the institutional review committee.

Result: The prevalence of MDR GNB was found to be at 22.30%. The prevalence of XDR GNB among the total isolated GNB was 9.41%. *Acinetobacter baumanii complex, Klebsiella spp., Escherichia coli* and *Pseudomonas aeruginosa* were the most common MDR and XDR pathogens encountered. For the antibiotics tested, a higher number of isolates were susceptible to carbapenem group (32%) followed by aminoglycosides (28.3%) doxycycline (26%) and piperacillin-tazobactam (17.4%).

Conclusion: More than 22% of patients with GNB infection were multidrug resistant. Aminoglycosides and carbapenems are good options for treatment of MDR-GNB infection however treatment should be based on culture and sensitivity reports. When treating XDR-GNB infection, adding doxycycline can also be considered.

Keywords: Antimicrobial; Gram Negative; Healthcare; Infection; Multidrug Resistance; Prevalence

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Introduction

Antimicrobial resistance (AMR) is a global health and development threat. Especially alarming is the rapid global spread of multi- and pan-resistant bacteria (also known as "superbugs") causing infections that are not treatable with existing antimicrobials.[1] Multi drug resistance (MDR) is defined as non-susceptible result obtained from in-vitro antimicrobial susceptibility testing to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. The antimicrobial categories used to define MDR and XDR are aminoglycosides, carbapenems, fluoroquinolones, antipseudomonal penicillin + b-lactamase inhibitors and extended-spectrum cephalosporins.[2]

In recent years, infections caused by MDR Gram-negative bacteria (MDR-GNB) have been reported to have increased significantly worldwide.[3,4] They include *Acinetobacter, Pseudomonas* and various *Enterobacteriaceae*.[5] Few studies from India showed prevalence of MDR-GNB ranging from 26% to 34% (12.1% being XDR) but one study from Pakistan showed it to be 64%.[3,6-8] In Nepal, MDR-GNB prevalence showed variation, ranging from 34% to 91%.[9-15]

Prevalence of these MDR and XDR GNB are in increasing trend and are a threat to public health. There is no single effective antibiotic to cover these pathogens and these pathogens need continuous surveillance aiming to develop relevant treatment protocols and early therapeutic approaches. In this study, we evaluated the changes in prevalence, etiology and antibiogram of MDR and XDR GNB isolated from various samples at a tertiary care hospital.

Method

The study was conducted in the Microbiology unit of pathology and lab medicine department and record section of Patan Hospital, Patan Academy of Health Sciences, Lalitpur, Nepal. The unit processes approximately 45,000 culture and sensitivity tests every year on different clinical samples. It was a hospital based descriptive cross-sectional study where all the records of the patient whose culture and sensitivity report yielded gram negative bacilli (GNB) from 1st January 2021 to 31st December of 2023 was analyzed. The hospital microbiology laboratory identified GNB using conventional methods like inoculation of samples on 5% sheep blood agar, MacConkey agar and performing Gram's stain and biochemical tests. Antibiotic Sensitivity testing (AST) was performed using the modified Kirby Bauer disk diffusion method and broth dilution of Colistin and polymyxin B to determine minimum inhibitory concentration for *Acinetobacter baumannii complex* and *Pseudomonas* as recommended in the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA) guidelines.[16] WHO AWaRe (Access, Watch and Reserve group of antibiotics) classification for antibiotic stewardship was also followed when antibiotics were used for sensitivity testing and reporting.[17]

All demographic profiles and laboratory culture results of the patients were acquired from the electronic database of the hospital information system. Subsequently, the data underwent conversion from open document spreadsheet (ODS) to EXCEL format and a thorough data cleaning process. Various clinical samples were categorized into streamlined sample groups such as blood, respiratory (For e.g.: sputum, bronchoalveolar lavage, tracheal aspirate, throat swab were all categorized into one respiratory sample) and urogenital, among others. Instances of multiple entries for the same patient within a 30-day period, each with a different laboratory number but featuring the same isolated organism, were meticulously addressed by eliminating duplicate entries within the EXCEL spreadsheet. The dataset encompassed antibiotic resistance patterns presented in tabulated form, alongside demographic data and unique inpatient hospital numbers. The total cost incurred from admission to discharge for the admitted patient whose culture report during the course of admission showed MDR or XDR GNB irrespective of cause of admission was analyzed. All the Records of GNB with already established intrinsic resistance to more than three different classes of antibiotic were also excluded from the study. The tested antibiotics were categorized into MDR defining categories and special formula series (=COUNTIF (A7:B7, "R"); =IF(K7>0, "R"); =COUNTIF (O7:R7, "R"); =IF(S7>2,"MDR","False"); =IF(T7>4,"XDR","False")) was designed in Excel and only the data set defining the MDR and XDR pathogens were sorted.

Data were exported and analyzed using the statistical software Stata v15.1 (StataCorp, College Station, Tx, USA). Data were summarized using frequencies and percentages.

The study obtained ethics approval from the Institutional Review Committee of Patan Academy of Health Sciences, Lalitpur, Nepal (Ref: 2107131552.

A1 /Date: 16 Feb 2024). As this study involved analyzing retrospective data from routine records stored in the hospital database system, the need for informed consent was waived; data confidentiality was maintained throughout the study.

Result

The microbiology unit of Patan Hospital, Patan Academy Health of Sciences received and processed 1,34,116 different clinical samples between the period of 1st January 2021 and 31st December of 2023 (34,169 in 2021; 48,958 in 2022 and 50,992 in 2023). Out of these, GNB were isolated from 11,390 samples (3070 in 2021, 4155 in 2022 and 4165 in 2023). The prevalence of MDR GNB was found to be 2540(22.30%) of 11390 isolates Among these MDR GNB, 1072(42.20%) were found to be XDR. The prevalence of XDR GNB among the total isolated GNB was 1072(9.41%), Table 1.

In those with GNB infection, the prevalence of MDR-GNB and XDR GNB was similar in both the male (52% in 2021; 50.40% in 2022 and 46.80% in 2023) and female (48% in 2021; 49.60% in 2022 and 53.22% in 2023). Majority of the MDR-GNB infection was seen in age 35 years and above but XDR-GNB were seen in the age group of <1Year and in patients above 50 years of age. Of the 2540 MDR-GNB isolates during the three-year period, 986(38.81%) were isolated from samples collected in out-patient department (OPD) while 1554(61.18%) originated from inpatients department (IPD). Of the total samples, 840(78.35%) of the XDR-GNB were isolated from samples that originated from inpatient departments while 232(21.64 %) were isolated from out-patient departments. Of the total IPD samples 539(38.77%) were from the Medicine department followed by Surgery, Obstetrics/gynecology, Pediatrics and Orthopedic department. Urine [1076(42.36%)] accounted for majority of the sample that yielded MDR-GNB followed by pus and wound swab, respiratory sample and blood but XDR-GNB was much higher in respiratory sample [(334(76.08%)] followed by urine, pus and wound swab and blood, Table 2. Out of the 1554 admitted patients suffering from MDR-GNB infection, 201(12.65%) died. Of the 840 patients admitted and infected with XDR-GNB, 149(17.61%) died during the course of their disease. Of the organism isolated, 482 (76.87%) *Acinetobacter baumannii* were MDR followed by *Klebsiella pneumoniae* [570 of 1738 (32.76%)]; *Klebsiella aerogenes* [98 of 367(26.70%)] *Klebsiella oxytoca* [70 of 310(22.58%)]; *Escherichia coli* [1169 of 6723(17.39%)] and *Pseudomonas aeruginosa* [94 of 697(13.49%)]. Of these MDR isolates, 408(84.65%) of 482 *Acinetobacter baumannii* complex were XDR followed by *Klebsiella pneumoniae* [393 of 570(68.95%)], *Klebsiella aerogenes* [35 of 98(35.71%)], *Klebsiella oxytoca* [23 of 70 (32.86%)] and *Escherichia coli* [203 of 1169(17.37%)] while no XDR *Pseudomonas aeruginosa* was reported, Table 3.

As per AWaRe classification of antibiotics by WHO, all the GNB isolates were tested for Amikacin, Gentamicin, trimethoprim-sulfamethoxazole, Ampicillin, Nitrofurantoin (for urinary isolates only), Chloramphenicol(for non-urinary isolates), and when applicable doxycycline, tobramycin, ampicillin-sulbactam belonging to the "Access group"; Cefixime, Ceftriaxone, Meropenem, imipenem, ofloxacin, ciprofloxacin, piperacillin-tazobactam, and when applicable ceftazidime, cefepime belonging to the "Watch group" and Colistin and polymyxin B when applicable belonging to the "Reserve group" of antibiotics.

For the antibiotics tested against MDR GNB, higher sensitivity was seen to carbapenem group (32.22%) followed by aminoglycosides (28.30%) doxycycline (26.00%), piperacillin-tazobactam (17.38%), cephalosporins (8.73%) and fluroquinolones (7.75%). The sensitivity of carbapenems and piperacillin-tazobactam shows gradual decline since last three years for the members of Enterobacteriaceae family (*Escherichia coli*; Meropenem: 61.33% in 2021, 66.58% in 2022, 54.48% in 2023; piperacillin-tazobactam: 25.23% in 2021, 21.16% in 2022, 13.33% in 2023) (*Klebsiella pneumoniae*; Meropenem: 16.67% in 2021 to 13.87% in 2023, piperacillin-tazobactam 6.54% in 2021 to 1.16% in 2023). A significant decrease in sensitivity percentage of piperacillin tazobactam was seen from 2021 to 2023. 36.11% of XDR GNB was sensitive to doxycycline (42.85% for *Acinetobacter*

Table 1. Results of total number of GNB isolated from different clinical sample submitted to Microbiology unit of department of pathology and lab medicine at Patan Hospital, Lalitpur, in the years from 2021 to 2023

Year	Gram Negative Bacilli (N)	MDR GNB(N%)	XDR GNB(N%)	XDR of MDR GNB(N%)
2021	3070	766(24.95)	350(11.40)	45.69
2022	4155	850(20.46)	324(7.80)	38.12
2023	4165	924(22.18)	398(9.56)	43.07
Total	11390	2540(22.30)	1072(9.41)	42.20

Table 2. Results of demographics of patients with MDR-GNB and XDR-GNB isolated from different clinical sample submitted to Microbiology unit of department of pathology and lab medicine at Patan Hospital, Lalitpur, in the years from 2021 to 2023

Demographic	2021		2022		2023		Total	
	MDR	XDR	MDR	XDR	MDR	XDR	MDR(N=2540)	XDR of MDR (N=1072)
Origin								
IPD	411	226	543	275	600	339	1554(61.18%)	840(78.36%)
OPD	355	124	307	49	324	59	986(38.81%)	232(21.64%)
Sex								
Female	368	154	422	151	492	181	1282(50.47%)	486(45.34%)
Male	398	196	428	173	432	217	1258(49.52%)	586(54.66%)
Age-Group								
<1 year	80	62	47	13	62	44	189(7.44%)	119(62.96%)
1-5 year	25	13	29	5	20	5	74(2.91%)	23(31.08%)
6-18 year	21	6	19	5	25	9	65(2.56%)	20(30.77%)
19-35 year	171	59	228	75	235	86	634(24.96%)	220(34.70%)
36-50 year	128	44	154	51	152	53	434(17.09%)	148(34.10%)
51-65 year	167	79	148	61	177	72	492(19.37%)	212(43.08%)
>65 year	174	87	228	114	253	129	652(25.67%)	330(50.61%)
Specimen								
Urine	332	103	349	63	395	89	1076(42.36%)	255(23.70%)
Respiratory	134	95	139	106	166	133	439(17.28%)	334(76.08%)
Pus	117	47	145	51	99	38	361(14.21%)	137(37.67%)
Wound swab	58	33	84	38	147	85	289(11.38%)	156(53.98%)
Blood	97	61	77	40	76	39	250(9.84%)	140(56.00%)
Body fluids	18	7	32	14	19	7	69(2.72%)	28(40.58%)
Genital sample	4	1	6	1	10	0	20(0.79%)	2(10.00%)
Tissue	3	0	5	2	8	4	16(0.63%)	6(37.50%)
Catheter	3	3	8	6	2	2	13(0.51%)	11(84.96%)
Bile	0	0	4	2	0	0	4(0.16%)	2(50.00%)
CSF	0	0	0	0	2	1	2(0.16%)	1(50.00%)
Discharge	0	0	1	0	0	0	1(0.00%)	0(0.00%)
Mortality	52	70	79	37	50	62	201(12.65%)	149(17.61%)

Table 3. Top pathogens causing MDR and XDR infections at Patan Hospital, Lalitpur, Nepal in the years from 2021 to 2023

Organism	2021		2022		2023		Total	
	MDR	XDR	MDR	XDR	MDR	XDR	MDR	XDR of MDR
Escherichia coli	335	68	399	59	435	76	1169 (17.39%)	203(17.37%)
Klebsiella pneumoniae	216	160	181	113	173	120	570 (32.80%)	393(68.95%
Acinetobacter baumannii complex	141	109	151	125	190	174	482(76.87%)	408(84.65%)
Klebsiella aerogenes	21	9	38	10	39	16	98(26.70%)	35(35.71%)
Pseudomonas aeruginosa	21	0	30	0	43	0	94 (13.49%)	0
Klebsiella oxytoca	21	4	32	12	17	7	70(22.58%)	23(32.85%)

Table 4. Antibiogram of MDR and XDR pathogens expressed in sensitivity % at Patan Hospital, Lalitpur, Nepal, in the years from 2021 to 2023

Organism	MDR-GNB						XDR-GNB				
	Escherichia coli	*Klebsiella pneumoniae*	*Acinetobacter baumannii complex*	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	*Klebsiella oxytoca*	*Acinetobacter baumannii complex*	*Escherichia coli*	*Klebsiella aerogenes*	*Klebsiella oxytoca*	*Klebsiella pneumoniae*
Amikacin	63.61	24.17	3.74	45.63	10.56	50.90	0.99	5.90	11.11	25.00	1.25
Gentamicin	40.59	17.32	4.84	17.72	7.78	22.75	0.91	0.00	0.00	0.00	4.37
Imipenem	59.43	19.62	2.75	35.16	12.76	48.52	1.13	0.00	0.00	3.80	7.70
Meropenem	60.80	18.10	2.97	40.48	3.77	46.93	0.00	0.00	0.00	0.00	0.00
Ceftriaxone	2.84	1.67	0.96	2.46	IR	9.18	0.00	0.00	0.00	0.00	0.00
Cefixime	0.40	0.37	NT	0.00	IR	2.15	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin	0.44	2.06	3.75	2.46	5.56	1.04	0.00	0.00	0.00	0.00	0.00
Ofloxacin	4.01	9.46	NT	26.23	8.50	11.21	0.00	0.00	0.00	0.00	0.00
Piperacillin-tazobactam	19.91	3.67	6.62	18.49	14.21	12.04	0.00	0.00	0.00	0.00	0.00
Nitrofurantoin	67.29	11.55	NA	20.37	NT	24.63	NA	48.60	0.00	0.00	4.68
Chloramphenicol	56.67	21.49	IR	34.26	NT	28.24	IR	20.00	28.50	0.0	8.08
Doxycycline	35.69	22.83	28.21	16.21	NT	33.33	42.85	52.63	0.00	50.00	35.07
Trimethoprim-sulfamethoxazole	24.05	8.82	12.53	18.49	NT	9.30	12.80	5.90	11.10	0.00	2.50
Ampicillin	1.57	IR	IR	NA	NA	NA	IR	0.00	NA	0.00	NA
Tobramycin	NT	NA	4.40	NT	10.03	NT	0.94	0.00	0.0	0.00	0.0
Colistin	NA	NT	100	NA	100	NA	100	NA	NA	NA	NA
Aztreonam	NT	NA	IR	NT	3.02	NT	IR	6.00	28.50	0.00	0.77
Polymyxin B	NA	NT	100	NA	100	NA	100	NA	NA	NA	NA
Ampicillin-sulbactam	NT	NT	6.86	NT	NT	NT	4.90	0.00	0.00	0.00	0.76
Ceftazedime	NT	NT	2.21	NT	11.99	NT	0.00	0.00	0.00	0.00	0.00
Cefepime	NT	NT	0.67	NT	3.00	NT	0.00	0.00	0.00	0.00	0.00

NA: Not applicable; IR: Intrinsic Resistant, NT: not tested for MDR

Table 5, Expense incurred during treatment of infection with MDR and XDR pathogens at Patan Hospital, Lalitpur, Nepal, in the year 2023 only

	GNB Treatment	MDR GNB treatment	XDR GNB Treatment
Total Expense	Rs. 17,23,83,192.17	Rs. 10,27,25,500.53	Rs. 6,69,21,537.54
Average Expense	Rs. 41,388.52	Rs. 1,11,174.78	Rs. 1,68,144.57
Difference in treatment	GNB to MDR GNB	+Rs. 69,786.26	
	MDR-XDR GNB		+Rs. 56,969.78

baumannii complex, 52.63% for *Escherichia coli* and 35.07% for *Klebsiella pneumoniae*), however, only 10.00% of the XDR GNB isolates were susceptible to the aminoglycosides, carbapenems, cephalosporins and fluroquinolones groups. Colistin and polymyxin B were tested only for *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* with no resistance was seen in any isolate, Table 4.

The total cost that was spent in 2023 only for the treatment of a person suffering from gram negative infection was Rs. 17,23,83,192.20 (Average: Rs 41,388.50). Patient suffering from MDR-GNB infection spent a total of Rs. 10,27,25,500.50 (Average: Rs 1,11,174.78) while those suffering from XDR-GNB spent Rs. 6,69,21,537.50 (Average: Rs 1,68,144.60) in their treatment. Patient suffering from MDR- GNB spent Rs 69,786.30 more than those suffering from GNB infection only while patient suffering from XDR-GNB spent Rs 56,969.80 more than those suffering from MDR-GNB infections (Rs 1,26,756.04 more than GNB infections only.

Discussion

This research is a component of diligent and ongoing monitoring of antimicrobial susceptibility, aimed at tracking the evolving antimicrobial resistance (AMR) profile across various organisms of interest within our hospital. MDR-GNB is among the primary reasons necessitating hospital admission, and now XDR-GNB is also progressively surfacing within this demographic, making these organisms of particular interest. The present article gives a review of the MDR and XDR GNB infections presented in Patan hospital and antimicrobial susceptibility from 2021 to 2023.

The prevalence of MDR-GNB among those with GNB infections accounted for 22.30% (24.95% in 2021; 20.46% in 2022 and 22.18% in 2023). Among these MDR GNB, 42.20% were found to be XDR (45.69% in 2021; 38.12% in 2022 and 43.07% in 2023). The prevalence of XDR GNB among the total isolated GNB was 9.41%. which is similar to the prevalence reported from few hospitals in India.[3,18] However, when these findings were compared to those from Nepal, a significantly higher prevalence rate was found in other studies compared to ours, ranging from 47% to 91%.[9-15] The higher rate of MDR-GNB prevalence in these studies can be attributed to the focused study site (ICUs), targeted organism group (*Acinetobacter* and *Pseudomonas*) and studies on stored isolates that were already suspected of being ESBL producers. Our study took a more holistic approach and looked into the overall MDR prevalence rate among all the isolated GNB. We also looked into the prevalence rate of XDR-GNB among identified GNB isolated and the percentage it accounts among the MDR-GNB isolates. We failed to identify any study in Nepal looking into the XDR-GNB population.

The study did not find any variation of prevalence among male and female but MDR-GNB infection was seen mainly in the age after 35 years. However, XDR-GNB infection was seen in extremes of ages (<1 year and above>50 years). These MDR and XDR-GNB infections seen after 35 years could be due to progressive and multiple episodes of antibiotic exposure and the XDR-GNB seen in extremes of age group could be attributed to healthcare associated infection. Our study also found that most of the MDR-GNB infection required hospital admission. One critical study finding is that although most of the XDR-GNB were isolated from sample originating form inpatients, about 21% of them were also isolated from outpatient samples, implying that the XDR-pathogens are now already circulating in the community and patient are presenting with difficult to treat infections in their first hospital visit as well. Most of the MDR-GNB infected patients in our study were admitted in the department of medicine (inclusive of Medical ICU) followed by surgery, Obstetrics/gynecology, pediatric and orthopedic department. Most of the other studies reviewed were focused on ICU setting but one study found that the surgical department had most MDR-GNB isolated than the medicine department.[19]

MDR-GNB was isolated from a wide array of clinical samples in our study. Majority of them were from urine followed by pus and wound swab, respiratory sample and blood which was similar to the findings of other studies as well.[10,19] XDR-GNB isolation was much higher from respiratory sample followed by urine, pus and wound swab and blood reflecting the higher possibility of device related incidence (Ventilator Associated Pneumonia, Catheter Associated Urinary Tract Infection). Out of total, 12.9% of patients suffering from MDR-GNB and 17.6% of those suffering from XDR-GNB succumb to these infections. In 2023 only a sum total of more than 10 crores Nepalese rupees were spent during the course of the treatment of these MDR infections with average spending of 1 lakh Nepalis rupees by a single patient. This was about 70 thousand more spending compared to treating patients with non MDR-GNB infection. Similarly for XDR-GNB treatment, patients spent a sum total of more than 6 crores Nepalis rupees, with average spending of more than 1 lakh 50 thousand by a single patient (about 50 thousand more than that required to treat MDR-GNB infection). With mere gross national income per capita (at then prices) estimated at Rs 1,83,060 (US$ 1,410) in the fiscal year 2022/23, this small reflection of a year alone suggests the huge economic burden the population of Nepal are facing due to these MDR and XDR infections.[20]

Majority of the *Acinetobacter baumannii* complex when isolated were MDR followed by *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Escherichia coli* and *Pseudomonas aeruginosa*. These are the usual MDR pathogens (Except with those pathogens that are intrinsically resistant to various antibiotics) encountered in the clinical settings.[7-11] While the percentage population of MDR *Pseudomonas* is less in our study, majority of the study found *Pseudomonas aeruginosa* to be one of leading MDR-GNB. When looked into, our study found that 84.65% of the MDR *Acinetobacter baumannii* complex were XDR, but no XDR *Pseudomonas aeruginosa* were reported. Our study also found that the XDR *Acinetobacter* baumanni complex showed an alarming increase in resistance

from 77.30% in 2021 to 91.58% in 2023. This shows an area of grave concern as most of the critically ill patients ending up in the ICUs have a high chance of colonization with this particular bacterium.

Our study found variable levels of sensitivity of MDR-GNB against the antibiotics that were tested as per CLSI guidelines. Carbapenems group showed the most sensitivity at 32 % (Meropenem (34.2%); Imipenem (30.3%) followed by aminoglycosides (28.3%; Amikacin (33.6%); Gentamicin (23%)) doxycycline (26%), piperacillin-tazobactam (17.4%). However, our study also found that for MDR-GNB, the cephalosporins and fluroquinolones were nowhere near good, probably due to non-rational use of antibiotics at the community level. It is also a grave concern to notice through our study that the carbapenems and piperacillin-tazobactam are steadily declining in sensitivity towards the members of the Enterobacteriaceae family, a major group of pathogens causing disease in humans.

We also found that doxycycline showed to be a good drug for treating XDR-GNB compared to other groups of antibiotics. Meanwhile, aminoglycosides, carbapenems, cephalosporins and fluroquinolones did not show any persuasive sensitivity for treatment of XDR-GNB. Colistin and polymyxin was not tested for other MDR and XDR-GNB as it is not recommended by CLSI and no interpretation criteria is available for Enterobacteriaceae family. However, it is recommended to be tested against *Acinetobacter baumannii* complex and *Pseudomonas* spp by using MIC methods only. We used the same methodology and could not find any resistance of colistin and polymyxin b in *Acinetobacter baumannii* complex and *Pseudomonas* spp.

This study utilized data that strictly adhered to all quality control protocols for conducting antimicrobial sensitivity testing. All the drug bug combinations and all the methods recommended to test antibiotic sensitivity as per CLSI guidelines were followed. We did not perform any molecular test to detect resistant genes on these isolates as these tests are relatively expensive and do not fall in the scope of routine microbiological testing. This could represent a new initiative that potentially provides deeper insights into prevalence at the molecular level.

A high volume of variable samples was analyzed during the study process with isolation of MDR-GNB from the majority of samples. With a high isolation rate and robust data quality, this study provides a clear reflection of the current prevalence rate and antibiotic sensitivity of MDR and XDR-GNB. We did see a high prevalence of *Acinetobacter* spp in our study similar to other studies and warrants good infection control practices in the health care setting to limit its transmissions. A significant decline in susceptibility to piperacillin-tazobactam from 2021 to 2023 also is an area of significant concern as it is one of the major drugs being prescribed by clinicians regardless of the disease. A rational use of antibiotics through tailored antibiotic regimen and antibiotic stewardship programs is needed to take a grip on this chaos. Our study did try to look into the economic burden in patients suffering from MDR and XDR GNB infection but the cost burden attributable to only treatment of such infection could not be analyzed and cost incurred for other services like surgery or non-infectious cause of admission was not excluded. This provides an opportunity to further look into in the future.

Prescribing clinicians should be encouraged to submit more clinical samples for microbiological studies. Increasing the yield of isolates could provide a clearer picture of actual prevalence and antibiotic susceptibility. Moreover, enhanced surveillance via comprehensive microbiological studies empowers healthcare facilities to implement targeted interventions and antimicrobial stewardship programs aimed at curbing the spread of antimicrobial resistance. Therefore, advocating for increased submission of biological samples for microbiological analysis is crucial in combating the escalating threat of antimicrobial resistance and ensuring optimal patient care.

Conclusion

The overall prevalence of MDR-GNB is at 22.30% in our hospital and 42.20% of these isolates are XDR. This prevalence has remained consistent yet gradually increasing over the past three years. Aminoglycosides and carbapenems remain the cornerstone for treatment of MDR-GNB infection but piperacillin-tazobactam must now be judicially used to refrain it from coming to the current status of cephalosporins and fluoroquinolones. Additionally, using doxycycline can be an alternative approach. For XDR-GNB treatment colistin, doxycycline can be the backbone of the treatment regimen plan when MDR or XDR GNB is suspected with adjustments made based on available culture and sensitivity testing results.

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Conflict of interest

None

Author's contribution

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References

1. Antimicrobial Resistance [Internet]. Geneva: World health Organization; 2020 Oct 6. 6p. Weblink
2. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81. DOI
3. Nagvekar V, Sawant S, Amey S. Prevalence of multidrug-resistant Gram-negative bacteria cases at admission in a multispeciality hospital. Journal of global antimicrobial resistance. 2020 Sep 1; 22:457-61. DOI
4. Jean SS, Harnod D, Hsueh PR. Global threat of carbapenem-resistant gram-negative bacteria. Frontiers in cellular and infection microbiology. 2022 Mar 15; 12:823684. DOI
5. Latest News from WHO: WHO publishes list of bacteria for which new antibiotics are urgently needed [Internet]. Geneva: World Health Organization; 2017 Feb 27; [about 3 screens]. Weblink
6. Alkofide H, Alhammad AM, Alruwaili A, Aldemerdash A, Almangour TA, Alsuwayegh A, et al. Multidrug-resistant and extensively drug-resistant enterobacteriaceae: prevalence, treatments, and outcomes – A retrospective cohort study. Infect Drug Resist. 2020; 13:4653-62. DOI
7. Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: a study. Journal of pathogens. 2016;2016(1):4065603. DOI
8. Abbas S, Sabir AU, Khalid N, Sabir S, Khalid S, Haseeb S, Numair Khan M, Ajmal WM, Azhar F, Saeed MT. Frequency of Extensively Drug-Resistant Gram-Negative Pathogens in a Tertiary Care Hospital in Pakistan. Cureus. 2020 Dec 5;12(12): e11914. DOI
9. Bhatta S, Pradhan M, Chaudhary R. Multidrug-resistant among Non-Fermenting Gram-negative Bacteria Isolated in the Department of Microbiology of a Tertiary Care Centre. J N Med Assoc. 2023 Nov;61(267): 868. DOI
10. Shilpakar A, Ansari M, Rai KR, Rai G, Rai SK. Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal. Tropical Medicine and Health. 2021 Dec; 49:1-9. DOI
11. Maki G, Kaljee L, Prentiss T, Olson E, Bajracharya D, Shrestha B, et al. Epidemiology of multi drug resistant gram-negative bacteria in Kathmandu, Nepal. International Journal of Infectious Diseases. 2019 Feb 1; 79:44-5. DOI
12. Manandhar S, Zellweger RM, Maharjan N, Dongol S, Prajapati KG, Thwaites, G et al. A high prevalence of multi-drug resistant Gram-negative bacilli in a Nepali tertiary care hospital and associated widespread distribution of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-encoding genes. Annals of clinical microbiology and antimicrobials. 2020 Dec; 19:1-3. DOI
13. Siwakoti S, Subedi A, Sharma A, Baral R, Bhattarai NR, Khanal B. Incidence and outcomes of multidrug-resistant gram-negative bacteria infections in intensive care unit from Nepal-a prospective cohort study. Antimicrobial Resistance & Infection Control. 2018 Dec; 7:1-8. DOI
14. Ghimire R, Gupte HA, Shrestha S, Thekkur P, Kharel S, Kattel HP, et al. High drug resistance among Gram-negative bacteria in sputum samples from an intensive care unit in Nepal. Public Health Action. 2021 Nov 1;11(1):64-9. DOI
15. Shakya S, Edwards J, Gupte HA, Shrestha S, Shakya BM, Parajuli K, et al. High multidrug resistance in urinary tract infections in a tertiary hospital, Kathmandu, Nepal. Public health action. 2021 Nov 1;11(1):24-31. DOI
16. CLSI. Performance standards for Antimicrobial Susceptibility Testing 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019. Full text
17. WHO. 2021 AWaRe classification [Internet]. Geneva, Switzerland: World Health Organization; 2021 Sep 30. Weblink
18. Saraswat D, Gawali D, Mishra A, Sharma DN, Rastogi AK, Ansari M. Prevalence of multi drug resistant gram-negative bacteria and its susceptibility patternisolated from admitted patientsin a hospital of central India; An emerging global problem. J Popl Ther Clin Pharmacol. 2023 Apr 10;30(8):517-24. DOI
19. Devi KD, Khandait M, Sharma M, Sardar M, Singh A, Saha R, et al. Prevalence of multidrug-resistant Gram-negative bacteria causing pyogenic infections at a tertiary care hospital in Haryana. MGM Journal of Medical Sciences. 2023 Jul 1;10(3):517-23. DOI
20. Ministry of Finance, Government of Nepal. Economic Survey 2022-2023. Singh Durbar, Kathmandu: Ministry of Finance, Government of Nepal; 2023 May.484p. Weblink