

Antimicrobial susceptibility patterns of bacterial isolates from routine clinical specimens of a tertiary hospital in Bangladesh

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Abstract

Background and objectives: To prevent the emergence and spreading of antimicrobial resistance, especially multidrug resistance in pathogenic bacteria, the selection of appropriate antibiotics is a prerequisite for the effective treatment of infection. This study aimed to analyze the prevalence and antimicrobial resistance patterns of bacterial isolates from various clinical samples in a tertiary care hospital.

Methods: This study was conducted at a teaching hospital of Dhaka city, Bangladesh from January 2020 to March 2021. The results of culture and antimicrobial susceptibility of bacterial isolates from various clinical samples were collected and analysed. Identification of bacteria and antimicrobial susceptibility test were performed according to the standard methods.

Results: A total of 1277 bacterial isolates was analyzed. Of them, 1072 (83.95%) were Gram-negative, and 205 (16.05%) were Gram-positive bacteria. Among the isolates, *Escherichia coli* (n=576), *Enterobacter spp.* (n=150), *Klebsiella spp.* (n=140), and *Staphylococcus aureus* (n=117) were predominant. The *Enterobacteriaceae* showed higher resistance to cephadrine (94.3%) and cefuroxime (76.7%), whereas least resistant to imipenem (10.1%) and meropenem (14.8%). *Pseudomonas spp.* was highly resistant to ceftioxone (80.2%), and colistin (70.8%), whereas least resistant to piperacillin-tazobactam (15.1%). Colistin was the most effective agent (resistance 6.7%) against *Acinetobacter spp.* Linezolid (resistance 1%) and vancomycin (resistance 2%) were highly effective against Gram-positive bacteria. Among the *Staphylococcus aureus*, 95.7% were methicillin-resistant (MRSA). A total of 889 (69.6%) bacterial isolates were identified as multidrug resistant. Multidrug resistance was more prevalent in Gram-positive isolates (79.5%) than that of Gram-negative bacteria (67.7%). Furthermore, 7.5% of Gram-negative bacterial isolates were resistant to all seven classes of antibiotics tested.

Conclusions: This study revealed presence of high rate of resistance to several antimicrobial agents in bacteria isolated from various clinical samples. The findings would help healthcare professionals to select appropriate antibiotics for the effective treatment of infections and to develop antibiotic stewardship protocol.

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Introduction

Discovery of antibiotics in the middle of twentieth century was considered a revolutionary breakthrough in medical science. Antibiotics revolutionized the treatment of infections caused by

bacteria, fungi and protozoa, resulting in transforming once deadly diseases into manageable health problems [1]. The emergence and spreading of antimicrobial-resistant pathogens, especially the acquired multidrug-resistant bacterial strains,

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posed a tremendous risk to public health around the world [2]. The US Centers for Disease Control and Prevention (CDC) estimated that around 700,000 people die each year worldwide as a result of infections by multidrug-resistant bacteria and also predicted that the death toll would increase to more than 10 million per year by 2050. In addition to causing death, antibiotic resistance increases the hospital stay and cost of treatment for bacterial infections, resulting in an economic burden for patients and the nation [3,4].

The use of antibiotics is increasing everyday. As a result, more than one million tons of antibiotics are produced each year throughout the world. Unfortunately, improper and overuse of antibiotics are leading to develop resistance in pathogenic bacteria [2,5]. Antimicrobial resistance issue is much higher in developing countries like Bangladesh compared to developed countries [6]. Improper prescribing of antibiotics by physicians and lack of strict regulations in the sale are the leading causes of overuse and misuse of antibiotics [7]. Moreover, the extensive utilization of antibiotics in veterinary and agriculture sectors leads to the development and accumulation of resistant microorganisms in animals, especially cattle, poultry, and swine. Upon consumption of these animals (such as egg, milk, and meat) and inadequate waste management have led to the spread of resistant organisms to humans and environment [1].

In recent times, the spectrum of antimicrobial resistance is changing significantly and it differs from one setting to another. The emergence of bacterial resistance to the existing antimicrobials creates difficulties in the treatment of infection. Analysis of antimicrobial resistance patterns of pathogenic bacteria helps to institute appropriate treatment of infectious diseases. The present study evaluated the antibiotic resistance patterns of bacterial isolates from routine clinical specimens in an urban tertiary hospital.

Materials and methods

This cross sectional study was conducted at a teaching hospital Dhaka city, Bangladesh from January 2020 to March 2021. The results of culture

and antimicrobial susceptibility tests of various clinical samples such as urine, wound swab, sputum, high vaginal swab (HVS), and tracheal aspirate were collected and analysed. Written permission to conduct this study was obtained from the respective authority of the hospital.

Identification of bacterial genus/species were done according to Bergey's manual of determinative bacteriology [8]. The antibiotic susceptibility tests were performed on Mueller-Hinton agar (Oxoid Ltd. Basingstoke, Hampshire, England) medium using the disk diffusion method (Kirby-Bauer technique) and interpreted according to NCCLS guidelines [9, 10]. Antibiotic discs used were amikacin (30 µg), amoxycillin (10 µg), amoxycylav (30 µg), azithromycin (15 µg), aztreonam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cephadrine (30 µg), ciprofloxacin (5 µg), cloxacillin (5 µg), colistin (10 µg), cotrimoxazole (25 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (30 µg), mecillinam (25 µg), meropenem (10 µg), methicillin (5 µg), nalidixic acid (30 µg), netilmicin (30 µg), piperacillin-tazobactam (110 µg), and vancomycin (30 µg). All the antibiotic discs were obtained from Oxoid Ltd. Basingstoke, Hampshire, England. Multidrug resistance was defined as non-susceptibility of a bacterium to ≥ 1 agent in ≥ 3 antimicrobial categories [11]. Seven frequently used classes of antibiotics against bacterial species were used to analyze multidrug resistance (Table-1).

Descriptive statistics, like frequencies and percentages, were used to determine the prevalence of bacterial isolates and antimicrobial resistance patterns.

Results

Distribution of bacterial isolates in different clinical samples

After analyzing the data of the microbiological culture tests result of various clinical samples, a total of 1277 bacterial isolates were included in the study. Of them, 1072 (83.95%) isolates were Gram-negative while 205 (16.05%) were Gram-positive bacteria. Of the total 1277 isolates, the most frequently isolated Gram-negative organisms were

Table-1: Antimicrobial class used to define multidrug resistance

Enterobacteriaceae	NFGNB	Gram-positive bacteria
Aminoglycoside (Amikacin), Carbapenem (Meropenem), Cephalosporin (Ceftriaxone), Fluoroquinolone (Ciprofloxacin), Monobactam (Aztreonam), Penicillin+ β -lactamase inhibitor (Amoxyclav) Sulfonamide (Cotrimoxazole)	Aminoglycoside (Amikacin), Carbapenem (Meropenem), Cephalosporin (Ceftriaxone), Fluoroquinolone (Ciprofloxacin), Monobactam (Aztreonam), Penicillin+ β -lactamase inhibitor (Piperacillin-tazobactam) Polymyxin (Colistin)	Aminoglycoside (Gentamycin), Fluoroquinolone (Ciprofloxacin) Glycopeptide (Vancomycin), Macrolide (Azithromycin), Oxazolidinone (Linezolid), Penicillin (Amoxycillin), Sulfonamide (Cotrimoxazole)

Note: NFGNB – non-fermentative Gram-negative bacteria.

Table-2: Distribution of bacterial isolates in various clinical samples (n=1277)

Organism	Types of samples (number)					Total n (%)
	HVS	Sputum	TA	Urine	Wound swab	
Gram-negative bacteria (n=1072)						
<i>Escherichia coli</i>	10	29	8	449	80	576 (45.1)
<i>Enterobacter spp.</i>	5	44	9	66	26	150 (11.7)
<i>Klebsiella spp.</i>	3	53	5	55	24	140 (11)
<i>Pseudomonas spp.</i>	5	26	3	43	29	106 (8.3)
<i>Acinetobacter spp.</i>	0	53	23	9	4	89 (7)
<i>Proteus spp.</i>	0	1	0	5	5	11 (0.9)
Gram-positive bacteria (n=205)						
<i>S. aureus</i>	0	7	1	13	96	117 (9.2)
<i>Enterococcus spp.</i>	0	0	0	83	0	83 (6.5)
CONS	0	0	0	5	0	5 (0.4)
Total	23	213	49	728	264	1277
	(1.8%)	(16.7%)	(3.8%)	(57%)	(20.7%)	

Note: HVS – high vaginal swab, TA – tracheal aspirate; CONS – coagulase negative *S. aureus*

Escherichia coli (45.1%), *Enterobacter spp.* (11.7%), *Klebsiella spp.* (11%) and *Pseudomonas spp.* (8.3%). *Staphylococcus aureus* was the most predominant Gram-positive bacteria (9.2%). Detail distribution of isolated bacteria is shown in Table-2, The highest percentage of bacterial isolates was reported in the urine samples (n=728; 57%), followed by wound swabs (n=264; 20.7%), and sputum (n=213; 16.7%). The most predominant isolate of urine was *Escherichia coli* (n=449; 61.7%). Among wound swab isolates, *Staphylococcus aureus* (n=96; 36.4%), and in sputum, both *Acinetobacter spp.* and *Klebsiella spp.* (both n=53; 24.9%) were the most common bacteria (Table -2).

Antibiotic resistance pattern of Gram-negative isolates

A total of 14 types of antibiotics (Figure-1) were tested against *Enterobacteriaceae* (n=877). *Enterobacteriaceae* were most resistant to cephadrine (n=827, 94.3%), followed by nalidixic acid (n=802, 91.4%), cefuroxime (n=673, 76.7%), and aztreonam (n=625, 71.3%), whereas least resistant to carbapenems (imipenem: n=89, 10.1%, meropenem: n=130, 14.8%), followed by aminoglycosides (amikacin: n=163, 18.6%; netilmicin: n=245, 27.9% and gentamicin: n=259, 29.5%).

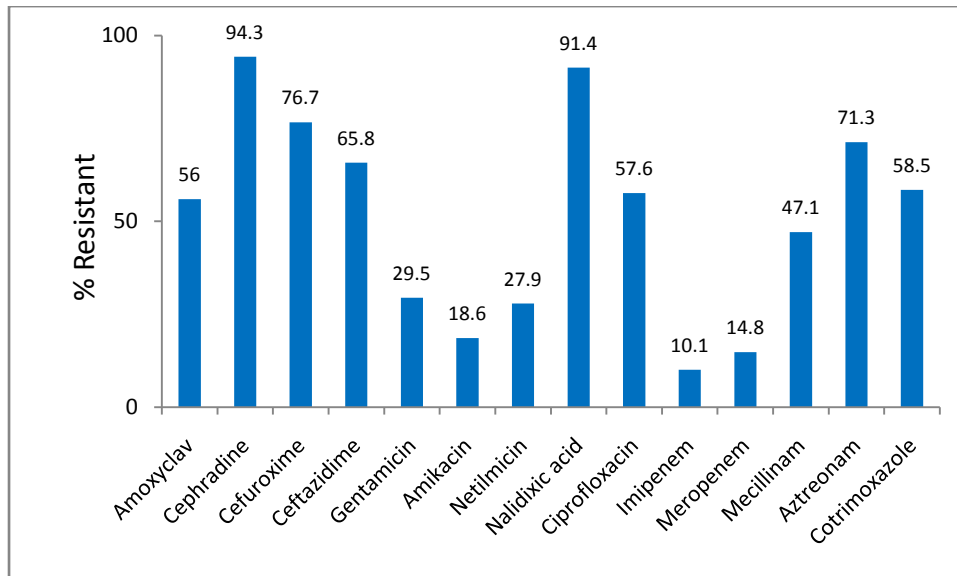


Figure-1: Antibiotic resistance pattern of the family *Enterobacteriaceae* (n=877)

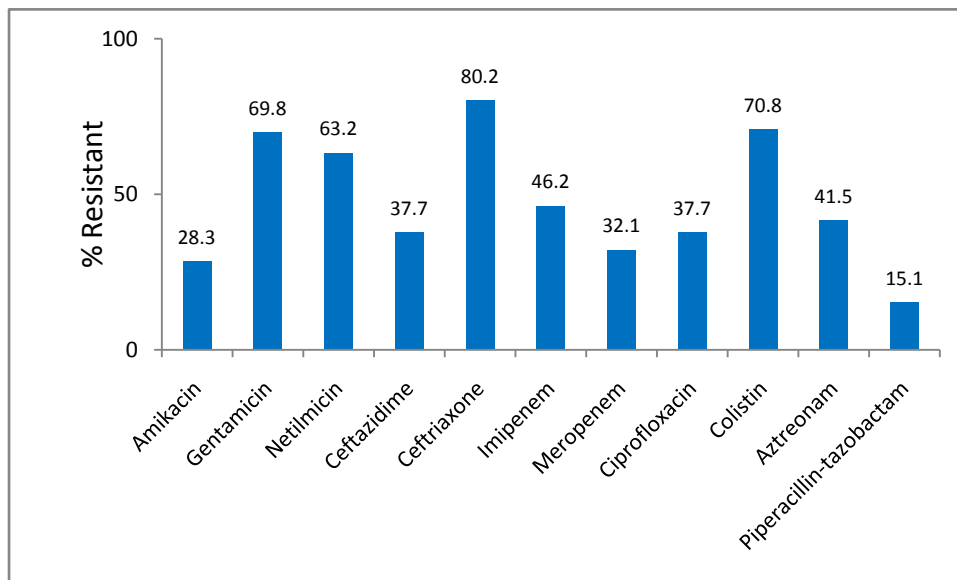


Figure-2: Antibiotic resistance pattern of *Pseudomonas spp.* (n=106)

Overall, 11 types of antimicrobials were tested for non-fermenting Gram-negative bacteria (NFGNB). Of them, *Pseudomonas spp.* (n=106) were most resistant to ceftriaxone (n=85, 80.2%) and colistin (n=75, 70.8%), whereas the isolates were least resistant to piperacillin-tazobactam (n=16, 15.1%),

followed by amikacin (n=30, 28.3%), and meropenem (n=34, 32.1%) (Figure-2). *Acinetobacter spp.* (n=89) showed a higher percentage of resistance (>90%) to almost all tested drugs except colistin (Figure-3). The sensitivity of *Acinetobacter spp.* to colistin (CT) was 93.3% (n=83).

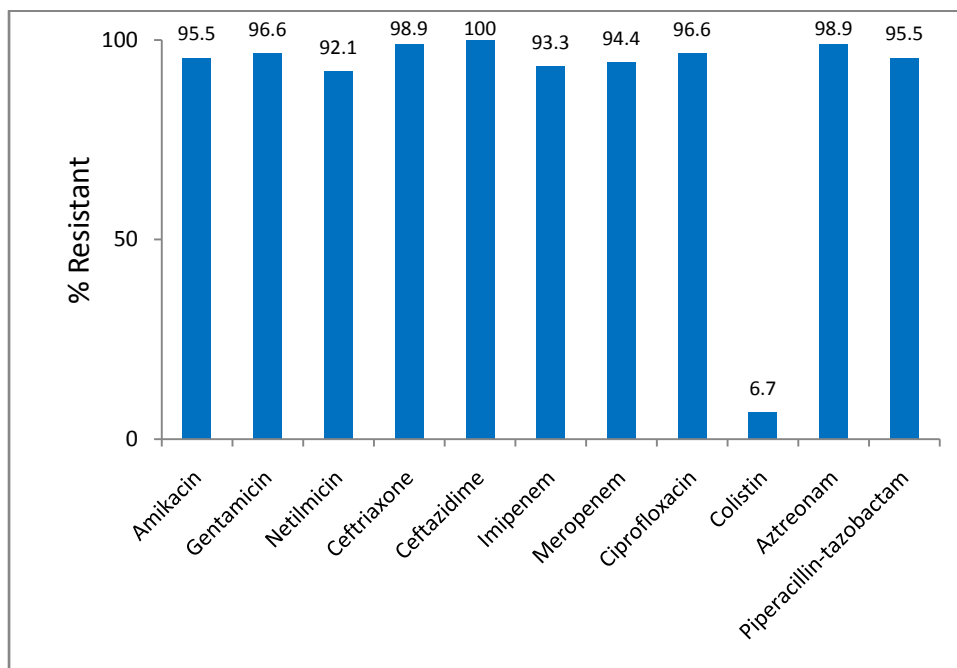


Figure-3: Antibiotic resistance pattern of *Acinetobacter* spp. (n=89)

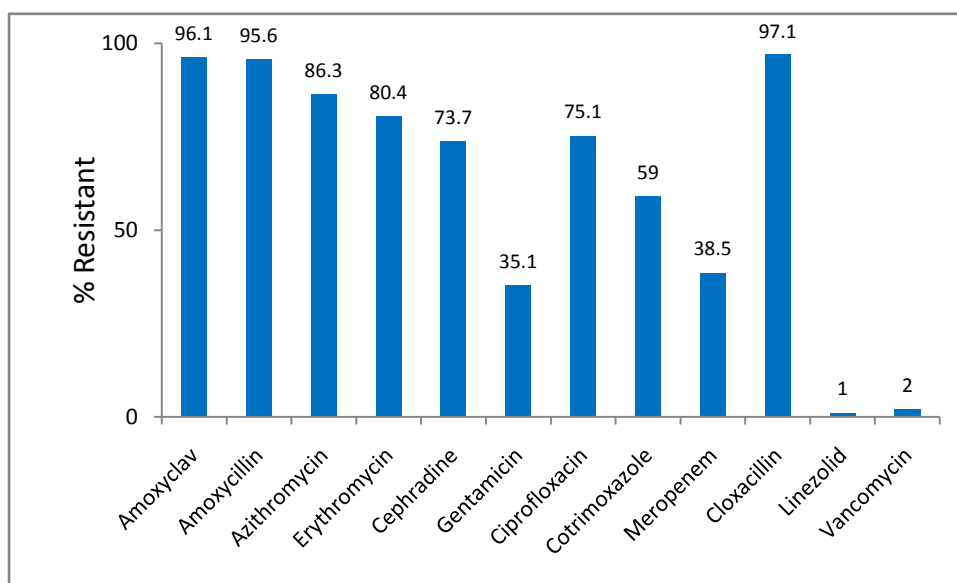


Figure 4: Antibiotic resistance pattern of Gram-positive isolates (n=205)

Antibiotic resistance pattern of gram-positive isolates

Figure-4 shows the antimicrobial resistance pattern of isolated Gram-positive bacteria. Twelve types of antimicrobial agents were tested for Gram-positive

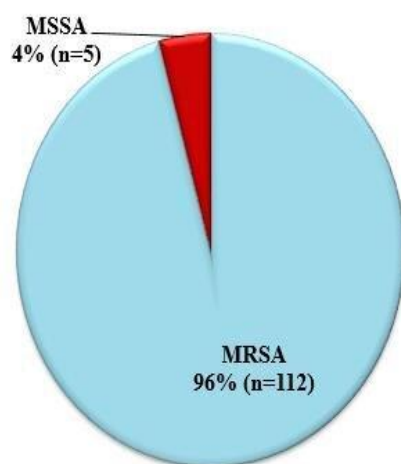
bacteria (n=205). Gram-positive bacteria were highly resistant to cloxacillin (n=199, 97.1%) followed by amoxyclav (n=197; 96.1%), amoxicillin (n=196, 95.6%), azithromycin (n=177, 86.3%), erythromycin (n=164, 80%), and ciprofloxacin

Table-3: Distribution of multidrug resistance patterns in gram-positive and gram-negative bacteria

Bacteria	Non-MDR (Resistant to <3 classes) n (%)	MDR (Resistant to 3 or more classes) n (%)	Resistant to all 7 classes of antibiotics n (%)
<i>Escherichia coli</i> (n=576)	167 (29)	409 (71)	43 (7.5)
<i>Enterobacter spp.</i> (n=150)	53 (35.3)	97 (64.7)	24 (16)
<i>Klebsiella spp.</i> (n=140)	70 (50)	70 (50)	9 (6.4)
<i>Proteus spp.</i> (n=11)	4 (36.4)	7 (63.6)	1 (9.1)
<i>Pseudomonas spp.</i> (n=106)	51 (48.1)	55 (51.9)	2 (1.9)
<i>Acinetobacter spp.</i> (n=89)	1 (1.1)	88 (98.9)	1 (1.1)
Total Gram-negative bacteria (n=1072)	346 (32.3)	726 (67.7)	80 (7.5)
<i>Staphylococcus aureus</i> (n=117)	32 (27.4)	85 (72.6)	0
<i>Enterococcus spp.</i> (83)	8 (9.6)	75 (90.4)	0
CONS (n=5)	2 (40)	3 (60)	0
Total Gram-positive bacteria (n=205)	42 (20.5)	163 (79.5)	0
Total isolates (n= 1277)	388 (30.4)	889 (69.6)	80 (6.3)

Note: CONS – Coagulase negative *S. aureus*; MDR – multidrug resistant

(n=154, 75.1%). Linezolid (n=2, 1%) and vancomycin (n=4, 2%) were found most effective against gram-positive isolates. Furthermore, methicillin was tested against *S. aureus* (n=117) to detect methicillin resistant *S. aureus* or MRSA. Out of 117 isolates, 112 (96%) were resistant to methicillin (Figure-5).

**Figure-5:** Distribution of methicillin resistant and sensitive *S. aureus* (MRSA, MSSA)

Multidrug resistance pattern of the bacterial isolates

Of the total bacterial isolates, 889 (65%) were multidrug resistant according to the definition used in this study (Table -1). Multidrug resistant bacteria was more prevalent in Gram-positive isolates (163/205, 79.5%) than Gram-negative bacteria (726/1072, 67.7%). The percentage of multidrug resistance was the highest among the *Acinetobacter spp.* (88/89, 98.9%) followed by *Enterococcus spp.* (75/83, 90.4%), *Staphylococcus aureus* (85/117, 72.6%), and *Escherichia coli* (409/576, 71%). Among the Gram-negative bacteria, a total of 80 (7.5%) isolates were resistant to all seven classes of antibiotics tested. Multidrug resistant bacteria was isolated from 72.3% (420/581) and 67.4% (469/696) samples obtained from male and female patients respectively.

Discussion

The selection of appropriate antibiotics is crucial for the treatment of infection and therefore analysis of the bacterial susceptibility pattern is helpful in this context. The objective of our study was to analyze antibiotic susceptibility pattern of

bacterial pathogens collected from various clinical samples in a tertiary hospital of Dhaka city. Our study revealed that *Escherichia coli*, *Enterobacter spp.*, *Klebsiella spp.*, and *Proteus spp.*, were highly sensitive to carbapenems (imipenem - 89.9% and meropenem - 85.2%). Previous studies from Bangladesh (susceptibility ranging from 85.6 to 100%) [12-15], India (susceptible 50% to 80%) [16], and Nepal (susceptible 87.8% to 95.3%) [17] also reported carbapenem as the most effective antibiotic against *Enterobacteriaceae*.

Our investigation revealed that *Pseudomonas spp.* were most sensitive to piperacillin-tazobactam (84.9%). A study from Nepal [18] also reported piperacillin-tazobactam (susceptibility 82.26%) as the most effective antibiotic against *Pseudomonas spp.* On the contrary, a study from Pakistan [19] reported piperacillin-tazobactam as the most resistant one (66.2%). Multiple studies from Bangladesh [6,20] showed colistin (susceptibility ranging from 96.5% to 100%) as the most effective antimicrobial against *Pseudomonas spp.* However, only 29.2% *Pseudomonas spp.* isolated in our study was susceptible to colistin. Khatun et al., from Bangladesh [21] reported aztreonam (susceptibility 75%) as the most effective antibiotic against *Pseudomonas spp.*, which was higher than our finding (susceptibility 58.5%). Contrary, another study from the same country observed the least susceptibility (12.5%) of *Pseudomonas spp.* to aztreonam [15].

Our study revealed that colistin was the only drug that was effective against *Acinetobacter spp.* with a sensitivity of 93.3%. Previously conducted studies from Bangladesh (sensitivity 95.1%) [6], Nepal (sensitivity 100%) [22], and Turkey (97.8%) [23] also reported colistin as the most effective drug against this bacterial species. One study from Nepal [18] reported piperacillin-tazobactam as the most effective drug against *Acinetobacter spp.* Furthermore, another study from Nigeria reported that *Acinetobacter spp.* as highly sensitive to meropenem and levofloxacin [24]. In contrast, our study revealed that *Acinetobacter spp.* was highly resistant to meropenem (94.4%), piperacillin-tazobactam (95.5%), and ciprofloxacin (96.6%).

Linezolid and vancomycin were found most active against gram-positive bacteria in this study.

Unfortunately, the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) causes an additional threat to public health as MRSA is more difficult to treat than methicillin-sensitive *S. aureus* (MSSA) [25]. In our study 95.7% *S. aureus* was MRSA which was much higher than that of several studies conducted in Nepal (15.4% and 21.5%) [26,18], Kenya (27.8%) [27], India (31.3%) [4], Bangladesh (34.16%) [6], Pakistan (66%) [28], and Tanzania (66.7%) [29].

Of the total 1277 isolates, 69.6% of isolates were multidrug resistant bacteria of which 7.5% was resistant to all seven classes of antimicrobials tested. Recently, two different studies from Bangladesh reported multidrug resistant bacteria as 62.5% and 67.1% respectively [30,6]. Our observation also showed a higher percentage of multidrug resistant bacteria than the findings of India (37.1%) [4] and Nepal (51.03%) [18]. The rate of multidrug resistant *Acinetobacter spp.* has been reported as 57% to 62.2%, 71.6% and 80.4% from Bangladesh, India and Nepal respectively [30,6,31,22], which are lower than our findings (98.9%). Contrary, a study from Ghana reported the prevalence of multidrug resistant *Acinetobacter spp.* as 100% [32].

The differences regarding the rate of antimicrobial and multidrug resistance found in our study compared to other studies might be due to differences in specimen source, antibiotic usage and settings. The study had some limitations. The study was conducted only in one tertiary care hospital where most of the patients come with complicated and prolonged ailments. Also, we did not verify the accuracy of the disk diffusion test by parallel minimum inhibitory concentration method. Therefore, study in different level of healthcare settings, locations and types of patients might yield different antimicrobial resistance profile.

The findings of our study would be helpful for healthcare professionals to select appropriate antibiotics for treating infections. Our study outcomes also focus on the need for national control programs to combat antimicrobial resistance. We expect that different awareness programs among public and healthcare professionals and enforcement of strict regulations to control the misuse and overuse of antibiotics

would be useful to prevent further increase of antimicrobial resistance in bacterial population.

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