

## ANTIBIOGRAM PROFILING OF CLINICAL ISOLATES FROM SEVERAL RENOWNED DIAGNOSTIC CENTERS IN DHAKA CITY, BANGLADESH

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**Antibiotic resistance is a global concern that has emerged in recent years due to inappropriate and indiscriminate use of antibiotics. It is important to follow the changes in this antibiotic resistance for the sake of better treatment and patient management. The aim of this study was to collect clinical isolates from some renowned diagnostic centers and to determine their susceptibility patterns in different age and sex groups of patients. Clinical samples from a number of patients with various infectious symptoms were selected and microbiologically analyzed. Antibiotic resistance patterns for 15 different antibiotics Amikacin, Azithromycin, Ciprofloxacin, Ceftriaxone, Cefuroxime, Erythromycin, Cefoxitin, Gentamicin, Imipenem, Linezolid, Meropenem, Norfloxacin, Piperacillin, Penicillin and Vancomycin were evaluated by the Agar-disc-diffusion method. The highest resistance of the studied pathogens to antimicrobials was seen against Azithromycin (100%) and Penicillin (100%) whereas the isolates were highly sensitive to Norfloxacin, Vancomycin, Linezolid and Cefoxitin.**

**Keywords:** Gram-negative bacteria, Gram-positive bacteria, clinical isolates, antibiotic resistance

### INTRODUCTION

The global spread of antimicrobial resistance (AMR) poses a serious threat to public health. Antibiotic resistance not only increases morbidity and mortality but also leads to a growing economic burden on health care. AMR occurs when pathogenic microorganisms develop resistance to the drugs used to control these microorganisms, made treatments less effective or ineffective (1). There are many documented cases of pathogens causing AMR, including methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant tuberculosis, and drug-resistant *Escherichia coli*, which are responsible for most deaths worldwide (2, 3). The World Health Organization (WHO) ranked AMR among the top 10 threats to global health in 2019 and evaluated that AMR could cause 10 million deaths annually by 2050 in the world (4, 5). WHO recently reported that around 700,000 people die each year out of drug-resistant infections (6). On the other hand, the supply of new antibiotics is insufficient to keep up with the increase in AMR pathogens, and unnecessary antibiotic use leads to selective accumulation of AMR pathogens around the world, increasing health risks. In addition, AMR jeopardizes effective treatments for other diseases, such as cancer chemotherapy, HIV and malaria treatment. It is important to understand the mechanism of AMR and develop rapid diagnostic tests at the point of care. Microbial resistance to antibiotics has been a constant concern since antibiotic development began. This is one of the major dangers posed by bacteria, as it

not only causes deadly infections, but also leads to prolonged sick leave, large budget expenditures, and increased morbidity. Inadequate management, unsanitary environments, untrained professionals, and overuse and abuse of antibiotics contribute to this state of panic as bacteria inherit and acquire resistance genes (7). The emergence of antibiotic resistance is a growing phenomenon and its successful containment requires periodic assessment and updating of the antibiotic susceptibility characteristics of the pathogen (8). The resistance patterns of different microorganisms vary by region of the world and are strongly influenced by the appropriate use of antimicrobials in that region. In 2017, WHO published a list of bacteria that needed urgent research and treatment. In the list, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Enterobacteriaceae* and ESBL-producing *Enterobacteriaceae* were listed as critical priority pathogens, while vancomycin-resistant *Enterococci* (VRE), Methicillin Resistant *Staphylococcus aureus* (MRSA) were listed as high priority pathogens. What's more, penicillin-non susceptible *Streptococcus pneumoniae* was listed as medium-priority pathogen. All of the antibiotic resistant organisms discussed are clinically important and have high morbidity and/or mortality risk (9). AMR has increased in low, middle and high-income countries around the world in recent years and this trend is expected to continue (10-12). The use of antibiotics is generally unchecked in developing countries such as Bangladesh, posing a major global

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and regional risk. Regular updating of antibiotic resistance patterns around the world can help prescribing appropriate antibiotics to patient and prevent the development of 'superbugs'. Therefore, research on antibiotic resistance can help establish appropriate empirical guidelines for antibiotic therapy, especially in countries with a high burden of antibiotic resistance. These guidelines can be used first to develop hospital policies on antibiotic use and then to develop national antibiotic policies based on World Health Organization (WHO). Based on these considerations, this study aimed to determine the antibiogram of clinical isolates collected from some well-known diagnostic centers in Dhaka city, Bangladesh.

### MATERIALS AND METHODS

**Collection of samples:** The samples were collected between October and December 2022. The samples were collected from several renowned diagnostic centers in Dhaka city, Bangladesh. A total of 30 bacterial isolates were found from urine, pus, sputum, wound swabs, blood, throat swabs, and umbilical swab from human patients of different ages and gender with different diseases. Sample processing and transportation were maintained as per WHO guidelines. All laboratory experiments were performed in the Department of Microbiology, Stamford University Bangladesh.

**Isolation and confirmation of bacterial isolates:** Samples were collected and aseptically transferred into the media for incubation. After an overnight incubation at 37°C for 24 hours, isolates were selected for further analysis.

**Microscopic analysis:** Microscopic analysis of the isolates was done through bacterial size, shape, and staining properties (13). Initial identification of selected isolates was performed by Gram staining procedure followed by biochemical tests. The cultural and morphological characteristics of selected isolates were identified according to standard microbiological protocols (14).

**Biochemical test for the confirmatory identification:** All isolated bacteria were identified by standard laboratory biochemical tests according to the methods described elsewhere (14). The biochemical tests for bacterial pathogens were indole test, MR-VP test, catalase test, oxidase test and citrate utilization test.

**Determination of antimicrobial susceptibility by disk diffusion method:** Pure culture from different clinical samples was selected for determining antibiotic susceptibility pattern against 15 different groups of antibiotics such as Amikacin (30µg), Azithromycin (30µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Cefuroxime (30µg), Erythromycin (30µg), Cefoxitin (30µg), Gentamicin (30µg), Imipenem (30µg), Linezolid (30µg), Meropenem (30µg), Norfloxacin (30µg), Piperacillin (110µg), Penicillin (30µg) and Vancomycin (30µg) by Kirby Bauer disc diffusion method (15). The plates were then inverted and incubated at 37°C for 24 h. After incubation, the plates were examined, and the zone of inhibition was measured in mm in diameter according to the standard guidelines as recommended by the Clinical and Laboratory Standards Institute (CLSI) guideline, 2020 (16).

### RESULTS & DISCUSSION

Nowadays antibiotic resistance is increasing day by day.

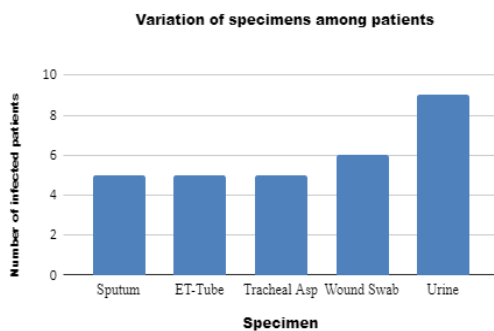


Figure 1: Sample categorization based on infected patients.

Various types of clinical samples were collected from different types of patients from October 2022 to December 2022 from some tertiary hospitals in Dhaka, Bangladesh to observe their resistance pattern. In Figure 1, there were 30 patients among which major portion of collected sample were urine (9). Wound swab was the second highest (6) sample type while sputum (5), ET-Tube (5), and Tracheal Asp (5) were in equal numbers.

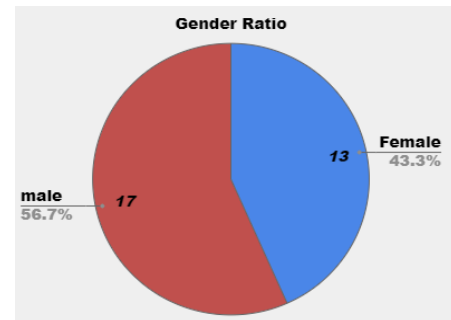


Figure 2: Distribution of patients based on their gender.

In this study, a total of 30 clinical samples from various patients with different infectious syndromes were obtained and subjected to microbiological culture for establishing a definitive etiological diagnosis. We found that male patients are likely to be more infected than female counterparts based on overall samples. Among the isolates tested 56.7% of them were obtained from male and about 43.3% were obtained from female subjects (Figure 2).

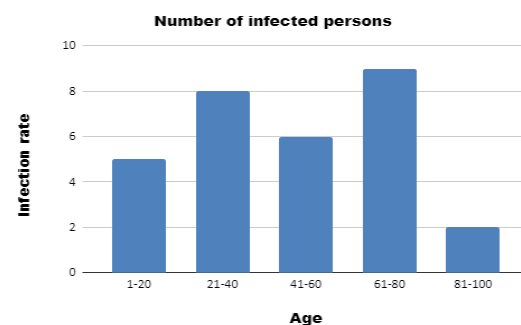


Figure 3: Distribution of age groups among respondents.

The incidence of infection appears to vary randomly with age, without any selective bias, although the tendency to be affected by various bacterial infections appears to be highest between the ages 61 to 80 for overall patients, while the second most common infection is at ages 21-40 years. It is noticeable that middle-aged patients were most infected during our investigation. The lowest prevalence rate was observed between the age group from 81-100 years according to our study findings (Figure 3). Another previous study showed that patients' ages ranged from 11-20 years and had the highest degree of susceptibility pattern (17). However, another report regarding clinical isolates showed that the incidence of infection was much higher in patients having age group 51 years to 70 years age

groups when compared to other age groups (18).

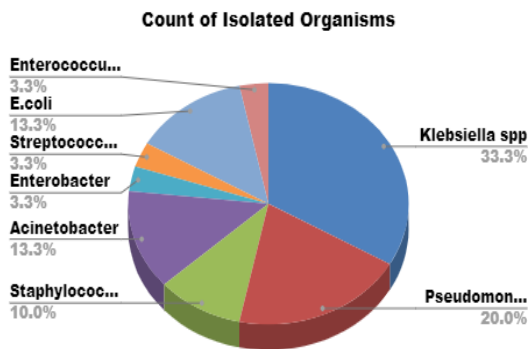


Figure 4: Number of the total isolates.

From Figure 4, the clinical isolates *Klebsiella* spp. (33.33%) was highest in amount to cause infections whereas *Streptococcus* spp. (3.3%) and *Enterobacter* spp. (3.3%) were the lowest. *Pseudomonas* spp. (20.0%), *Acinetobacter* (13.3%), *Staphylococcus* (10.0%), *Enterococcus* spp. (3.3%) and *E. coli* (13.3%) were found.

Gram-negative organisms were higher in ratio than Gram-positive organisms in our findings. Another similar kind of study from our country also produced the same kind of observation (19).

Different biochemical tests such as MR-VP, Indole, Citrate, Catalase and Oxidase tests were done to identify the isolates. However, *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter* spp., *Streptococcus* spp. and *Staphylococcus* spp. were presumptively identified through standard biochemical testing (Table 1). A total of 30 isolates were checked by using 15 different antibiotics. Here the highest susceptibilities of the pathogens to antibiotics were seen towards Norfloxacin (100%) (Figure 5). Moreover, it was found to be highly sensitive to Vancomycin, Linezolid, and Cefoxitin. A recent study showed the sensitivity percentage of Gentamicin (41%), Nitrofurantoin (68%), Imipenem (78%), and Meropenem (67%) against gram (+) uropathogens. In the case of Bangladesh, the findings for antimicrobial resistance were the same in the time period of 2016-2020 in another case report (20).

Table 1: Biochemical testing of isolated organisms.

Sample no.	MR Test	VP Test	Citrate Test	Indole Test	Catalase Test	Oxidase Test	Presumptive Organism
W <sub>3,4</sub> , U <sub>7,28</sub>	+	-	-	+	+	-	<i>E. coli</i>
S <sub>1,2,5,18,20,25</sub> , U <sub>26,27,29,30</sub>	-	+	+	-	+	-	<i>Klebsiella</i> spp.
E <sub>6,8,10</sub> , T <sub>15,17</sub> , W <sub>19</sub>	-	-	+	-	+	-	<i>Pseudomonas</i> spp.
T <sub>11,12</sub> , W <sub>14,21</sub>	-	-	+	-	+	-	<i>Acinetobacter</i> spp.
U <sub>9,13</sub>	-	+	+	-	+	-	<i>Enterobacter</i> spp.
W <sub>24</sub>	-	-	-	-	-	-	<i>Streptococcus</i> spp.
W <sub>16,22,23</sub>	+	+	+	-	+	-	<i>Staphylococcus</i> spp.

Note: S=sputum, E= ET-Tube, T=Tracheal Asp, W=Wound Swab, U=Urine, MR= Methyl Red, VP= Voges Proskauer.

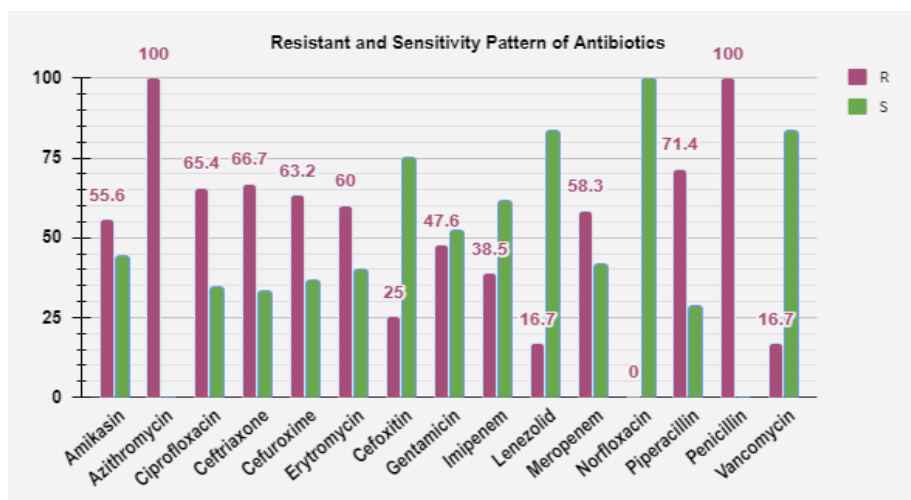


Figure 5: Antimicrobial resistance patterns of commonly observed isolates (%).

In comparison, the highest resistance of the studied pathogens to antimicrobials was seen against Azithromycin (100%) and Penicillin (100%) followed by Piperacillin (71.4%), Ceftriaxone (66.7%), Ciprofloxacin (65.5%), Cefuroxime (63.2%), Erythromycin (60%), Meropenem (58.3%), Gentamicin (47.6%), Imipenem (38.5%), Cefoxitin (25%), Linezolid (16.7%) and Vancomycin (16.7%). These findings were also justified by another study that was conducted against Gram (-) ve organisms (21, 22). A relevant study on Gram-positive bacteria showed a lower rate of resistance to azithromycin (83.7%) than our study, indicating that the antibiotic resistance mechanism is increasing at a higher level (23).

### CONCLUSION

Antibiotic resistance in pathogenic bacteria is a global challenge associated with high morbidity and mortality. This study provides insight into local resistance patterns and prevalence of resistant organisms across a variety of infections to further guide appropriate antibiotic prescribing practices and coordinate robust antibiotic stewardship programs. Significant increases in drug resistance are emerging, and when combined with poor infection control measures, drug-resistant bacteria can easily spread to other patients and the environment. Up-to-date and regular monitoring of the burden of resistance is essential to guide clinicians in selecting appropriate empiric antibiotics, leading to reduced overall resistance rates and effective outcomes. Good knowledge of local epidemiology and antibiograms of bacterial isolates will help design empirical coverage and provide adequate and appropriate antibiotic stewardship.

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### CONFLICT OF INTEREST

The authors have no conflict of interest.

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