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Antibiogram and Antimicrobial Susceptibility Pattern of Bacterial Isolates from A Tertiary Care Hospital in Dhaka

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Abstract

Antimicrobial resistance (AMR) has emerged as one of the most significant public health challenges worldwide, posing a serious threat to the effective treatment of infectious diseases. This study aimed to identify bacterial isolates among various clinical samples and to determine their antimicrobial susceptibility profile. This observational study was carried out from January to December, 2023 in the Department of Microbiology at a tertiary care hospital in Dhaka, Bangladesh. Clinical samples were collected from both outpatients and inpatients who visited the hospital within the study period. The specimens included urine, stool, sputum and blood samples, as well as swabs from wounds, ears and the vaginal area. These samples were sent to the microbiology laboratory for processing, identification and antimicrobial susceptibility testing (AST). Standard microbiological protocols were followed. Among 8554 clinical samples only 941 (11%) yielded bacterial growth. Out of culture-positive cases, Escherichia coli was the most predominant one which accounted for 397 (42.19%) of all the bacterial isolates, followed by Salmonella Typhi 174 (18.49%) and Klebsiella species 142 (15.10%). In case of Escherichia coli increased level of susceptibility were observed in case of meropenem 97%, nitrofurantoin 83%, amikacin 82%, gentamicin 80% and piperacillin-tazobactam 75% respectively. In case of Klebsiella species elevated level of sensitivity were seen in case of meropenem 85%, amikacin 78% and gentamicin 70% respectively. All the 2nd, 3rd and 4th generation of cephalosporins showed reduce level of sensitivity in case of Escherichia coli and Klebsiella species. All the isolates of Salmonella Typhi and Paratyphi were susceptible to ceftriaxone and meropenem. Almost all the strains of Salmonella Typhi and Paratyphi were resistant to ciprofloxacin. Gram-positive organisms observed increased level of sensitivity towards linezolid, vancomycin and nitrofurantoin. In conclusion, the study highlights the concerning trends in antimicrobial resistance among bacterial isolates, emphasizing the need for continuous surveillance, antibiogram, rational antibiotic use and the implementation of effective infection control measures to combat this growing public health threat.

Keywords: Antibiogram, Antimicrobial susceptibility pattern, antimicrobial resistance, Bacterial pathogens

Introduction

Antimicrobial resistance (AMR) has emerged as one of the most significant public health challenges worldwide, posing a serious threat to

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the effective treatment of infectious diseases [1]. AMR leads to prolonged illness, increased morbidity and mortality and escalates healthcare costs [2]. The widespread prevalence of resistant microorganisms in humans, animals, foods and the environment coupled with inadequate infection control practices, poor sanitation and improper food handling contribute to the relentless spread of AMR [3]. In many settings, lack of clinical microbiology laboratories to identify the specific etiologic agents and their antimicrobial susceptibility testing causes increased empirical therapy leading to the emergence of AMR. Additionally, the unrestricted availability of over-the-counter (OTC) antibiotics worsens the situation, enabling inappropriate use and resistance development [4]. AMR causes an estimated 700,000 deaths annually worldwide and if not properly addressed, the number could grow to 10 million per year alongside a cumulative cost of $100 trillion by 2050 [5]. A recent Lancet study analyzing AMR data from 204 countries estimated that 4.95 million deaths occurred in 2019 due to infections caused by antibiotic-resistant organisms. The highest death rates were found in lower-middle income countries [6]. The magnitude of AMR infection among humans is high which has been observed in several studies during the time period of 2017 to 2020 [7].

AST (Antimicrobial Susceptibility Testing) helps to determine AMR pattern of various bacterial isolates, make the treatment helpful by proper antibiotic selection and forecast therapeutic outcome. AST results are interpreted using the CLSI (Clinical and Laboratory Standards Institute) guidelines and it helps the clinicians to choose a cost-effective antibiotic to the patient. As the AMR pattern exposed an increase in the frequency of antibiotic resistant bacteria in various health-care settings, the perception of antibiogram was introduced [8]. The World Health Organization (WHO) emphasizes the key role of the microbiology laboratory in antimicrobial stewardship (AMS) by informing the appropriate use of antibiotics through development of antibiograms [9].

An antibiogram, a periodic summary of antimicrobial susceptibility test of bacterial isolates submitted by a hospital's clinical microbiology laboratory can serve as the primary source of validated data to be used by clinicians to assess local antimicrobial susceptibility patterns of pathogens and guide empirical therapy or selection of antimicrobials [2].

The susceptibility rates of the commonly isolated organisms to their commonly prescribed antibiotics are obtainable separately in the antibiogram [8]. The antibiogram report can be generated using a software called WHONET which gives uniform guidelines in performing the AST [2]. Various studies conducted show that antimicrobial resistance is increasing and need to study the local resistance trends of institutions through constructing an antibiogram. There is a lack of such robust data to guide pathogen-directed therapy and empirical antibiotic therapy [10]. Given these challenges, understanding the local antimicrobial susceptibility patterns of bacterial pathogens is crucial to guiding effective treatment strategies and curbing the rise of resistance. This article aims to analyze pathogen resistance and sensitivity patterns in a hospital setting, identify trends in antibiotic resistance and highlight the importance of developing a hospital-specific antibiogram. Such a resource will support evidence-based prescribing of empirical antibiotics, improving treatment outcomes and aiding efforts to combat antimicrobial resistance.

Materials and Methods

Study Design

This observational study was conducted from January to December 2023 in the Department of Microbiology at Uttara Adhunik Medical College, a tertiary care hospital in Dhaka, Bangladesh. All culture-positive samples submitted during the study period were included, excluding repeat isolates from the same patient to avoid duplication.

Sample Collection

Clinical samples were obtained from both outpatients and inpatients visiting the hospital. The specimens included urine, stool, sputum, and blood samples, as well as swabs from wounds, ears, and the vaginal area. These samples were sent to the microbiology laboratory for processing, identification, and antimicrobial susceptibility testing (AST). Standard microbiological protocols were followed, and data were retrospectively evaluated using patient case records with a standardized data collection format.

Sample Processing and Isolation

The collected clinical specimens (urine, stool, exudates, sputum samples along with swabs from wounds, ears) were initially cultured on blood agar and MacConkey agar plates. Blood agar allows for the growth of fastidious organisms and can reveal hemolytic patterns, while MacConkey agar is selective for Gram-negative bacteria and differentiates lactose fermenters from non-fermenters. Plates were incubated aerobically for 18-24 hour at 35°C. Blood specimens were inoculated into blood culture bottles at the collection site immediately after collection, carried to the microbiology laboratory and BD BACTEC FX40 automated blood culture method was used. In case of a growth, the BD BACTEC FX40 automatically gives an alert. The positive bottles were subculture on MacConkey's, blood agar and chocolate agar media. The chocolate agar plates were incubated inside a candle jar to provide 5-10% CO2, whereas the other two agar plates (blood agar and MacConkey agar) were incubated aerobically for 18-24 hour at 35°C

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to allow bacterial growth according to SOP. Bacterial species were identified following laboratory SOPs. After the incubation period, individual bacterial colonies were observed and picked for identification. The isolates were then subjected to a series of biochemical tests following standard identification procedures to identify the bacterial species. All isolates were tested for AST by the standard Kirby-Bauer disc diffusion method. 3-5 fresh colonies of test organism were picked up with a sterile loop, to make a direct suspension in normal saline and turbidity is adjusted to 0.5 McFarland standard which ensures a standardized bacterial load for the inoculation. It was then inoculated on the surface of a Muller Hinton agar (MHA) plate using sterile cotton swab. After inoculating the MHA plate, antibiotic discs were placed on the surface. The following antibiotics were included in the study: Penicillin (P), Ampicillin (AMP), Amoxicillin-Clavulanic Acid (AMC), Cloxacillin (OB), Piperacillin-Tazobactam (TZP), Aztreonam (ATM), Meropenem (MEM), Cefuroxime (CXM), Cefixime (CFM), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefepime (FEP), Amikacin (AK), Gentamicin (CN), Netilmicin (NET), Ciprofloxacin (CIP), Vancomycin (VA), Erythromycin (E), Clindamycin (CD), Azithromycin (AZM), Nitrofurantoin (F), Linezolid (LZD), Sulfamethoxazole -Trimethoprim (SXT), Doxycycline (DO). The MHA plates were incubated at 35°C for 18-24 hours. After incubation, the zone of inhibition around each antibiotic disc was measured in millimeters using a calibrated ruler. The interpretation of the zone of inhibition was based on the CLSI guidelines (2023) [11]. For each specific antibiotic Mueller Hinton agar media and antimicrobial discs were procured from Oxoid Ltd.,UK.

Quality Control and Standards

Quality control was ensured using reference strains, including Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, and Staphylococcus aureus ATCC 25923.

Data Collection Tool

Data were collected using the WHONET desktop software [12], a standardized tool for microbiology data management. The software facilitated the structured documentation of patient demographics, specimen types, bacterial species, and antimicrobial susceptibility profiles. Information was systematically recorded to ensure consistency and accuracy across all data points. The collected data included important variables such as patient age, sex, clinical specimen type (e.g., blood, urine, sputum, wound swabs), and results of antimicrobial susceptibility testing (AST). The susceptibility outcomes, categorized as Resistant (R), Intermediate (I), or Susceptible (S), were directly entered into WHONET, ensuring alignment with established data collection protocols. This standardized approach enabled the integration and subsequent analysis of data to identify antimicrobial resistance patterns effectively.

Data Analysis

The collected data were meticulously cleaned, and analyzed using WHONET and the Quick Analysis of Antimicrobial Patterns and Trends (QAAPT) software's [13]. The process involved configuring laboratory-specific data, updating susceptibility interpretations as Resistant (R), Intermediate (I), or Susceptible (S), and generating resistance profiles categorized by bacterial species, antibiotics tested, and designated time periods. QAAPT enhanced the analysis by aggregating and visualizing susceptibility data, enabling streamlined computation of susceptibility percentages for each organism. To ensure accuracy, organisms with fewer than 30 isolates were excluded from the dataset, reducing the risk of unreliable statistical inferences. This integrated approach provided robust and high-quality data for further analysis and reporting.

Antibiogram Development

Using the data processed through WHONET and QAAPT, comprehensive antibiograms were generated to present antimicrobial susceptibility patterns for bacterial isolates that met the study's inclusion criteria. The antibiograms detailed the percentage of isolates classified as susceptible, intermediate, or resistant to each antibiotic, offering critical insights into resistance trends across various bacterial species. The analysis incorporated AST results from diverse clinical specimens, including blood, urine, sputum, and wound swabs, processed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines to ensure methodological rigor. QAAPT's functionality enabled dynamic visualization and efficient reporting of susceptibility data, highlighting resistance patterns and identifying emerging resistance hotspots.

Results

A total of 8554 clinical samples were collected and subjected to culture and sensitivity, of which 941 (11%) yielded bacterial growth (Table 1).

Table 1: Frequency of bacterial growth pattern (n=8554)

Bacterial growth	Frequency	Percentage (%)
Growth	941	11
No growth	7613	89
Total	**8554**	**100**

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Pattern of organisms isolated were shown in Table 2. Out of culture-positive cases, Gram-negative organisms were mostly isolated in comparison to Gram-positive one. Among them Escherichia coli was the most predominant one which accounted for 397 (42.19%) of all the bacterial isolates, followed by Salmonella Typhi 174 (18.49%) and Klebsiella species 142 (15.10%). The least isolated organisms were Enterococcus species 46 (4.88%), Salmonella Paratyphi A 41 (4.36%), Enterobacter species 41 (4.36%), Staphylococcus aureus 40 (4.25%), Acinetobacter species 36 (3.82%) and Pseudomonas aeruginosa 24 (2.55%) respectively.

In case of Escherichia coli increased level of susceptibility were observed in case of meropenem 97%, nitrofurantoin 83%, amikacin 82%, gentamicin 80% and piperacillin-tazobactam 75% respectively. In case of Klebsiella species elevated level of sensitivity were seen in case of meropenem 85%, amikacin 78% and gentamicin 70% respectively. Sensitivity was higher for meropenem 97%, amikacin 75% and piperacillin-tazobactam 72% in case of Enterobacter species. All the 2nd, 3rd and 4th generation of cephalosporins showed reduce level of sensitivity in case of Escherichia coli, Klebsiella and Enterobacter species. In case of Acinetobacter species meropenem and piperacillin-tazobactam exhibited raised level of susceptibility and the percentage was 80% and 71% respectively. In case of Pseudomonas aeruginosa both meropenem and piperacillin-tazobactam exhibited 80% sensitivity individually and 75% susceptibility was observed in case of netilmicin. All the isolates of Salmonella Typhi and Paratyphi were susceptible to ceftriaxone and meropenem. Susceptibility percentage of cefixime, sulfamethoxazole-trimethoprim and ampicillin were 99%, 87% and 91% respectively in case of Salmonella Typhi and 100%, 88% and 85% respectively in Salmonella Paratyphi. In case of Salmonella Typhi only 2% strains were sensitive to ciprofloxacin (Table 3).

Table 3: Antibiotic susceptibility pattern of isolated major Gram-negative bacteria (n=855)

Name of antibiotics	Gram-negative bacteria susceptible percentage (%)						
	E.coli (n=397)	*Klebsiella spp.(n=142)*	*Enterobacter* spp.(n=41)	*Acinetobacter spp. (n=36)*	*P.aeruginosa (n=24)*	*S.*Typhi *(n=174)*	*S.*Paratyphi (n=41)
Ampicillin	NT	IR	IR	IR	IR	91	85
Amoxyclav	21	36	IR	IR	IR	NT	NT
Aztreonam	24	53	42	IR	50	NT	NT
Piperacillin-Tazobactam	75	63	72	71	80	NT	NT
Cefuroxime	13	24	IR	NT	NT	NT	NT
Cefixime	22	45	14	NT	IR	99	100
Ceftazidime	36	53	38	20	68	NT	NT
Ceftriaxone	40	55	51	31	IR	100	100
Cefepime	41	47	38	24	65	NT	NT
Meropenem	97	85	97	80	80	100	100
Gentamicin	80	70	70	68	NT	NT	NT
Amikacin	82	78	75	NT	67	NT	NT
Netilmicin	NT	NT	NT	NT	75	NT	NT
Ciprofloxacin	36	42	54	50	62	2	1
Sulfamethoxazole-Trimethoprim	49	55	61	51	IR	87	88
Nitrofurantoin	83	46	50	NT	IR	NT	NT
Azithromycin	IR	IR	NT	NT	NT	62	NT

(Note: IR - Intrinsic Resistance, NT - Not Tested)

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Table 4: Antibiotic susceptibility pattern of isolated major Gram-positive bacteria (n=86)

Name of antibiotic	Gram-positive bacteria susceptible percentage (%)	
	S.aureus (n=40)	*Enterococcus spp.(n=46)*
Penicillin	9	23
Ampicillin	0	86
Cloxacillin	81	NT
Clindamycin	80	IR
Vancomycin	NT	100
Doxycycline	92	NT
Linezolid	100	100
Gentamicin	93	IR
Nitrofurantoin	100	77
Ciprofloxacin	49	20
Sulfamethoxazole-Trimethoprim	83	IR
Azithromycin	40	NT
Erythromycin	45	NT

(IR - Intrinsic Resistance, NT - Not Tested)

Antibiotic susceptibility pattern of Gram-positive isolates was shown in Table 4. All the isolates of Staphylococcus aureus were susceptible to linezolid and nitrofurantoin and increased level of sensitivity were marked in case of gentamicin 93%, doxycycline 92%, sulfamethoxazole-trimethoprim 83%, cloxacillin 81% and clindamycin 80% respectively. All the isolates of Enterococcus species were sensitive to vancomycin and linezolid and elevated level of susceptibility was marked in case of ampicillin and nitrofurantoin where the percentage was 86% and 77% respectively.

Discussion

Increased in antimicrobial resistance has made it necessary in recent time to an up to date information on antibiotic susceptibility and resistance patterns of bacterial isolates through antibiogram in order to determine appropriate empirical therapy [14]. Antibiograms served as invaluable tools for guiding empirical therapy decisions, informing hospital infection control policies, and supporting broader antimicrobial resistance surveillance efforts. By aligning with global standards for data reporting and interpretation, this study leveraged QAAPT's capabilities to generate actionable insights critical for clinical and public health decision-making. In current study, the frequency of bacterial growth rate among various clinical samples was 11% (Table 1). In contrary to our finding, higher growth rate was observed in a study done in Northwest Ethiopia by Yitayeh et al. and the rate was 18.7% [15]. In our study, the rate of growth was relatively low in comparison to above study and the reason might be due to prior antibiotic therapy before submitting the clinical samples.

In present study among the culture-positive cases, Gram-negative organisms were mostly isolated in comparison to Gram-positive one. Among them Escherichia coli was the most predominant one which accounted for 397 (42.19%) of all the bacterial isolates, followed by Salmonella Typhi 174 (18.49%) and Klebsiella species 142 (15.10%) (Table 2). A study done in Ghana by Dodoo et al. [16] and two studies done in India by Dikkatwar et al. and R et al. [17,18] also found Escherichia coli and Klebsiella species as predominant organisms like our study. In conflict with current study, Aika and Enato of Nigeria [19] observed Staphylococcus aureus and Coliforms as predominant organisms in their study. The difference in the pattern of bacterial isolates might be due to difference in study subjects, study design, identification method, geographic variation and variation within a study population [14]. In this study, in case of Escherichia coli increased level of susceptibility was observed in case of meropenem (97%), nitrofurantoin (83%), amikacin (82%), gentamicin (80%) and piperacillin-tazobactam (75%) respectively and decreased level of sensitivity was marked in case of 2nd, 3rd and 4th generation of cephalosporins and ciprofloxacin (Table 3). Like present study, higher level of sensitivity was marked in case of meropenem (94%), nitrofurantoin (98%), amikacin (93%), gentamicin (88%) and piperacillin-tazobactam (88%) respectively in a study done in India [18]. A study done in Pakistan by Iftikhar et al. also noted elevated level of susceptibility towards meropenem (82%) and amikacin (93%) like present study [20]. In identical with present study, lower level of susceptibility was noted in a study where in case of cefuroxime (34%), ceftriaxone (39%) and ciprofloxacin (41%) sensitivity was noted [18]. Diminished level of sensitivity towards ciprofloxacin and 2nd, 3rd and 4th generation of cephalosporins might be due to irrational use of these drugs by clinicians, paramedics and other personnel in hospitals and other clinical settings.

In current study, in case of Klebsiella species raised level of sensitivity were seen in case of meropenem (85%), amikacin (78%) and gentamicin (70%) respectively (Table 3). In case of meropenem (89%), amikacin (84%) gentamicin (83%) susceptibility was observed in a study done in India [18] and these observations were almost similar with the present study. In opposite to current study, diminished level of sensitivity was marked in a study done by Dikkatwar et al. where in case of meropenem (10%), gentamicin (27%) and amikacin (18%) susceptibility was noted [17]. In this study, in case of Enterobacter species, susceptibility rate was higher in case of meropenem (97%), amikacin (75%), piperacillin-tazobactam (72%) and gentamicin (70%) respectively. In case of 3rd and 4th generation of cephalosporins reduced level of sensitivity was marked in current study (Table 3). A study done in Ethiopia by Amsalu et al. observed 66.7% resistant in case of gentamicin and sulfamethoxazole-trimethoprim

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individually and in case of ceftriaxone and ciprofloxacin 75% resistant individually was observed [14].

In present study, both meropenem and piperacillin-tazobactam exhibited 80% sensitivity in case of Pseudomonas aeruginosa (Table 3). In case of meropenem (93%) and piperacillin-tazobactam (85%) susceptibility was observed in a study done by R et al. [18], but in contrast to present study, 51% sensitivity was observed in case of meropenem in a study done by Iftikhar et al. [20]. In case of Acinetobacter species, in this study 80% susceptibility was observed in case of meropenem and like present study almost similar rate of sensitivity was observed in a study done in Pakistan [20]. In current study, all the isolates of Salmonella Typhi were susceptible to ceftriaxone and meropenem, 99% and 87% susceptibility was observed in case of cefixime and sulfamethoxazole-trimethoprim respectively and only 2% strains were susceptible to ciprofloxacin (Table 3). In similar to our findings, a study done in Nepal showed 98.9% strains were sensitive to ceftriaxone and cefixime individually and all the strains were sensitive to sulfamethoxazole-trimethoprim [21].

In present study in case of Staphylococcus aureus, all the isolates were sensitive to linezolid and nitrofurantoin and increased level of susceptibility were observed in case of gentamicin (93%) followed by doxycycline (92%), sulfamethoxazole-trimethoprim (83%), cloxacillin (81%) and clindamycin (80%) (Table 4). Like present study, in case of Staphylococcus aureus higher level of sensitivity was observed in a study done in India by R et al. [18] where the susceptibility rate in case of nitrofurantoin was (100%), gentamicin (83%), clindamycin (74%). In current study, in case of ciprofloxacin and erythromycin the sensitivity rate was low and the percentage was 49% and 45% respectively and susceptibility percentage 38% in case of ciprofloxacin and 43% in case of erythromycin was observed by the above mentioned study done by R et al. [18]. In contrast to our finding, a study done in Nigeria observed lower rate of sensitivity in case of cloxacillin (2%), clindamycin (42%), gentamicin (36%), nitrofurantoin (24%), ciprofloxacin (18%) and erythromycin (7%) [19]. Increased susceptibility in current study might be due to lesser use of these drugs in the hospital. In this study, all the isolates of Enterococcus species were sensitive to vancomycin and linezolid and elevated level of susceptibility was marked in case of ampicillin and nitrofurantoin where the percentage was 86% and 77% respectively (Table 4). Like present study, in India a study done by R et al. observed higher level of sensitivity towards vancomycin and nitrofurantoin in case of Enterococcus species and the percentage was 82% and 75% respectively [18].

Conclusion

Antibiograms and antimicrobial susceptibility testing remain indispensable tools in the fight against bacterial infections, offering essential data to inform treatment decisions. This study reinforces the importance of targeted and evidence-based antibiotic therapy, as well as the need for public health initiatives that promote responsible antibiotic use. Results of this study will help in providing useful guidelines for choosing an effective antibiotic in our hospital. With the rising prevalence of antibiotic-resistant bacteria, it is imperative that healthcare systems adapt by implementing stricter guidelines on antibiotic usage, enhancing diagnostic capabilities, and investing in the development of new therapeutic options. The data derived from antibiograms serve as a cornerstone for such initiatives, enabling clinicians to make informed decisions and tailor treatments to the specific needs of patients. The fight against antimicrobial resistance requires a collective, sustained effort to preserve the efficacy of antibiotics for generations to come.

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Data Availability

The data is contained within the manuscript and supplementary material

Competing Interests

The authors declare no conflict of interest.

Author's Contribution

MN and FA drafted the manuscript. MN provided statistical analysis of the data. MFB, RK, MMB, NN, MSA and MJS validated the results and revised the manuscript.

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