



Research Article

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Microbial Pathogens and Antibiotic Resistance in Healthcare-Associated Infections: Insights from Primary Care Hospitals, Sirajganj, Bangladesh

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Abstract

Background: Healthcare-Associated Infections (HAIs) are a significant concern in Bangladesh that contribute to prolong stays in a hospital and increases healthcare costs.

Objectives: Understanding the microbial profiles (pathogens) and their antimicrobial resistance patterns of HAIs which is very essential for the effective control measurements.

Methods: Clinical swab samples were collected from various sites of Primary Care Hospitals (PCHs), including Operation Theater (OT) rooms, Information Desks (ID), Patient Waiting Chairs (PWC), and Patient Beds (PB). Bacterial cultures were performed on different medias, such as MacConkey agar, chromogenic agar, and blood agar. Different biochemical tests were conducted for the isolation of bacteria. Antimicrobial susceptibility test was performed using Kirby-Bauer disc diffusion method with a panel of five (amoxicillin, ceftazidime, gentamicin, ceftriaxone, and cefixime) antibiotics.

Results: Out of eighty-nine (89) swab samples, 79 (89.0%) showed positive bacterial growth. *Pseudomonas aeruginosa* was the most frequently pathogen isolated, followed by *Acinetobacter spp.*, *Staphylococcus aureus*, and *Escherichia coli*. Antibiotic susceptibility testing revealed that a significant resistance occurred in isolates against amoxicillin, ceftazidime, gentamicin, ceftriaxone, and cefixime. Gentamicin showed the highest efficacy, while meropenem exhibited the least effectiveness.

Conclusion: This study underscores the dominance of gram-negative bacteria, especially *Pseudomonas aeruginosa*, in causing HAIs in PCHs in Sirajganj. The findings highlighted an urgent essential for robust infection control measures and antibiotic stewardship programs to combat HAIs effectively.

Keywords: Healthcare-associated infections, Bacterial profiles, Antibiotic resistance, Primary care hospitals, Sirajganj, Bangladesh



Introduction

Healthcare-Associated Infections (HAIs), commonly referred to as nosocomial infections, are a significant global public health concern. These infections occur in hospital and healthcare environments, often affecting patients undergoing medical procedures or treatments. The World Health Organization (WHO) estimates that millions of people worldwide are impacted by HAIs annually, with significant morbidity and mortality rates, especially in low- and middle-income countries like Bangladesh [1]. HAIs are primarily caused by bacterial, viral, or fungal pathogens that thrive in hospital environments and are often transmitted through healthcare workers, medical devices, and contaminated surfaces [2].

In recent years, the rise of antibiotic resistance among pathogens responsible for HAIs has exacerbated the challenges associated with infection control and treatment. Pathogens have become increasingly resistant to commonly used antibiotics, including beta-lactams, carbapenems, and aminoglycosides [3]. This resistance significantly limits treatment options, contributing to increased hospital stays, higher healthcare costs, and greater mortality rates [4]. In Bangladesh, the rapid spread of Multidrug-Resistant (MDR) organisms in healthcare facilities has heightened the public health risk, placing additional strain on an already overburdened healthcare system [5].

Factors contribute to the high prevalence of antibiotic-resistant HAIs in Bangladesh, including overcrowded hospitals, poor infection control practices, overuse and misuse of antibiotics, and inadequate diagnostic facilities [6]. As a result, multidrug-resistant strains of *Klebsiella pneumoniae*, *E. coli*, and *Acinetobacter* have become dominant in healthcare settings, particularly in Intensive Care Units (ICUs), where immunocompromised patients are at greater risk of acquiring infections [2]. Despite ongoing efforts to combat antibiotic resistance, the burden of HAIs in Bangladesh continues to rise, underscoring the need for enhanced infection control protocols and antibiotic stewardship programs.

This study aims to investigate the association between human pathogens and antibiotic resistance in healthcare-associated infections in Bangladesh. By analyzing the prevalence of common HAI-causing pathogens and their resistance patterns, this study seeks to provide a comprehensive understanding of the current HAI landscape in the country and propose potential strategies for mitigating the spread of antibiotic-resistant infections. Understanding the epidemiology of these infections is critical for guiding public health interventions and optimizing treatment approaches in hospital settings.

Methods and Materials

Study Design

This descriptive cross-sectional study was conducted at the Department of Microbiology, Khwaja Yunus Ali University (KYAU), Sirajganj, Bangladesh, from August 1, 2022, to June 30, 2023.

Clinical Sample Collection

The swab samples were obtained from primary care hospitals located in Belchuchi upazila and Sirajganj Sadar, Sirajganj district, Bangladesh. Samples were collected from highly touched surfaces in hospital area such as OT rooms, information desks, patient waiting chairs, and patient beds.

Sterile swab sticks were soaked in sterile normal saline (0.85%) to moisten them, which aids in better sample adherence during swabbing. After soaking, the swab sticks were used to collect samples from the target surfaces or tissues by gently rotating them over the sampling area. Following the collection, the swab sticks were carefully placed back into sterile transport tubes to prevent contamination before proceeding with bacteriological culture.

Sample Transport

After collection of swab samples were immediately placed in an ice-cooler box (previously placed a thermometer to monitor temperature at 4°C) and, transported to the laboratory of Microbiology at KYAU for microbiological analysis.

Bacteriological Culture

Swab samples were inoculated on selective media including MacConkey agar, chromogenic agar, EMB agar and blood agar plates for bacterial isolation. Plates were incubated at 37°C for 24 hours to support optimal bacterial growth.

Bacterial Identification

A set of biochemical tests were performed to confirm the bacteria's species i.e. Kligler's Iron Agar (KIA) test, Motility Indole Urease (MIU) test, Simmon's citrate test, oxidase test, coagulase test, and catalase test following Bergey's Manual of Systematic Bacteriology (9th Edition). Finally confirmed with BD Phoenix™ M50 system.

Antimicrobial Susceptibility Testing

The Antimicrobial Susceptibility Testing (AST) was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA), following the standard guidelines provided by the Clinical and Laboratory Standards Institute-2023 (CLSI). Five antibiotics such as Amoxicillin (10µg), Cefotaxime (10µg), Ceftriaxone (30µg), Cefixime (10) and Gentamicin (30µg) were used against four bacterial isolates. Zones of inhibition surrounding the antibiotic discs were measured and interpreted into three conditions such as intermediate, resistance and sensitive.

Data Analysis

The data found from this study was analyzed using Microsoft Excel and SPSS-2021 for descriptive statistics.

Results

This study investigated the prevalence of Healthcare-Associated Infections (HAIs) and the antibiotic resistance patterns of common pathogens isolated from various hospital environments

in Bangladesh. A total of 89 samples were collected from different high-contact areas, including information desks, operating theaters, patient beds, and waiting chairs, across multiple healthcare settings. The results demonstrated a high frequency of microbial contamination and a significant occurrence of Multidrug-Resistant (MDR) pathogens.

Microbial Growth from Sample Sites

Of the 89 total samples collected, 79 (88.8%) showed microbi-

al growth, while 10 (11.2%) exhibited no growth. The distribution of microbial contamination across different sample sites is summarized in Table 1. The highest growth frequency was observed in the Operating Theater (OT) (95.6%) and Waiting Chair (WC) (95.4%), followed by the Patient Bed (PB) and information desk (ID) (both 81.8%). These findings indicate that hospital surfaces and high-contact areas serve as significant reservoirs for pathogen transmission (Table 1).

Table 1: Growth rate of different swab samples isolated from primary care hospitals in Sirajganj.

Sample Sites	Total Samples	Growth	Growth Frequency (%)	No Growth	No growth Frequency (%)
Information Desk (ID)	22	18	81.8	4	18.2
Operating Theater (OT)	23	22	95.6	1	4.4
Patient Bed (PB)	22	18	81.8	4	18.2
Waiting Chair (WC)	22	21	95.4	1	4.6
Total	89	79	88.8	10	11.2

Identification of Pathogens

The distribution of the identified bacterial pathogens across sample sites is shown in Table 2. The high prevalence of *Pseudo-*

monas aeruginosa (67.1%) and *Acinetobacter baumannii* (17.7%) aligns with their known roles in healthcare-associated infections (Table 2).

Table 2: Total frequency of bacterial isolates and their growth rate with percentage.

Bacterial Isolates	Total Number	Frequency (%)
<i>Pseudomonas aeruginosa</i>	53	67.1
<i>Staphylococcus aureus</i>	8	10.1
<i>Acinetobacter baumannii</i>	14	17.7
<i>Escherichia coli</i>	4	5.1

The results presented in Table 3 reflect the distribution of different bacterial species isolated from various sample sources within a healthcare environment. The overall findings suggest a sig-

nificant presence of bacterial pathogens, especially *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli*, across all sample types (Table 3).

Table 3: Different bacteria isolated from different samples and their growth rate in percentage.

Sample Sources	Bacterial Isolates	Number (n)	Percentage (%)
Information Desk (ID)	<i>Pseudomonas aeruginosa</i>	14	78
	<i>Staphylococcus aureus</i>	2	11
	<i>Acinetobacter baumannii</i>	1	6
	<i>Escherichia coli</i>	1	6
Operating Theater (OT)	<i>Pseudomonas aeruginosa</i>	15	68.2
	<i>Acinetobacter baumannii</i>	4	18.2
	<i>Staphylococcus aureus</i>	3	13.6

Patient Bed (PB)	<i>Pseudomonas aeruginosa</i>	12	66.7
	<i>Acinetobacter baumannii</i>	4	22.2
	<i>Escherichia coli</i>	2	11.1
Waiting Chair (WC)	<i>Pseudomonas aeruginosa</i>	12	57.1
	<i>Acinetobacter baumannii</i>	5	23.8
	<i>Staphylococcus aureus</i>	3	14.3
	<i>Escherichia coli</i>	1	4.8

Antimicrobial Susceptibility Pattern

Three bacterial isolates among four evaluated in this bioassay (*Acinetobacter baumannii*, *Staphylococcus aureus*, and *Escherichia coli*) were grown on Mueller-Hinton agar medium against five anti-

biotics (Amoxicillin, Ceftazidime, Ceftriaxone, Cefixime and Gentamicin) that suggested 100% resistant. Only a bacterium *Pseudomonas aeruginosa* showed 47.2% sensitive against five antibiotics but showed resistance to more than 50% (Table 4).

Table 4: Most of the bacteria showed resistance against five different antibiotics (commercially available).

Bacterial Species	Antibiotics Tested	Resistant (%)	Intermediate (%)	Sensitive (%)
<i>Pseudomonas aeruginosa</i>	Amoxicillin, Ceftazidime, Gentamicin, Ceftriaxone, Cefixime	52.8	0	47.2
<i>Acinetobacter baumannii</i>	Amoxicillin, Ceftazidime, Gentamicin, Ceftriaxone, Cefixime	100	0	0
<i>Staphylococcus aureus</i>	Amoxicillin, Ceftazidime, Gentamicin, Ceftriaxone, Cefixime	100	0	0
<i>Escherichia coli</i>	Amoxicillin, Ceftazidime, Gentamicin, Ceftriaxone, Cefixime	100	0	0

Discussion

In this study, we investigated the microbial contamination, and their antibiotic resistance pattern of bacteria isolated from different areas namely information desk, waiting chair, operation tables and patient beds of Primary Care Hospital located in Sirajganj, Bangladesh. The patient can be infected by pathogens, and it can occur in 48 h or more after admission in a hospital, or in thirty (30) days after received health care [7]. *Pseudomonas aeruginosa* emerged as the predominant pathogen across all samples we isolated from hospital sources. Khan and Faiz, (2016) [8] reported that the pathogen *Pseudomonas aeruginosa* found in hospital environments in Saudi Arabia that can cause human disease, and our findings support their statement. Abedin et al., (2020) [9] mentioned that *Pseudomonas* spp. can cause disease in patients after post-operative condition and our finding agrees with their results.

The results of this study indicate a widespread presence of bacterial pathogens in healthcare-associated environments in Bangladesh, with contamination rates as high as 95.6%. This aligns with previous studies showing that healthcare environments serve as reservoirs for various opportunistic pathogens, particularly in high-traffic areas such as waiting rooms and operating theaters [2].

The high contamination rates in Operating Theaters (OT) are of particular concern, as these environments are meant to be sterile. Contaminated surfaces, equipment, and hands of healthcare workers can easily transmit infections to vulnerable patients undergoing surgery, resulting in severe HAIs [4].

The total number of bacterial isolates identified during the study included *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli*. *Pseudomonas aeruginosa* accounted for the majority of the isolates, comprising 67.1% of all samples. This is consistent with findings from other studies in Healthcare-Associated Infection (HAI) environments in Bangladesh, where *Pseudomonas aeruginosa* has been reported as a predominant pathogen, particularly in hospital-acquired infections [10].

This pathogen is known for its intrinsic resistance to many antibiotics and is a leading cause of HAIs, particularly in immunocompromised patients. In Bangladesh, the incidence of multi-drug-resistant *Pseudomonas aeruginosa* is a growing concern due to its resistance to beta-lactams, aminoglycosides, and carbapenems [11].

Staphylococcus aureus, including methicillin-resistant *Staphylococcus aureus* (MRSA), accounted for 10.1% of isolates. This pathogen is a common cause of surgical site infections, bloodstream infections, and pneumonia. The detection of *Staphylococcus aureus* in this study highlights its role in HAIs, as it is often associated with contact transmission, making it a critical pathogen in infection control [12].

Acinetobacter baumannii is another significant pathogen, responsible for 17.7% of isolates in this study. It is well known for its ability to acquire resistance to a wide array of antibiotics and survive in hospital environments for extended periods. *Acinetobacter baumannii* has been implicated in outbreaks in intensive care units (ICUs) worldwide and is associated with ventilator-associated pneumonia, septicemia, and wound infections [5]. The high prevalence of this pathogen in this study underscores the need for stringent hygiene and sterilization protocols in healthcare settings to prevent its spread.

Escherichia coli, which represented 5.1% of the isolates, is a common pathogen associated with Urinary Tract Infections (UTIs) and bloodstream infections in healthcare settings. Although its frequency was lower compared to other pathogens in this study, the emergence of multidrug-resistant strains, such as Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli*, has raised concerns about the effective treatment of HAIs [13].

The significant presence of gram-negative bacteria, particularly *Pseudomonas aeruginosa* (78.0%) and *Acinetobacter baumannii* (68.2%), underscores the challenges posed by multidrug-resistant organisms in hospital settings. The distribution of bacterial isolates varied by hospital sources, with higher proportions observed in areas with frequent patient contact, such as patient information desk and operating theaters [14]. The high prevalence of bacterial isolates aligns with global trends in healthcare settings, where these pathogens are increasingly associated with multidrug resistance, complicating treatment options and leading to higher morbidity and mortality rates [15].

Antimicrobial susceptibility testing suggested an alarming level of resistance among the bacteria isolated. *Pseudomonas aeruginosa* exhibited resistance to different type of antibiotics, such as amoxicillin, ceftazidime, gentamicin, ceftriaxone, and cefixime, commonly prescribed by doctors in Bangladesh. In this study, more than 52% of isolates collected showed resistance against five antibiotics. Centers for Disease Control and Prevention (CDC) reported (2016) [16] human pathogens acquired resistance against different antibiotics. Our results showed that pathogens are resistant to different antibiotics that support the statement of CDC.

Acinetobacter baumannii, *Staphylococcus aureus*, and *Escherichia coli* showed complete resistance (100%) to different antibiotics tested in our study that can lead nosocomial infections. Our finding strongly suggested taking a step by which antibiotics can be applied in specific conditions of diseases. Only, the appropriate use of anti-

biotics can prevent the formation of multi-drug resistance in human pathogens [17]

Our findings are consistent with a previous study that identified *Pseudomonas aeruginosa* as a predominant pathogen in hospital-acquired infections [8]. The high prevalence of antimicrobial resistance observed in *Acinetobacter baumannii*, and *Staphylococcus aureus* aligns with global reports of increasing resistance trends among gram-negative and gram-positive bacteria [18]. However, our study contrast with other findings that reported varying patterns of bacterial dominance and resistance profiles across different healthcare settings [19]. These variations underscore the importance of local surveillance and tailored infection control strategies to address specific microbial challenges within regional healthcare environments.

Conclusion

In conclusion, this study highlights the prevalence of multi-drug-resistant bacteria, particularly *Pseudomonas aeruginosa* can cause for healthcare-associated infections in Sirajganj, Bangladesh. Our findings demonstrated that health ministry of Bangladesh can take different steps to control the formation of multidrug resistance in human pathogens from primary care hospitals.

Ethical Issue

Ethical issues were granted to this study from the ethical grant committee of Khwaja Yunus Ali University. Prior consent was obtained from the authorities of the primary care hospitals (PCHs) before sample collection.

Conflicts of Interest

Nothing to declare.

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Authors's Contribution

All authors contributed equally to this study and participated in revising the final manuscript. Abedin MZ, Shilpi RY, and Ahmed

AA designed the study and supervised the project. Both Abedin MZ, and Khalil MMR wrote the manuscript and corrected the final version. Prity SS, Mia S, Jarin L, Mahatab MF, Hossain MS and Hossain MM contributed to the sample processing and conducted the experiments.

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