

Isolation, Identification and Antibiotic Resistance of Different Clinical Isolates in Savar Area, Bangladesh

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Abstract: Introduction: The rise of multidrug-resistant (MDR) microorganisms, which pose a grave threat, has made choosing antibiotics to treat bacterial infections incredibly challenging. The prescription antibiotics have to consistently be effective against the identified related infections. Thus the research team wanted to find the antibiotic sensitivity profiles and pathogenic bacterial isolates in various patient specimens. **Methods:** The collection of 403 clinical samples of throat swabs, sputum, blood, stool, and urine from people of both genders and various ages was performed aseptically. Identification was carried out by microscopic, cultural, biochemical, and serological analysis. Finally, the disk diffusion method by Kirby-Bauer was employed to determine antibiotic responsiveness profiling. **Results:** A total of 93 samples (23.08%) were identified as positive isolates comprising 28.74% (25/87) of urine culture, followed by 24.32% (27/111) of blood, 23.08% (15/65) of stool, 20% (07/35) of throat swabs, and 18.1% (19/105) of sputum culture. Gram-negative bacteria 81(87.1%) showed more prevalence than Gram-positive bacteria 12 (12.90%). The most frequently identified isolates were *Klebsiella* spp. (24%), *Salmonella* spp. (23%), *E. coli* (17%), *Pseudomonas* spp. (5%), and *Enterococcus* spp. (3%). The infections showed more prevalence within the age group of 16-46 years (60.2%) and among males (48%) than females (46%). **Conclusions:** It was found that *E. coli* was the most abundant bacteria isolated from urine, *Klebsiella* spp. was the most abundant bacteria isolated from sputum and throat swabs, and Shiga toxin-producing *Escherichia coli* was the most abundant bacteria isolated from stool. Amikacin, Ciprofloxacin, Gentamicin, Imipenem, and Levofloxacin were considerably effective antibiotics, whereas Cefotaxime, Ceftazidime, Cefuroxime, Mecillinam, and Meropenem were least effective.

Keywords: Clinical isolates, Antibiotics susceptibility, Multidrug-resistant (MDR), Savar area.

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Introduction

Medications used to treat and cure bacterial infections are known as antibiotics. Microorganisms that were previously vulnerable to antimicrobial drugs develop resistance to them, a phenomenon known as antimicrobial resistance (AMR) [1]. A particular evolutionary push to create a counterattack mechanism against a particular antimicrobial or class of antimicrobials led to the principal mechanism of antimicrobial resistance [2]. Due to the rise in AMR bacteria, hospital-



acquired infections and other medical issues are on the rise globally, but the healthcare industries have not given the problem much attention [3]. Antimicrobial resistance is a widespread issue with a long history in underdeveloped nations like Bangladesh where there are significant infrastructure and regulatory obstacles [4].

In isolates from urine, stools, throats, throat swabs, and blood samples it has been reported that AMR is higher in gastrointestinal pathogens as well as increased rates of resistance to antibiotics such as erythromycin, amoxicillin, or tetracycline. Antimicrobials are threatened by the emergence of resistance as well as other forms of resistance. Resistance has also been transmitted among individuals and particularly among healthcare facilities. Transmission may be due to the day's interrelations between humans or the movement of animals or different types of packed foods and drinks. In countries such as Bangladesh, where the hygiene situation is so bad that bacteria can be easily transmitted, this problem will only get worse.

Most bacterial infections are primarily treated empirically in Bangladesh, where etiological agents have rarely been identified. Therefore, optimizing treatment and reducing morbidity and mortality related to the disease would be of great value if it were possible to identify the more common bacterial pathogens and their corresponding AMR profile. Thus, for the purpose of being aware and helping people to cope with this issue, a study has been conducted at Lab One Hospital, Savar, Dhaka where pathogenic bacteria isolates are assessed and their antimicrobial resistance profiles analyzed from different types of clinical samples.

Methodology

Ethical Consideration

The Gono Bishwabidyalay, Savar, Dhaka, Bangladesh Institutional Ethical Grant Committee agreed to this proposal.

Study Population and Biological samples

The data were collected during the period from January 2021, to January 2022, for both indoor and outdoor patients at Lab One Hospital in Savar, Bangladesh. Randomly, 403 samples, i.e., urine (87), sputum (105), throat swabs (35), stool (65), and blood (111), were collected aseptically from suspected patients and then transferred to the Department of Microbiology at Gono Bishwabidyalay by a cool chain system.

Sample Collection and Processing

Blood samples: From suspected bloodstream infection patients of all ages, blood samples have been collected aseptically. Only aerobic culture has been used for this study. For children aged 0 to 12 years, 1 to 5 ml of blood samples were properly inoculated into peds plus vials/F, and for adults older than 12 years, 8 to 10 ml of blood samples were correctly inoculated into aerobic vials/F. After that, the clinical samples were incubated fast at 35°C for a maximum of 72 hours in the automated BD Bactec™ FX40 system, unless the outcome was indicated as positive. Following regular CLSI microbiological procedures, the vials that produced a positive result were then subculture on Blood Agar and MacConkey Agar [5].

Urine samples: Urine samples were obtained from patients who were instructed to collect midstream clean catch urine in a wide, sterile container supplied by the laboratory. All patients were advised to maintain a proper aseptic procedure before urine collection.

Isolation and identification of uropathogens

By phenotypically examining the organisms on culture media designed for uropathogens, bacteria were identified. One loop of urine samples was inoculated onto blood agar, MacConkey agar, and chromogenic UTI agar media that had been sterilized and solidified. These media were then incubated aerobically for 24 hours at 37°C and counted the colonies [6].



Criteria for significant bacterial numbers [7]

The estimation of the number of bacteria, or colony-forming units (CFU), per milliliter of urine was performed. The following are reports on the bacterial counts: 1) Less than 10^4 organisms per milliliter, insignificant; 2) Doubtful significant, 10,000-100,000 organisms/mL (10^4 - 10^5 /mL), 3) Significant bacteriuria, >105 organisms/mL (more than 100,000 organisms/mL).

Throat swabs, sputum, and stool samples

These samples were collected in secure containers with a tight lid and leak-resistant containers to decrease specimen loss and healthcare worker exposure to the specimen. The containers were clean and free of particles and interfering substances to protect the specimen from contaminants in the sterile and transported containers. Prior to the start of the antibiotic treatment, all samples were taken. Blood agar (7% sheep blood) and MacConkey agar plates were used to aseptically inoculate samples of sputum, throat, and stool, respectively. XLD agar, SS agar, and MacConkey-sorbitol agar were used to inoculate stool samples [8]. The CLSI recommendations were followed when performing the isolation, detection, characterization, Gram staining, microscopic properties, colony properties, and biochemical assays.

Antibiotic Sensitivity Test

Following the Kirby-Bauer disc diffusion method, antimicrobial susceptibility testing was performed on Mueller-Hinton agar [9], against a panel of 12 antibiotics (Biomaxima, Poland), including Ampicillin (25µg), Amikacin (30µg), Azactam (30µg), Cefepime (30µg), Ceftriaxone (30µg), Cephalexin (30µg), Cefotaxime (30µg), Colistin (50µg), Imipenem (10µg), Tazobactam Piperacillin (100/10µg), Cefixime (5µg), Ceftazidime (30µg), Cotrimoxazole (25µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Meropenem (10µg), Netilmicin (30µg), Levofloxacin (5µg) and Gatifloxacin (5µg) as per the Clinical Laboratory Standard Institute (CLSI) guidelines [10], susceptibility was noted as sensitive, resistant, and intermediate based on the diameter of zone of inhibition.

Table 1: Biochemical tests of the isolated bacterial species from different specimens

SL	Result (slant/butt)	Symbol	Interpretation
1.	Red/Yellow	K/A	Glucose fermentation only, peptone catabolized.
2.	Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation.
3.	Red/Red	K/K	No fermentation, Peptone catabolized.
4.	Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
5.	Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
6.	Red/Yellow with bubbles and black precipitate	K/A,G,H ₂ S	Glucose fermentation only, Gas produced, H ₂ S produced.
7.	Yellow/Yellow with bubbles and black precipitate	A/A,G, H ₂ S	Glucose and lactose and/or sucrose fermentation, Gas produced, H ₂ S produced.
8.	Red/Yellow with black precipitate	K/A, H ₂ S	Glucose fermentation only, H ₂ S produced.
9.	Yellow/Yellow with black precipitate	A/A, H ₂ S	Glucose and lactose and/or sucrose fermentation, H ₂ S produced.



Results

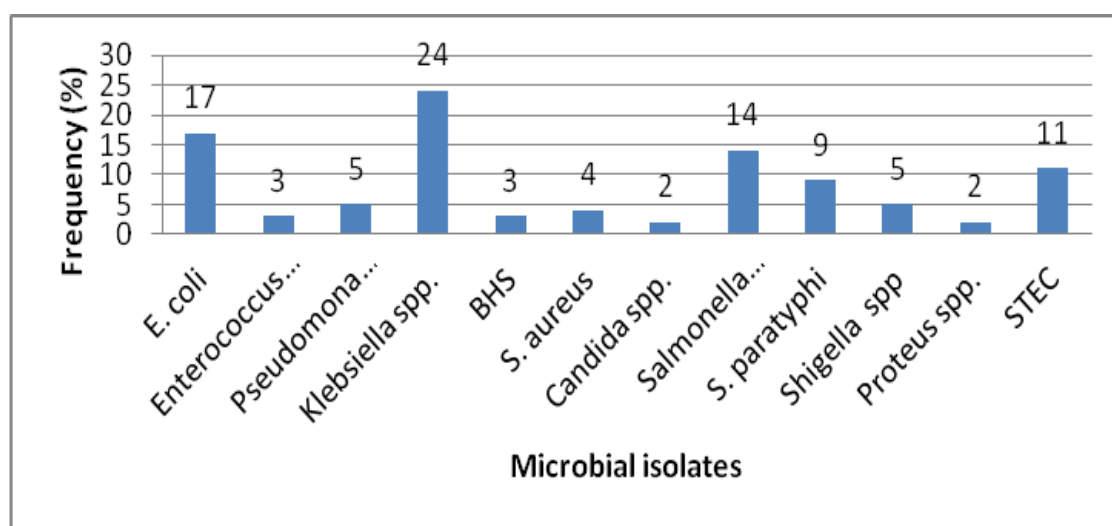
Microbiological infection is now exceedingly difficult and can even be fatal. Because of this, prompt detection, identification, and testing of the antibiotic sensitivity of bacterial pathogens in the diagnostic microbiology laboratory are crucial. Antimicrobials are prescribed by doctors in impoverished nations like Bangladesh at a rate that exceeds their real necessity. Every type of antibiotic is freely accessible at any pharmacy, and anyone can purchase it without a prescription. These medications are mostly to blame for the emergence of bacterial resistance as well as for the unfavourable outcomes of cultures [11].

Table 2: Bacterial cultures and its frequency

Specimens	Total Samples	Culture Positive	Culture Positive Frequency (%)	Culture Negative	Culture Negative Frequency (%)
Urine	87	25	28.7	64	71.3
Sputum	105	19	18.1	86	81.9
Throat swabs	35	07	20.0	28	80.0
Stools	65	15	23.08	50	76.9
Blood	111	27	24.3	84	75.1
In total	403	93	23.1	310	76.9

Table 3: Gender and age-wise distribution of infected patients (n=93).

Age Group	Sex		
	Male	Female	Total
<15	9 (9.6 %)	6 (6.5%)	15 (16.1%)
16-45	25 (26.9%)	31 (33.3%)	56 (60.2%)
>46	14 (15.1%)	8 (8.6%)	22 (23.7%)
	48 (51.6%)	45 (48.3%)	93(100%)



Note: BHS=Beta haemolytic *Streptococcus*, STEC= Shiga toxin-producing *E. coli*

Figure 1: Graphical representation of identified pathogens from different clinical samples.

Isolation of Microorganisms from Different Specimens

For urine, females (18, 72%) were the most affected by the infection. Among the 25 samples, 23 (92%) were likely to suffer from a bacterial infection, and 2 (8%) were likely to suffer from a



fungal infection. Among the 25 specimens, adults (17, 68%) were most likely to suffer, followed by the elderly (5, 20%) and children (3, 12%). The most abundant isolated pathogen from urine is *E. coli* 11 (44%).

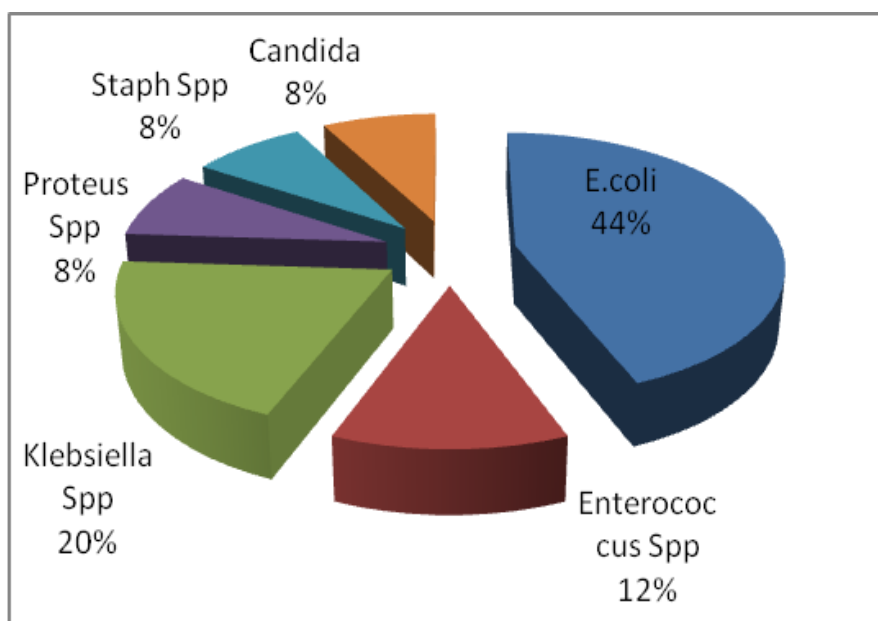


Figure 2: Frequency of bacterial isolates in suspected urine samples

For blood, males (18, 66.7%) were the most likely to suffer from the infection. Among the 27 specimens, adults (17, 63%) were most likely to suffer, followed by the elderly (7, 25.9%) and children (3, 11.1%). The most abundant isolated pathogen from urine is *Salmonella typhi* 21 (78%).

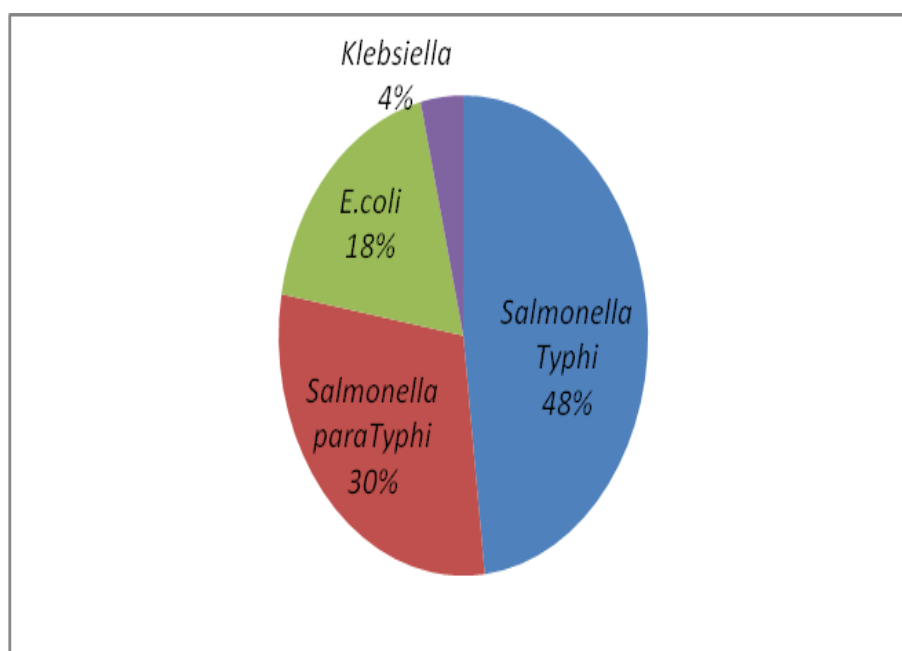


Figure 3: Frequency of bacterial isolates in suspected blood samples

For sputum, males (11, 57.9%) were the most likely to suffer from the infection. Among the 19 specimens, adults (13, 68.4%) were most likely to suffer, followed by the elderly (5, 26.3%) and

children (1, 5.3%). The most abundant isolated pathogen from sputum is *Klebsiella spp.*, 12 (63%).

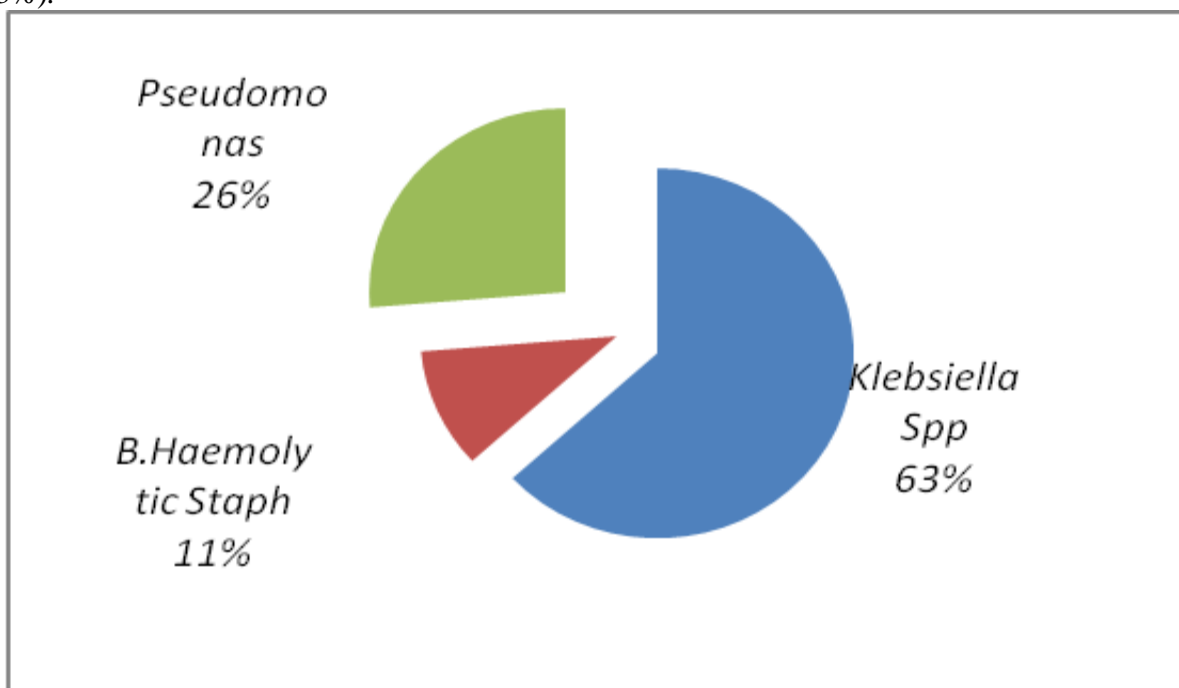


Figure 4: Frequency of bacterial isolates in suspected sputum samples

For throat swabs, males (4, 57.1%) were the most likely to suffer from the infection. Among the 7 specimens, adults (5, 71%) were most likely to suffer, followed by the elderly (2, 29%). The most abundant isolated pathogen from urine is *Klebsiella spp.*, 4 (57%).

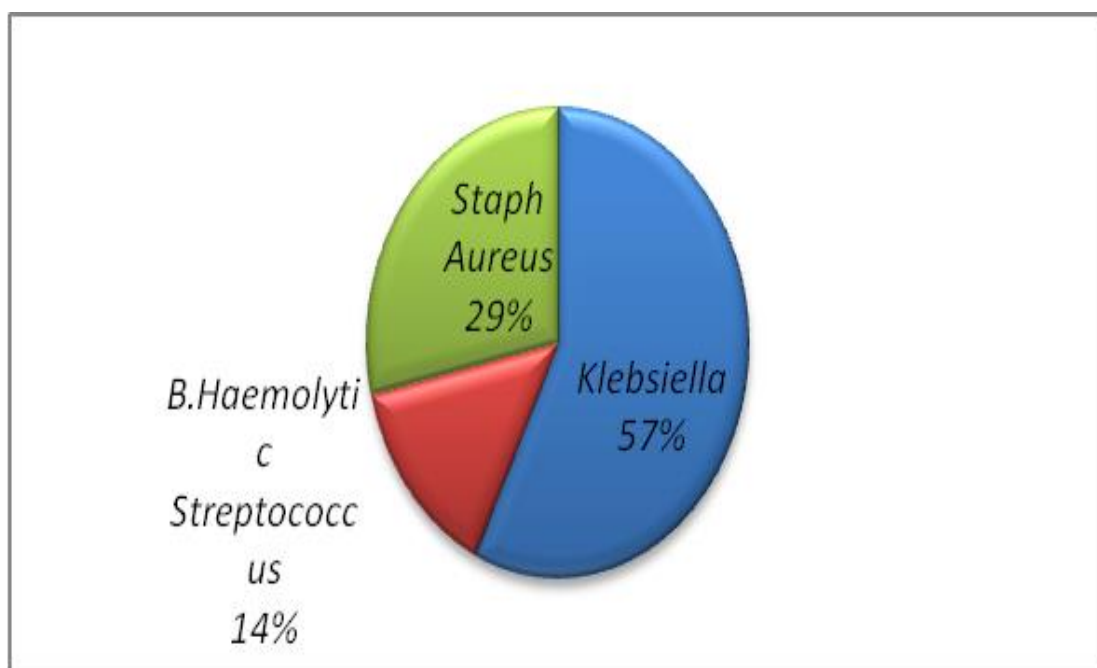


Figure 5: Frequency of bacterial isolates in suspected throat swab samples

For stools, males (n = 11; 73%) were the most likely to suffer from the infection. Among the 15 specimens, adults (7, 46.7%) were most likely to suffer, followed by the elderly (4, 26.7%) and children (4, 26.7%). The most abundant isolated pathogen from urine is *STEC-10* (67%).

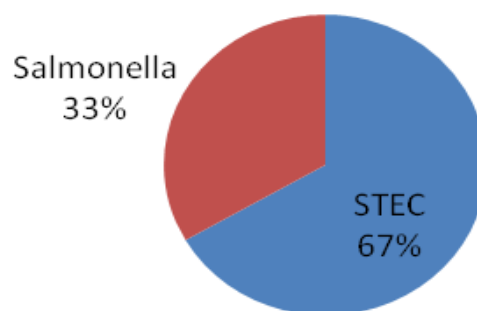


Figure 6: Frequency of bacterial isolates in suspected stool samples

The most frequently identified isolates were *Klebsiella spp.*, 22 (24%), *E. coli* 16(17%), followed by *Salmonella* species 21(23%), *Pseudomonas spp.*, 5 (5%) and *Enterococcus spp.*, 3 (3%).

Table 4: The diversity of bacterial pathogens that have been found in various clinical samples

Microbial isolates	Different specimens						Frequency (%)
	Urine	Blood	Stool	Sputum	Throat swab	Total	
<i>E. coli</i>	11	5	0	0	0	16	17%
<i>Enterococcus spp.</i>	3	0	0	0	0	3	3%
<i>Pseudomonas spp.</i>	0	0	0	5	0	5	5%
<i>Klebsiella spp.</i>	5	1	0	12	4	22	24%
<i>B.Haemolytic Streptococcus</i>	0	0	0	2	1	3	3%
<i>S. aureus</i>	2	0	0	0	2	4	4%
<i>Candida spp.</i>	2	0	0	0	0	2	2%
<i>Salmonella typhi</i>	0	13	0	0	0	13	14%
<i>S. paratyphi</i>	0	8	0	0	0	8	9%
<i>Shigella spp.</i>	0	0	5	0	0	5	5%
<i>Proteus mirabilis</i>	2	0	0	0	0	2	2%
Shiga toxin-producing <i>E.coli</i> (STEC)	0	0	10	0	0	10	11%
Total	25	27	15	19	7	93	100%

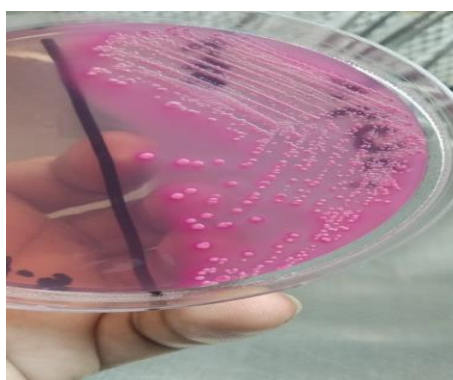


Fig. 7: *E.coli* on MacConkey Agar



Fig.8: *E.coli* on UTI agar



Fig 9: Beta haemolytic staph on blood agar



Fig. 10: *E.coli* on Blood Agar



Fig. 11: *Klebsiella* spp. on UTI agar



Fig.12. Antibiotic Resistance patterns

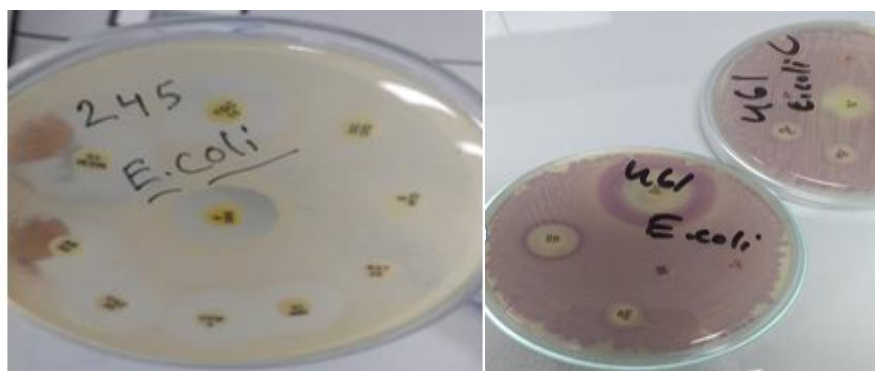


Fig.13: Antibiotic sensitivity patterns

DISCUSSION

The emergence and spread of multi-drug resistant infections is one of the main obstacles to the delivery of high-quality healthcare in hospitals in the majority of resource-constrained situations. Identification of bacterial pathogens and wise selection of antimicrobials that are effective against the organisms are essential for the successful therapy of patients with various infectious disorders. This investigation was carried out at a Lab One Hospital in Bangladesh to determine the distribution of bacterial pathogens and, consequently, to analyze their antibiotic resistance profile from various clinical specimens, such as urine, blood, throat, stool, and septum.

In our current study, 91 (22.6%) outcomes were culture-positive. Gram-negative isolates made up 81 (89.01%) more of all isolates, which is understandable given that they frequently cause serious illness and are a significant source of nosocomial infections (sepsis, pneumonia, and meningitis).

The bulk of the clinical isolates were found in throat, blood, urine, stool, and sputum samples. Particularly, *E. coli* and *Klebsiella* spp. were the most recognized etiologic agents among the isolates from urine culture. The majority of the patients ranged in age from 16 to 45. The isolated pathogens were 93 in number and showed growth of *Klebsiella* spp. at 22 (24%), *E. coli* at 16 (17%), followed by *Salmonella* species at 21 (23%), *Pseudomonas* spp. at 5 (5%), and *Enterococcus* spp. 3 (3%). *Escherichia coli*, *Staphylococcus aureus*, *Citrobacter freundii*, *Citrobacter koseri*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus* spp., and *Yersinia enterocolitica* were uncovered from similar kinds of specimens by Gashe et al. (2018) [12]. Badulla et al. (2020) also stated that *Staphylococcus* spp., *E. coli*, *Pseudomonas* spp., and *Klebsiella pneumoniae* contaminated such specimens [13].



It was found that most isolated bacteria showed antimicrobial resistance to ciprofloxacin, ceftazidime, and levofloxacin. Likewise, Gashe *et al.* (2018) stated Ceftriaxone and Ceftazidime as resistant to considerably high portions of total isolates [12]. Trimethoprim-sulfamethoxazole, Ceftazidime, Erythromycin, and Gentamicin were found to be significantly resistant to similar kinds of isolates by Hailemariam *et al.* (2021) [14]. Abebe *et al.* (2019) described all clinical isolates' resistance against Ampicillin [15].

E. coli was most prominent in urine and blood and was resistant to Cefixime (31.25%), Cefuroxime (18.75%), Ceftazidime (50%), Ciprofloxacin (25%), Levofloxacin (18.75%), Imipenem (18.75%), Meropenem (25%), and Ampicillin (43.75%). Jain *et al.* (2021) stated that clinical *E. coli* isolates exhibited resistance to Amoxicillin (98%), followed by Cefuroxime (75%), and Cotrimoxazole (62%). According to Hailemariam *et al.* (2021), Cotrimoxazole and Ciprofloxacin were found to be less effective against *E. coli* infections, whereas Meropenem exhibited the highest efficiency [5,6,7,8 &14].

Klebsiella spp. was most prominent in urine, sputum, and throat and was resistant to Doxycycline (27.3%), Cefixime (13.6%), Cefuroxime (13.6%), Ceftazidime (13.6%), Ciprofloxacin (13.6%), Levofloxacin (13.6%), Ampicillin (13.6%), Imipenem (13.6%), and Meropenem (13.6%). Aminul *et al.* (2021) stated *Klebsiella* isolates from clinical specimens exhibited significant resistance against aminoglycosides, β -lactam antibiotics, Carbapenem, Ciprofloxacin, Cotrimoxazole, Piperacillin, and Tazobactam [17]. Clinical *Klebsiella* isolates were found to be resistant, ranging from 65 to 79%, against Cefepime, Cefixime, Cefuroxime, Cefotaxime, Ceftazidime, and Ceftriaxone by Tanniet *et al.* (2021) [18].

STEC and *Shigella* were most prominent in stool and were resistant to Meropenem (30%), Azactam (30%), Tetracycline (30%), Ceftazidime (60%), and Levofloxacin (60%). Amoxicillin, Ampicillin, Chloramphenicol, and Tetracycline were found noticeably ineffective against clinically isolated *Shigella* by Teferi (2020) [19]. Jain *et al.* (2021) described *STEC* as exhibiting significant resistance against Amoxicillin (98%), Cefuroxime (75%), and Cotrimoxazole (62%) [20].

Salmonella spp. was the most prominent bacteria found in the blood and was most resistant to Naladic Acid (100%) and Cotrimoxazole (43%). Ciprofloxacin and Pefloxacin were found to be significantly ineffective (99%) against *Salmonella* infections by Katiyar *et al.* (2020) [21]. Ampicillin, Azithromycin, Chloramphenicol, and Trimethoprim-sulfamethoxazole exhibited notable resistance against *Salmonella* isolated from diarrheal cases in China by Li *et al.* (2023) [22].

In comparison to comparable studies conducted in Bangladesh's other cities, this finding is substantially higher. This study also matches the result by Hailemariam M. *et al.* (2015) in southern Ethiopia. A regular and thorough study of urogenital pathogens and their antibiotic susceptibilities is needed in order to treat and recommend antimicrobials. After successfully passing a standard test to identify the infection and determine the pattern of antibiotic resistance, patients should be given the proper antimicrobial treatments. The results of this study will be crucial in helping patients and doctors choose the best antimicrobial treatments for empiric care.

CONCLUSION

Infectious diseases are getting alarmingly dangerous because of the significant emergence of multidrug-resistant (MDR) pathogenic microorganisms. Finding the most suitable antimicrobial therapeutics against infectious diseases is not well practiced in most cases in third-world countries like Bangladesh, which accelerates frequent drug-resistant strain development. Most of the isolates showed significant resistance to Ciprofloxacin, Ceftazidime, and Levofloxacin, which should not be prescribed as therapeutics against such infections by physicians and practitioners. Such investigations are needed to be accomplished regularly to guide the health care service in prescribing proper antimicrobial medications to treat infectious diseases effectively.



ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals and human samples were used in this study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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