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Research Article

STUDY OF ANTIBIOTIC SENSITIVITY PATTERN OF INFECTIOUS MICROBES ISOLATED FROM DIAGNOSTIC LABORATORY SPECIMENS IN HISAR, HARYANA

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ABSTRACT

This study aims to assess the antibiotic sensitivity pattern of pathogens isolated from clinical specimens collected between January 2023 and March 2023 using standard microbiological methods. Bacteria were isolated from urine, stool, sputum, pus, swabs, blood, and fluids. It was found that *Escherichia coli* was the most identified pathogen, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida spp.*, *Acinetobacter baumannii*, and *Proteus mirabilis/vulgaris*. Sensitivity pattern data revealed that Tigecycline was the most effective antibiotic against gram-negative microbes, whereas Vancomycin and Linezolid were effective against gram-positive microbes. *Candida spp.* was found to be resistant to Clotrimazole. The high prevalence of *Escherichia coli* as the most identified pathogen raises concerns about the spread of urinary tract infections and the need for effective treatment options. The sensitivity pattern data highlights the importance of choosing the appropriate antibiotics based on the type of microbe to effectively combat these infections and prevent further resistance development.

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INTRODUCTION

Antibiotic resistance is a major issue in India as well as around the world, causing serious health issues. While antibiotic resistance is indeed a global concern, it is important to note that India faces unique challenges due to its high population density, misuse of low-cost antibiotics and inadequate healthcare infrastructure, making it particularly vulnerable to the spread of antibiotic-resistant infections. The situation is of great concern and requires prompt attention and action to address the issue and prevent the further spread of antibiotic resistance. (Baker *et al*, 2022). This study investigates the antibiotic sensitivity pattern of infectious microbes isolated from diagnostic laboratory specimens in Hisar, Haryana. The region's unique epidemiological factors and healthcare practices necessitate a focused exploration of microbial resistance patterns to inform evidence-based clinical decisions. The increasing prevalence of antibiotic-resistant strains calls for continuous monitoring, given the potential for treatment failures and compromised patient outcomes. This research contributes to the ongoing global effort to combat antibiotic resistance and underscores the significance of regional surveillance in this critical endeavour.

In 1929, Alexander Fleming made the significant discovery of the first antibiotic, penicillin. This discovery played a crucial role in saving the lives of millions of people during and after World War II (Tan and Tatsumura, 2015). However, the use of

antibiotics over natural compounds in treating infectious diseases has resulted in the development and spread of antibiotic resistance. This is when microorganisms, particularly bacteria, become resistant to the effects of antibiotics, which poses a significant challenge in treating bacterial infections effectively (Van *et al*, 2011).

Antibiotic resistance is a critical issue that we must address. It arises from a range of factors, including misdiagnosis, inappropriate antibiotic usage, and incorrect prescribing practices (Laxminarayan and Heymann 2012). Resistance can be either natural or acquired. Natural resistance occurs when bacteria have inherent mechanisms that enable them to withstand the effects of antibiotics. Acquired resistance, on the other hand, is due to genetic mutations or exchange of genetic material with other bacteria. Both types of resistance allow bacteria to survive and multiply in the presence of antibiotics that would otherwise be effective against them (Chanda *et al*, 2019; Hollenbeck and Rice, 2012). It is crucial to diagnose and treat infections correctly to reduce the use of ineffective antibiotics. This not only saves costs but also helps prevent the development of antibiotic resistance. As responsible citizens, we must take this issue seriously and work towards reducing antibiotic resistance.

Muhie (2019) reviewed the fact that bacterial infections are the primary reason for hospitalization of patients. The alarming increase in antibiotic resistance and the limited treatment

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options available pose a significant threat to the effective management of bacterial diseases worldwide. During the hospitalisation, nosocomial infections by multidrug-resistant strains further deteriorate the patient's condition.

In this study, we conducted an analysis of antibiotic sensitivity and resistance patterns using data from various microbial cultures collected in different hospitals across the Hisar region, Haryana state, from January 2023 to February 2023. This analysis enables us to discern prevalent pathogen trends within our region. Such insights empower us to make well-informed choices when selecting empirical antibiotic treatments for our patients, thereby enhancing the likelihood of treatment success. Furthermore, maintaining this dataset as a resource will facilitate ongoing monitoring of pathogen prevalence, enabling proactive measures to prevent potential outbreaks and combat antibiotic resistance.

MATERIAL AND METHODS

The study was carried out at the Microbiology Department of Manglam Diagnostics in Hisar. We collected approximately 1018 samples from a total of 1018 patients (Table 1). To identify the pathogens, we employed standard microbiological techniques, which included colony morphology assessment, gram staining, and subsequent biochemical testing. Stringent precautions were implemented to ensure result accuracy. We conducted susceptibility testing using the Kirby Bauer-Disk Diffusion Method, and the results were interpreted in accordance with the guidelines set by the Clinical and Laboratory Standards Institute (CLSI, 2022). The specimens from which organisms were isolated included blood (n = 114), sputum/ET aspirate (n = 284), urine (n = 362), pus (n = 184), and fluids (n = 82).

Table 1 Total number of samples collected

Months	Blood	Urine	Sputum/Tracheal	Fluid	Pus
Jan	50	199	154	50	108
Feb	64	163	130	32	76
Total	114	362	284	82	184

RESULTS

During the study period, it was observed that microbial growth was detected in various samples obtained from 1018 patients. Out of these samples, 331 (32.3%) tested positive for microbial growth, whereas the remaining 694 (67.6%) were tested negative. The major categories of specimens included urine (10.3%), sputum/tracheal swabs (9.8%), pus (9.1%), blood (1.5%), and fluids (1.4%). *E. coli* was the most common cause of urinary tract infection (58%) and *Klebsiella pneumoniae* (34%) for respiratory tract infection. Whereas, *Staphylococcus aureus* (38.2%) was extensively found in pus samples and *Pseudomonas aeruginosa* (29%) was commonly found in blood and body fluid (Table 2).

Table 2 Type of samples and organisms isolated

Microorganisms isolated	Urine	Sputum/Tracheal	Pus	Fluids	Blood
<i>Escherichia coli</i>	62	15	19	3	2
<i>Klebsiella pneumoniae</i>	15	34	13	3	4
<i>Pseudomonas aeruginosa</i>	11	21	15	4	5
<i>Staphylococcus aureus</i>	1	7	36	1	1
<i>Proteus spp</i>	3	2	6	0	0

<i>Acinetobacter baumannii</i>	0	8	1	1	1
<i>Salmonella typhi</i>	0	0	0	0	2
<i>Serratia marcescens</i>	0	1	0	0	1
<i>Candida spp</i>	11	11	1	0	0
<i>Streptococcus pyogenes</i>	0	0	2	2	0
<i>Enterococcus faecium</i>	3	1	1	1	0
Total (%)	106 (10.3%)	100 (9.8%)	94 (9.1%)	15 (1.4%)	16 (1.5%)

Among the 331 positive samples gram negative, gram positive and fungal isolates constituted 252 (76.1%), 56 (16.91%) and 23 (6.9%) respectively. It is important to note that *Escherichia coli* (9.8%) was predominant among the gram-negative isolates, followed by *Klebsiella pneumoniae* (6.7%), and *Pseudomonas aeruginosa* (5.4%). Similarly, among the Gram-positive organisms, *Staphylococcus* species (4.4%) was the most isolated. Finally, *Candida species* (2.2%) was the most isolated fungus (Table 3).

Table 3 Frequency of Organisms isolated out of Total samples

S. No.	Microorganism	Frequency (%)
1	<i>Escherichia coli</i>	101 (9.8%)
2	<i>Klebsiella pneumoniae</i>	69 (6.7%)
3	<i>Pseudomonas aeruginosa</i>	56 (5.4%)
4	<i>Staphylococcus aureus</i>	46 (4.4%)
5	<i>Candida spp.</i>	23 (2.2%)
6	<i>Acinetobacter baumannii</i>	11 (1.0%)
7	<i>Proteus mirabilis/vulgaris</i>	11 (1.0%)
8	<i>Enterococcus faecium</i>	6 (0.5%)
9	<i>Streptococcus pyogenes</i>	4 (0.3%)
10	<i>Salmonella typhi/paratyphi A</i>	2 (0.2%)
11	<i>Serratia marcescens</i>	2 (0.2%)
	Total	331

The antibiotic sensitivity pattern revealed that among the Gram-negative bacteria, *Escherichia coli* showed highest resistance to Ampicillin, Cefotaxime and Ceftriaxone and least resistant to Tigecycline and Amikacin. *Klebsiella pneumoniae* were highly sensitive against Tigecycline, but shown resistance against Ampicillin, Ceftazidime, Cefepime, Cefotaxime and Ceftriaxone. *Pseudomonas aeruginosa* shown least resistance to Amikacin, Piperacillin/Tazobactam and Gentamicin, but highly resistant to Amoxyclave, Ampicillin, Co-Trimoxazole, Doxycycline, Cefotaxime, Ceftriaxone as well as Tigecycline. *Acinetobacter baumannii* was shown least resistant to Imipenem, Tigecycline and Doxycycline.

Besides this, it was found that *Proteus* had the lowest resistance to Imipenem, Piperacillin/Tazobactam, and Ceftriaxone, but exhibited high resistance to Tigecycline, Doxycycline, and Ampicillin. In contrast, *Serratia marcescens* was found to be the least resistant to Piperacillin/Tazobactam, Tigecycline, and Amikacin, but highly resistant to Cefotaxime, Ceftriaxone, Ampicillin, Amoxyclave, Ceftazidime, Cefepime, Levofloxacin, and Doxycycline. Lastly, *Salmonella typhi/paratyphi A* was observed to be least resistant to Cefotaxime, Ceftriaxone, and Co-Trimoxazole, while demonstrating high resistance to Imipenem, Tigecycline, Piperacillin/Tazobactam, Gentamycin, Ampicillin, Amoxyclave, and Doxycycline.

Table 4 Antibiotic sensitivity pattern of gram-negative isolates

S. No.	Antibiotics	Percentage of sensitive strains						
		<i>E. coli</i>	<i>Kleb.</i>	<i>Pseudo.</i>	<i>Acineto.</i>	<i>Proteus</i>	<i>Serr.</i>	<i>Salmo.</i>
1	AK	84.1	27.5	96.4	9.09	54.5	100	0
2	AMC	44.5	14.4	0	0	45.4	0	0
3	AMP	7.9	1.4	0	0	0	0	50
4	CAZ	28.7	13.04	41.07	0	45.4	0	0
5	CPM	42.5	13.04	50	0	54.5	0	0
6	CIP	20.7	24.6	51.7	0	63.3	50	0
7	LE	22.7	23.1	46.4	0	45.4	0	0
8	COT	33.6	27.5	0	18.1	63.3	50	100
9	DO	65.3	42.02	0	81.8	9.09	0	0
10	GEN	54.5	28.9	64.2	36.3	54.5	50	0
11	IMP	80.1	31.8	58.9	100	100	50	0
12	CTX	18.8	14.4	0	0	54.5	0	100
13	CTR	19.8	13.04	0	0	81.8	0	100
14	PIT	78.2	46.3	71.4	54.5	90.9	50	0
15	TGC	97.02	98.5	0	100	18.8	50	0

Abbreviations: AMP-Ampicillin; AMC-Amoxyclove; AK-Amikacin; CAZ-Ceftazidime; CTX-Cefotaxime; CTR-Ceftriaxone; CPM-Cefepime; CIP-Ciprofloxacin; COT-Co-Trimoxazole; DO-Doxycycline; GEN-Gentamycin; IMP-Imipenem; LE-Levofloxacin; PIT-Piperacillin/Tazobactam; TGC-Tigecycline.

In this study, it is observed that all the gram-positive pathogens were highly sensitive to Doxycycline, Gentamycin and Linezolid. It was found that *Staphylococcus aureus* was highly resistant to Amoxyclove, Cefotaxime, Penicillin, Erythromycin and Ciprofloxacin. Whereas *Enterococcus faecium* was highly resistant to Amoxyclove, Gentamycin, Azithromycin and Cefotaxime but least resistant to Linezolid, Vancomycin and Penicillin (Table 5). *Streptococcus pyogenes* shown highest resistance to Amoxyclove and Gentamycin only. *Candida spp.* was commonly isolated from urine and respiratory tract specimens. The fungal pathogen shown resistance to Clotrimazole and Fluconazole (Table 6).

Table 5 Antibiotic sensitivity pattern of gram-positive isolates

S. no.	Antibiotics	Percentage of sensitive strains		
		<i>Staphy. Aureus</i>	<i>Entero. Faecium</i>	<i>Strept. pyogenes</i>
1	AMC	0	0	0
2	CTX	0	0	100
3	CIP	32.6	33.3	75
4	COT	50	16.6	100
5	DO	97.8	33.3	100
6	GEN	55.5	0	0
7	VA	97.8	66.6	100
8	LZ	100	83.3	100
9	E	36.9	16.6	75
10	AZM	50	0	75
11	P	4.34	50	50

Abbreviation: AMC-Amoxyclove; AZM-Azithromycin; CTX-Cefotaxime; CIP-Ciprofloxacin; COT-Co-Trimoxazole; DO-Doxycycline; E-Erythromycin; GEN-Gentamycin; LZ-Linezolid; VA-Vancomycin and P- Penicillin.

Table 6 Antibiotic sensitivity pattern of fungal isolate

S. no.	Antibiotics	Percentage of sensitive strains <i>Candida spp.</i>
1	Vancomycin	100
2	Amphotericin-B	100
3	Clotrimazole	65.2
4	Fluconazole	78.2
5	Voriconazole	100

DISCUSSION

The high frequency of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* in the study is concerning as these bacteria are commonly associated with healthcare-associated infections. The identification of amikacin as the most effective antibiotic for *E. coli* and *P. aeruginosa* suggests that it could be a valuable treatment option for infections caused by these bacteria. Additionally, the high sensitivity of tigecycline across all the relevant bacteria indicates its potential as a broad-spectrum antibiotic. However, the presence of antimicrobial resistance in both Gram-negative and Gram-positive bacteria highlights the importance of appropriate antibiotic use to prevent further development of resistance.

In the present study, positivity rate in samples collected was found to be 32.3%. The prevalence of culture positive cases as indicated by our findings, is consistent with previous research conducted by Agrawal and Ranjan (2019) (26.69%), Gill and Sharma (2016) (24.8%) and Trivedi and Nagendra, (2022) (28%). This suggests a consistent trend in the rate of culture positive cases across multiple studies. Also, this study is comparable with that of Savanur and Gururaj (2019), who performed a study on the sensitivity pattern of bacterial pathogens in ICU patients. Isolated bacteria were mostly gram-negative, with *Escherichia coli*, *Acinetobacter*, *Klebsiella*, *Pseudomonas*, and *Proteus* being the most common. Fungal growth was observed in some samples.

Similarly, Sriram *et al* (2013) studied the antibiotic sensitivity patterns of the patients admitted general medicine and pulmonary department in an Indian tertiary care teaching hospital. They observed that *Escherichia coli* was the major organism identified in 36.4% of the isolated specimens, followed by *Klebsiella sp.* (18.9%). Amikacin was found to be the most sensitive antibiotic against *E. coli* (99.3%) infection, which shows consistency with our results.

In another study, sensitivity pattern exhibited by urinary tract infection causing microbes was examined and found that *Escherichia coli* was the most frequently isolated class of pathogen followed by *Klebsiella species* (Majumder *et al*, 2022). This was in contradiction with a study conducted on 385 patients admitted to the intensive care unit of a hospital, out of these samples, 249 were positive (64.7%). *P. aeruginosa* was the most isolated bacteria followed by *K. pneumoniae* and *Staphylococcus epidermidis*. Amikacin was highly sensitive to *P. aeruginosa* (Radji *et al*, 2010).

CONCLUSION

The microbiological environment and trends of antibiotic resistance in our area are usefully revealed by our recent study on antibiotic sensitivity. In the study, standard microbiological techniques such as Kirby Bauer-Disk Diffusion, were used to analyze 1026 samples from 1018 patients to identify cases of

antibiotic resistance. The clinical specimens showed the presence of various pathogens, with blood, sputum/ET aspirate, urine, pus, and fluids as specimens used. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, ensuring the reliability and consistency of the data. The study will be helpful for healthcare professionals to make informed decisions when prescribing empirical antibiotic therapies. The study emphasizes the need for continuous monitoring and ethical use of antibiotics to combat resistance.

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