VOLUME - 15, ISSUE - 02, FEBRUARY - 2026 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

ASSESSMENT OF ANTIBIOTIC RESISTANCE PATTERN OF HUMAN PATHOGENS ISOLATED FROM DIFFERENT CLINICAL SPECIMEN

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ABSTRACT The increase in antimicrobial resistance is considered as serious threats to the public health. Antibiotic resistance occurs when bacteria develop defense mechanism against the antibiotic designed to kill them. The present study was conducted to determine the antibiotic resistance pattern of various clinical specimens. In this study, total 60 samples were collected from various clinical specimens viz. blood, urine, stool and pus provided by pathology laboratory situated in Washim city area. The samples were subjected to enrichment and isolation and were further subjected for virulence study. The virulent isolates were proceed for antibiotic susceptibility test by Kirby-Baur disk diffusion method against twelve frequently used antibiotics. Total 391 isolates were obtained out of which, 26 isolates showed positive virulence test. All the virulent isolates were found to be resistant to multiple antibiotics. Total 16 isolates showed MAR Index 1 indicating their origin from a high risk source of contamination where antibiotics are often used. Hence, present study focuses on the increasing incidences of antibiotic resistance in the human pathogens indicating high risk in the field of medical Science with reference to treatment regimen.

KEYWORDS : Antimicrobial resistance, Kirby-Baur disk diffusion, MAR Index

INTRODUCTION

Antibiotic resistance by human pathogen is rapidly increasing all over the world. According to World health organization the multiple drug resistant pathogens is currently considered as one of the serious threats to the public health. Due to the high consumption and misuse of antibiotics human pathogens become resistant to various types of antibiotics (O'Bryan *et al.,* 2018). Drug resistance affects the treatment of common infectious diseases sometimes becoming complicated to treat (Keenan *et al.,* 2015). It is predicted that if no action is taken then mortality rate could rises to 10 million annually (WHO, 2000).Drug resistance affects the treatment of common infectious diseases sometimes becoming complicated to treat (Keenan *et al.,* 2015). According to GBD, 33 bacterial diseases were responsible for 7.7 million deaths globally (Ikuta *et al.*, 2022). It is predicted that if no action is taken then mortalityrate couldrises to 10 million annually (WHO, 2000). The top six antibiotic resistant bacteria causing human deaths are *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, Acinetobacter baumannii* and *Klebsialla pneumonia* (CDC, 2019). Methicillin resistant *staphylococcus aureus* (MRSA), DRUG RESISTANT *Mycobacterium tuberculosis* and extended spectrum- -lactamase (ESBL) are considered as serious threats to public health. Bai *et al*., (2022) reported that, MRSA-related death rate of 32.1% in high income countries and 39.1% in middle income countries. Self medication, High use of antibiotics, fixes dose combination, lack of hygienic condition, access to antibiotics without doctor prescription and poor management of industrial effluents treatment plants resulted in high antibiotic resistance (Gandra *et al.,* 2017).

Colistin-resistant *K. pneumoniae and carbapenem* was responsible for 69% death rate during 2011-2015 in india (Kaur *et al*., 2017). Bacteria isolated from water and soil sample in hyderabad near pharmaceutical industrial area revealed upto 70% resistanace against Cephalosporin antibiotic (Britto *et al.,* 2019). *E. coli* isolated from hospital and domestic waste was observed to be resistant to third generation cephalosporin (Akiba *et al.,* 2015). Hence the present study aimed to provide antibiotic resistance pattern of 26 bacterial and fungal pathogens isolated from clinical specimen.

METHOD

The test human pathogens were isolated from different clinical samples (n= 60) viz. Blood, urine, pus, and stool. The virulence detection among the isolated strains was done adopting the method recommended by Behara *et al.,* (2013) with slight modifications for bacterial isolates and phospholipase activity for fungal isolates.

The antibiotic S/R pattern of clinical isolates was determined by Kirby- Bauer Disk diffusion technique recommended by CLSI, 2025.The well isolated colonies were selected from 18 to 24 hours agar plate and inoculated in nutrient broth. The turbidity of suspension is adjusted to match the 0.5 McFarland turbidity standards.After adjusting the turbidity of the inoculum, a sterile cotton swab is dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.The agar plates were inoculated by spreading the swab over the entire sterile agar surface. Then antimicrobial discs were dispensed onto the surface of the inoculated agar plates. Each disc was pressed down to ensure complete contact with the agar surface.

The antibiotic sensitivity resistance pattern was determined against the currently recommended antibiotic regimen viz. Ampicillin (10μg), Azithromycin (15μg), Streptomycin (10μg), Chloramphenicol (30μg), Ciprofloxacin (5μg), Tetracyclin (30μg),Ofloxacin (5μg), Amoxicillin-clavulanate (20μg), Amikacin (30μg), Vancomycin (30μg), Fluconazole (FLC) and Amphotericin B (AMB).The plates were incubated at 35-37 °C and examined after 16- 18 hrs. for bacteria and at 28°C after 24-48 hrs for fungal culture. The strains which had showed resistance to more than one antimicrobial agent were considered to be MDR and were screened out for further studies. Further, MAR index was calculated using the formula (Subramani *et al.,* 2012):

$$\text{MAR index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

RESULT

In the present research study, total 391 clinical isolates were isolated from four different clinical samples (n=60). Out of which 24 bacterial isolates showed positive test for India ink degradation test and 02 fungal isolates showed positive Phospholipase activity.

All the 26 isolates were found to be resistant to multiple

antibiotics. Out of which, 16 isolates showed MAR Index 1. From the Figure it was found that, out of 9 *E. coli* isolates, (04) isolates viz. E1, E4, E7 and E9 showed MAR index 1, E5 and E6 showed MAR index 0.6 and remaining two E2 and E3 showed MAR index 0.4 and 0.5 respectively. In case of *S. aureus,* all the three isolates viz. S1, S2 and S3 showed MAR index 1. In case of *Salmonella typhi,* Sa2 isolate showed MAR index 1 and remaining three isolates viz. Sa1, Sa3 and Sa4 showed MAR index of 0.3, 0.9 and 0.5 respectively. In case of *Shigella* isolates, Sh1 showed MAR index 1. In case of isolates belonging to *Klebsiella* species, K1 showed MAR index 1 and K2 showed MAR index 0.8. In case of *Proteus vulgaris* isolates, Pv1 showed MAR index 0.4 and Pv2 showed MAR index 1.

All the four *Pseudomonas aeruginosa* isolates, P1, P2, P3 and P4 showed MAR index 1. Similarly,*C. albicans* isolates C1and C2 showed MAR index 1. All the isolates were found to be MDR however, 16 isolate viz. E1, E4, E7, E9, S1, S2, S3, Sa2, Sh1, K1, Pv2, P1, P2, P3, P4, C1andC2 showed MAR index equal to 1 which indicate their origin from a high risk source of contamination where antibiotics are often used (Wei and Wee, 2011).

Table 1: Antibiotic Sensitivity/resistant Pattern Of Isolated Human Pathogens

Sr.No.	Test isolates	Mean zone of inhibition (in mm)												MAR Index
		AMP 10 µg	AZM 15 µg	C 30µg	CIP 5 µg	TE 30 µg	OFX 5 µg	AMC 20 µg	AK 30 µg	V 30 µg	S 10 µg	FLC 10 µg	AMB 10 µg	
1	E1	13(R)	11(R)	12(R)	13(R)	11(R)	11(R)	10(R)	11(R)	12(R)	10(R)	_	_	1
2	E2	21(S)	14(R)	12(R)	11(R)	21(S)	20(S)	16(I)	22(S)	20(S)	11(R)	_	_	0.4
3	E3	15(I)	12(R)	11(R)	10(R)	NI(R)	21(S)	15(I)	21(S)	21(S)	11(R)	_	_	0.5
4	E4	11(R)	11(R)	12(R)	11(R)	NI(R)	10(R)	NI(R)	10(R)	12(R)	10(R)	_	_	1
5	E5	10(R)	13(R)	10(R)	17(I)	13(I)	NI(R)	10(R)	21(S)	NI(R)	NI(R)	_	_	0.6
6	E6	22(S)	17(I)	11(R)	12(R)	10(R)	11(R)	13(R)	23(S)	11(R)	11(R)	_	_	0.6
7	E7	11(R)	11(R)	12(R)	13(R)	NI(R)	NI(R)	NI(R)	13(R)	10(R)	10(R)	_	_	1
8	E8	23(S)	12(R)	10(R)	18(I)	NI(R)	12(R)	11(R)	22(S)	12(R)	11(R)	_	_	0.7
9	E9	12(R)	12(R)	11(R)	10(R)	NI(R)	11(R)	13(R)	13(R)	13(R)	10(R)	_	_	1
10	S1	10(R)	11(R)	10(R)	12(R)	10(R)	NI(R)	10(R)	11(R)	12(R)	9(R)	_	_	1
11	S2	11(R)	NI(R)	11(R)	10(R)	9(R)	11(R)	NI(R)	13(R)	13(R)	10(R)	_	_	1
12	S3	13(R)	13(R)	11(R)	12(R)	NI(R)	12(R)	12(R)	12(R)	11(R)	NI(R)	_	_	1
13	Sa1	12(I)	13(R)	12(R)	22(S)	NI(R)	23(S)	17(I)	20(S)	10(R)	25(S)	_	_	0.3
14	Sa2	10(R)	12(R)	NI(R)	13(R)	NI(R)	10(R)	10(R)	11(R)	10(R)	NI(R)	_	_	1
15	Sa3	9(R)	11(R)	25(S)	14(R)	10(R)	11(R)	10(R)	11(R)	11(R)	NI(R)	_	_	0.9
16	Sa4	21(S)	17(I)	24(S)	14(R)	21(S)	26(S)	NI(R)	13(R)	NI(R)	11(R)	_	_	0.5
17	Sh1	11(R)	11(R)	24(R)	12(R)	NI(R)	12(R)	12(R)	10(R)	13(R)	10(R)	_	_	1
18	K1	13(R)	13(R)	10(R)	10(R)	9(R)	12(R)	10(R)	11(R)	11(R)	9(R)	_	_	1
19	K2	22(S)	11(R)	NI(R)	12(R)	NI(R)	11(R)	10(R)	23(S)	12(R)	20(R)	_	_	0.8
20	Pv1	20(S)	11(R)	23(S)	11(R)	9(R)	19(S)	15(I)	12(R)	20(S)	24(S)	_	_	0.4
21	Pv2	11(R)	14(R)	12(R)	13(R)	10(R)	11(R)	10(R)	12(R)	10(R)	10(R)	_	_	1
22	P1	10(R)	13(R)	10(R)	13(R)	11(R)	12(R)	11(R)	13(R)	NI(R)	10(R)	_	_	1
23	P2	9(R)	15(R)	10(R)	10(R)	NI(R)	11(R)	NI(R)	10(R)	12(R)	NI(R)	_	_	1
24	P3	NI(R)	12(R)	11(R)	14(R)	NI(R)	11(R)	11(R)	12(R)	11(R)	NI(R)	_	_	1
25	C1	_	_	_	_	_	_	_	_	_	_	NZ(R)	10(R)	1
26	C2	_	_	_	_	_	_	_	_	_	_	10(R)	9(R)	1
	No. of isolates and values in parasynthesis is % S	6(25)	0(0)	3(12.5)	1(4.1)	2(8.3)	5(20)	0(0)	7(27)	3(12.5)	2(8.3)	0(0)	0(0)	
	No. of isolates and values in parasynthesis is % I	2(8.3)	2(8.3)	0(0)	2(8.3)	1(4.1)	0(0)	3(12.5)	0(0)	0(0)	0(0)	0(0)	0(0)	
	No. of isolates and values in parasynthesis is % R	16(66)	22(91)	21(87.5)	21(87.5)	21(87.5)	19(80)	21(87.5)	17(70)	21(87.5)	22(91)	2(100)	2(100)	

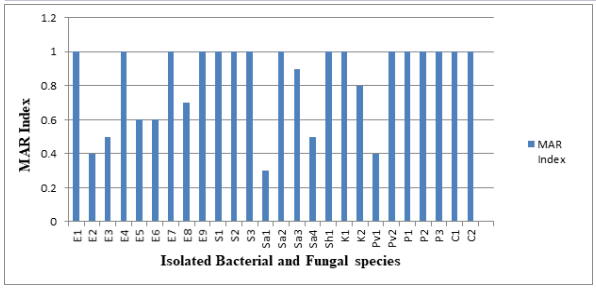

Figure 1: Antibiotic Sensitivity/resistant Pattern Of Isolated Human Pathogens

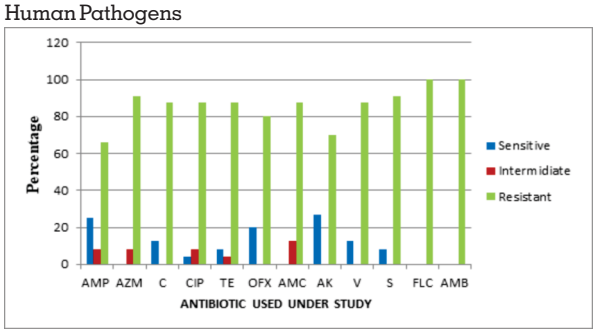

Figure 2: Percentage Distribution Of Antimicrobial Susceptibility

CONCLUSION

In the present research study, antibiotic sensitivity resistance pattern were evaluated. It is revealed that, total 26 isolates were found to be resistant to antibiotics. Out of which 16 isolates were showed MAR Index 1 indicating their origin from a high risk source of contamination where antibiotics are often used. This research study has demonstrated very high level of resistance to different common antibiotics in different classes of bacteria.

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