

Original Research Article

Profile of pathogens isolated from different clinical samples and their antimicrobial pattern: a retrospective study

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ABSTRACT

Background: Since there is a significant rise in resistant bacteria to different antimicrobial agents, there is a need to study the resistance pattern of different isolates from different clinical samples for effective use of available antimicrobials by clinicians. The aim of the present study was to detect the resistance pattern of various antimicrobials against different clinical isolates in hospitalised patients in out setting.

Methods: This is a retrospective study involving the collection of the data from the records of microbiology laboratory. All clinical specimens were processed as per standard microbiological procedures. Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method on Mueller Hinton agar plate as per CLSI guidelines.

Results: A total of 153 isolates were recovered from 219 clinical samples accounting for 69.86% of total positivity. Which includes gram negative bacilli 107/153 (69.93%) gram positive cocci 36/153 (23.53%) and yeast 10/153 (6.54%). Among the total isolates gram negative bacilli account for major number of isolates 69.93% followed by gram positive cocci 23.53% and yeast 6.54%. Gram positive cocci and gram-negative bacilli showed a significant level of antimicrobial resistance. Nitrofurantoin is highly effective against urinary isolates of *Escherichia coli*. vancomycin and linezolid are most effective antimicrobials against gram positive cocci. Among gram negative bacilli meropenem and amikacin are most effective antimicrobials. Statistical significance of occurrence of *Escherichia coli* as predominant isolate as compared to other isolates were analysed by chi square test by using GraphPad online calculator. A p value < 0.001 was obtained.

Conclusions: Significant rise in antimicrobial resistant pathogens were observed. Local antimicrobial policy should be developed for effective selection of available antimicrobials which are the need of the day to reduce the burden of diseases on global health care system.

Keywords: Antimicrobial resistance, Gram negative bacilli, Gram positive cocci

INTRODUCTION

Infections with microbes has a drastic effect on human health.¹ Microbial infections are important cause of morbidity and mortality across the globe with increase resistance of pathogens to different antimicrobial agents posing great concern to public health.² Antimicrobials are being used to overcome the drastic effect of microbial agents. Their wide spread use has led to emergence of

multidrug resistant (MDR) pathogens. Antimicrobial resistance (AMR) may be due to natural, acquired/clinical resistance.¹

High morbidity and mortality are seen in infections caused by drug resistant pathogens. The pattern of resistance to various antimicrobial agents may change over a period of time.³ Nosocomial infections pose a great challenge to the well fare of the patient

management, since most of them are MDR strains. This increases the hospital stay of in patients and increases the health care cost.¹

AMR is a great health care problem in India.⁴ The burden of infectious disease is more in India because of increased drug resistant bacteria due to indiscriminate use of antimicrobials. Frequent cause of hospitalisation is associated with different microbial infections. The choice of selecting an effective antimicrobial agent has been reduced because of their resistance to different pathogens causing hospital and community acquired infections.⁵

Patients infected with drug resistant pathogens are at high risk of serious clinical outcomes and require more health care services. Resistance to different antimicrobials is seen because of indiscriminate use of different antimicrobial agents, which in turn leads to mutations and results in drug resistance. MDR bacteria pose great threat for patients. since, it becomes very difficult to treat such patients and requires use of broad-spectrum antibiotics.⁶

METHODS

This was a retrospective study which involves analysis of medical microbiology test results of different clinical samples collected over a period of 6 months between July 2019 to December 2019. Only IPD patients were included in the study. The study was carried out at Ananta Institute of Medical sciences and research center, Rajsamand. This is a tertiary care hospital.

Data collection and testing

Type of clinical samples, isolates, age, sex and their antimicrobial susceptibility pattern were collected from the records. All clinical specimens were processed as per standard microbiological procedures. The isolates were first identified by standard biochemical techniques and then subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton agar plate as per CLSI guidelines.⁵

Antibiotic discs

The following antibiotic discs containing amikacin AK 30 mcg, amoxicillin/clavulanic acid AMC 30 mcg, ampicillin/sulbactam A/S 10/10 mcg, azithromycin AZM 15 mcg, aztreonam AT 30 mcg, cefoxitin CX 30 mcg, ceftizoxime CZX 30 mcg, ceftriaxone CTR 30 mcg, cefuroxime CXM 30 mcg, ciprofloxacin CIP 5 mcg, clindamycin CD 2 mcg, colistin CL 10 mcg, erythromycin E 15 mcg, gentamicin GEN 10 mcg, imipenem IPM 10 mcg, levofloxacin LE 5 mcg, linezolid LZ 30 mcg, meropenem MRP 10 mcg, piperacillin/ tazobactam PIT 100/10 mcg, polymyxin-B PB 300 units, trimethoprim TR 5 mcg, vancomycin VA 30 mcg, cefoperazone/ sulbactam CFS 75/10 mcg, ceftazidime/clavulanic acid CAC 30/10 mcg,

nitrofurantoin NIT 300 mcg were used as per manufacturer (Himedia) instructions.

Statistical analysis

Statistical analysis was done by using GraphPad online calculator. Chi square test was employed. P value<0.05 is considered as statistically significant

RESULTS

A total of 153 isolates were recovered from 219 clinical samples accounting for 69.86% of total positivity. Which includes gram negative bacilli 107/153 (69.93%) gram positive cocci 36/153 (23.53%) and yeast 10/153 (6.54%). Among the total isolates gram negative bacilli account for major number of isolates 69.93% followed by gram positive cocci 23.53% and yeast 6.54% (Table 1).

Among the gram-negative bacilli, the major isolates are *Escherichia coli* 35.29% followed by *Klebsiella* species 12.41% and *Pseudomonas aeruginosa* 12.41%. Among the gram-positive cocci, the major isolates are coagulase negative *Staphylococcal species* (CONS) 12.41% followed by coagulase positive *Staphylococcus aureus* 6.53% and *Enterococcus faecalis* 4.57%. Among the yeast the major isolate is *Candida albicans* 4.57% followed by *Candida tropicalis* 1.96% (Antifungal agents were not tested).

Among different clinical samples received urine samples account for highest number of isolates 62/119 (52.10%). Among which gram-negative bacilli accounts for 53/62 (85.48%) followed by gram positive cocci 5/62 (8.06%) and yeast 4/62 (6.45%). *Acinetobacter* species were predominant in clinical aspirates 10/21 (47.61%). *Pseudomonas aeruginosa* was predominant in pus 11/48 (22.91%) (Table 1). A total of 10 co-infections were detected among the total isolates (Table 1).

Antimicrobial resistance

Antimicrobial resistance pattern of gram-negative bacilli and gram-positive cocci varied from nil to 100% for different isolates. The details of each isolate and their antimicrobial resistance pattern is given in Table 2 and 3.

Among gram negative isolates, meropenem and amikacin are more effective against *E-coli* with 9.25% and 18.51% resistance respectively each. Meropenem and piperacillin/ tazobactam are more effective against *Klebsiella* species with 36.84% and 47.36% resistance respectively each. Amikacin and ciprofloxacin are more effective against *P. aeruginosa* with 15.78% and 31.57% resistance respectively each. Amikacin, ampicillin/sulbactam and levofloxacin are more effective against *Acinetobacter* species with 41.66% resistance respectively each. Polymyxine-B and colistin showed no resistance when used against *P. aeruginosa* and *Acinetobacter* species. Piperacillin/ tazobactam is more effective against proteus species with 33.33% resistance (Table 2).

Among gram positive isolates ampicillin/sulbactam, cefoxitin/clindamycin are more effective against cons with 21.05% and 26.31% resistance respectively each. Clindamycin and trimethoprin/ sulphamethoxazole are more effective against *S. aureus* with 30% and 60% resistance respectively each. Gentamicin and Trimethoprin/ sulphamethoxazole more effective against *Enterococcus faecalis* with 14.28% and 57.14% resistance respectively each. Gentamicin is nil resistant to CONS and *S. aureus*. Linezolid and vancomycin are nil resistant to *Enterococcus faecalis*, CONS and *S. aureus* (Table 3). Among total number of clinical isolates, males accounted for (54.25%) positivity and females accounted

for (45.75%) positivity. Among the male patients the predominant isolates are *E-coli* (30.12%), followed by *P. aeruginosa* (16.86%) and *Acinetobacter species* (14.45%). Among the female patients the predominant isolates are *E-coli* (41.42%), CONS (15.71%) and *Klebsiella species* (12.85%). Details of each isolate in males and females are mentioned in Table 4.

Statistical significance of occurrence of *Escherichia coli* as predominant isolate as compared to other isolates were analysed by chi square test by using GraphPad online calculator. A p value<0.001 was obtained.

Table 1: Different microbial agents isolated from various clinical samples.

Isolated micro-organism (%)	Clinical sample	Single infection	Co-infection	Total	
Gram negative Bacilli (A)	<i>Acinetobacter species</i> (7.84)	Aspirates	9/21	1	10/21
		Pus	2/48	-	2/48
		Stool	1/3	-	1/3
		Others (tips)	1/2	-	1/2
	<i>Escherichia coli</i> (35.29)	Urine	39/119	1	40/119
		Vaginal swab	4/15	-	4/15
		Pus	7/48	-	7/48
		Drain fluid	1/1	-	1/1
	<i>Klebsiella species</i> (12.41)	Aspirates	3/21	1	4/21
		Pus	-	1	1/48
		Sputum	3/5	-	3/5
		Stool	1/3	-	1/3
	<i>Pseudomonas aeruginosa</i> (12.41)	Urine	7/119	-	7/119
		Vaginal swab	3/15	-	3/15
		Aspirates	2/21	2	4/21
		Pus	10/48	1	11/48
	Proteus species (1.96)	Others (tips)	1/2	-	1/2
Urine		2/119	1	3/119	
Total (%)	Urine	3/119	-	3/119	
		99/107* (92.52)	08/107* (7.48)	107/153 (69.93)	
Gram positive cocci (B)	Coagulase negative <i>Staphylococci</i> (12.41)	Aspirates	1/21	-	1/21
		Pus	11/48	-	11/48
		Stool	1/3	-	1/3
		Urine	2/119	-	2/119
		Vaginal swab	4/15	-	4/15
	COPS (6.53)	Pus	10/48	-	10/48
	<i>Enterococcus faecalis</i> (4.57)	Urine	3/119	-	3/119
		Vaginal swab	4/15	-	4/15
Total (%)		36/36# (100)	-	36/153 (23.53)	
Yeast (C)	<i>Candida albicans</i> (4.57)	Aspirates	-	1	1/21
		Pus	1/48	-	1/48
		Sputum	1/5	-	1/5
		Urine	3/119	-	3/119
		Vaginal swab	1/15	-	1/15
	<i>Candida tropicalis</i> (1.96)	Aspirates	-	1	1/21
		Urine	1/119	-	1/119
	Vaginal swab	1/15	-	1/15	
Total (%)		08/10@ (80)	2/10@ (20)	10/153 (6.54)	
Total (A+B+C) (%)		143/219\$ (65.30)	10/219\$ (4.56)	153/219\$ (69.8)	

*Total number of gram-negative bacilli isolated, # Total number of gram-positive cocci isolated, @Total number of yeast isolated, \$ Total number of samples tested, COPS-Coagulase positive *Staphylococci* p<0.001

Table 2: Antibiotic resistance pattern of gram-negative bacilli isolated from different clinical samples.

Antimicrobial agent	Resistance percentage of gram-negative bacilli				
	<i>Escherichia coli</i> , (n=54)	<i>Klebsiella</i> species, (n=19)	<i>Pseudomonas aeruginosa</i> , (n=19)	<i>Acinetobacter</i> species, (n=12)	<i>Proteus</i> species, (n=3)
Ceftizoxime	85.18	68.42	84.21	100	66.66
Piperacillin/tazobactam	29.62	47.36	42.10	75	33.33
Cefuroxime	92.59	73.68	100	100	100
Polymyxin B	NT	NT	0	0	NT
Ceftriaxone	83.33	73.68	73.68	83.33	100
Cefaperazone/sulbactam	59.25	63.15	73.68	66.66	66.66
Levofloxacin	79.62	63.15	47.36	41.66	66.66
Aztreonam	75.92	73.68	42.10	100	66.66
Imipenem	68.51	52.63	42.10	66.66	100
Meropenem	9.25	36.84	26.31	58.33	66.66
Amikacin	18.51	78.94	15.78	41.66	66.66
Ampicillin/ sulbactam	51.85	73.68	84.21	41.66	66.66
Amoxicillin/clavulanic acid	90.74	78.94	73.68	75	100
Ciprofloxacin	81.48	52.63	31.57	58.33	66.66
Trimethoprim/sulphamethoxazole	68.51	63.15	84.21	66.66	100
Colistin	NT	NT	0	0	NT
Ceftazidime/clavulanic acid	42.59	63.15	57.89	91.66	66.66

NT-Not tested

Table 3: Antibiotic resistance pattern of gram-positive cocci isolated from different clinical samples.

Antimicrobial agent	Resistance percentage of gram-positive cocci		
	CONS, (n=19)	<i>Staphylococcus aureus</i> , (n=10)	<i>Enterococcus faecalis</i> , (n=07)
Gentamicin	0	0	14.28
Ciprofloxacin	36.48	60	85.71
Levofloxacin	42.10	60	85.71
Erythromycin	47.36	60	100
Linezolid	0	0	0
Clindamycin	26.31	30	100
Vancomycin	0	0	0
Cefuroxime	42.10	70	100
Ceftizoxime	42.10	70	100
Ceftriaxone	47.36	80	100
Azithromycin	89.47	70	100
Cefoxitin	26.31	70	NT
Ampicillin/sulbactam	21.05	80	85.71
Amoxicillin/clavulanic acid	73.68	80	85.71
Trimethoprim/ sulphamethoxazole	47.36	60	57.14

Table 4: Different clinical isolates in males and females.

Isolate	Male	Female	Total
<i>Acinetobacter</i> species	12	-	12
<i>Escherichia coli</i>	25	29	54
<i>Klebsiella</i> species	10	9	19
<i>Pseudomonas aeruginosa</i>	14	5	19
<i>Proteus</i> species	1	2	3

Continued.

Isolate	Male	Female	Total
Coagulase negative <i>Staphylococci</i>	8	11	19
COPS	7	3	10
<i>Enterococcus faecalis</i>	1	6	7
<i>Candida albicans</i>	4	3	7
<i>Candida tropicalis</i>	1	2	3
Total (%)	83/153, (54.25)	70/153, (45.75)	153, (100.00)

DISCUSSION

Different pathogens are associated with human infections. Management of this infectious diseases by timely identification and selection of effective antimicrobials against the causative agents help in early recovery of the patients and also helps in reducing the hospital costs as well as the stay time in hospital. Emergence and spread of MDR pathogens are a major challenge for better health care management. MDR pathogens increase morbidity and mortality in hospitalised patients. This study present various pathogens isolated from different clinical samples and their antimicrobial activity in hospitalised patients.

The total positivity of the clinical isolates of the present study were 69.86% with predominance of gram-negative bacilli 69.93% as compared to gram positive cocci 23.53%. An earlier study has reported a total positivity of 64.70% which correlates with our study.⁷ Abebe et al and Masyeni et al reported gram-negative bacilli as the most predominant isolate. Similar findings are observed in the present study. Most of the nosocomial infections are associated with gram negative bacilli causing severe form of the disease. These strains are mostly MDR.^{3,8}

The present study has reported a high-level resistance to various class of antimicrobials against gram negative bacilli and gram-positive cocci. Antibiotic resistance is a threat to the world and is a major public problem in India. since, India harbours great burden of bacterial diseases. Emergence of MDR strains from India for gram negative bacilli and gram-positive cocci has been reported.⁹ This coincides with the present study which shows high resistance of bacteria to various antimicrobials.

Urine samples accounted for majority (54.33%) of the samples in the present study. similar findings have been observed with other studies.^{1,5} The most common pathogen isolated from all clinical samples in our study was *Escherichia coli* 35.29% with predominance of females (57.05%) as compared to males (42.95%). This is followed by *Klebsiella* species, CONS and *Pseudomonas aeruginosa* 12.41% each. These findings are in correlation with other studies.^{2,9,10} Among the uropathogens isolated, *Escherichia coli* and *Klebsiella* species were the predominant isolates in our study which correlates with earlier studies.^{5,12}

Among the antimicrobials used against *Escherichia coli*, meropenem showed 09.25% resistance, amikacin showed 18.51% resistance and piperacillin/ tazobactam showed 29.62% resistance. Nitrofurantoin showed nil resistance against *Escherichia coli* isolates from urine. similar findings were also reported by other studies with decreased potency of ciprofloxacin and co-trimoxazole among uropathogens.^{5,6,10} Among *Klebsiella* species isolated meropenem is more effective which showed 36.84% resistance. This finding is different from an earlier study which reported 100% resistance.⁴ In *Pseudomonas aeruginosa* nil resistance was observed for polymyxine-B and colistin. Amikacin showed least resistance 15.78% to *Pseudomonas aeruginosa* followed by meropenem 26.31%. Higher rate of resistance for amikacin (78.00%) and meropenem (100.00%) was reported by an earlier study.⁴ *Acinetobacter* species showed nil resistance to polymyxine-B and colistin and 41.66% resistance to amikacin, ampicillin/ sulbactam and levofloxacin respectively each. Piperacillin/tazobactam was the most effective antimicrobial against proteus species with 33.33% resistance. This finding is different from a previous study which reported 100.00% resistance to piperacillin/ tazobactam.⁴

Among the gram-positive isolates nil resistance was observed for vancomycin, linezolid. Gentamicin showed nil resistance against CONS and *Staphylococcus aureus* but showed 14.28% resistance against *Enterococcus faecalis*. Among CONS, ampicillin/sulbactam was the most effective antimicrobial with a resistance of 21.05% followed by cefoxitin and clindamycin with 26.31% resistance. This finding is different from an earlier study which reported 100% sensitivity to cefoxitin and clindamycin.⁵ *Staphylococcus aureus* showed least resistance to clindamycin (30%). This finding is different from an earlier study which reported 100% resistance to Clindamycin.⁵ Trimethoprin/ sulphamethoxazole showed a least resistance of 57.14% when used against *Enterococcus faecalis*. Khatun et al reported a higher resistance of 75% to trimethoprin/ sulphamethoxazole. The difference in the resistance pattern of different antimicrobials for gram negative bacilli and gram-positive cocci in different studies may be attributed to the usage of that particular antimicrobial in different settings.

High rate of resistance to various antimicrobials in our study may be due to inclusion of only hospitalised patients. Since nosocomial infections are seen in hospitalised patients and the strains are generally resistant to most of the commonly used antimicrobials. Similar

findings were observed by earlier studies which included IPD and OPD patients.^{5,6} The high rate of antimicrobial resistance in IPD patients may indicate the need for surveillance studies on nosocomial infections to identify the source of infection. Incorrect diagnosis may lead to irrational use of antibiotics which may lead to overuse or misuse of antimicrobials resulting in dissemination of antibiotic resistance.

CONCLUSION

The most predominant isolate from different clinical samples in our study was *Escherichia coli*. Among gram negative isolates in the present study, the most effective antimicrobials are colistin, meropenem, amikacin, piperacillin/tazobactam, ampicillin/sulbactam. Among urinary isolates of *Escherichia coli*, nitrofurantoin is very effective. Gentamicin, vancomycin and linezolid are most effective antimicrobials against gram positive cocci in our study. Significant rise in resistance to antimicrobials was observed in this study. Local antimicrobial policy should be developed for effective selection of available antimicrobials which are the need of the day to reduce the burden of diseases on global health care system.

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