

Antimicrobial resistance pattern of pathogenic bacterial isolates from lower respiratory tract infections in South Trivandrum district, Kerala

Dr. L. Suresh Babu^{1*}, Dr. Shoba Kurien²

¹Professor, ²Professor and Head, Department of Microbiology, Dr. Somervell Memorial CSI Medical College and Hospital Karakonam, Trivandrum district, Kerala, INDIA.

Email: sureshbabu6831@yahoo.in

Abstract

Background: The lower respiratory tract infections are among the most common infectious diseases of human, worldwide. They account for 3.5% deaths in adults. As per World Health Organisation (WHO), among the infectious disease deaths in India, LRTIs have been attributed to almost 20% mortality. There were only very few reports available on the antibiotic resistance pattern of bacterial pathogens isolated from LRTIs in Kerala, that too none in south Trivandrum district. **Aims and objectives:** 1. To isolate and identify the pathogenic bacteria from sputum or endotracheal aspirate/tube specimens from various cases of LRTIs. 2. To elucidate the antimicrobial susceptibility pattern for all the pathogens thus isolated. 3. To update the antibiotic pattern at regular intervals to the clinicians by conducting more such resistance pattern studies. **Materials and methods:** The work was a retrospective study done between November 2019 and April 2021, after getting clearance from the ethics committee. 400 cases of LRTIs were included in the study. One sample from each case, of either, sputum or endotracheal aspirate/tube specimen were collected in a sterile wide mouth screw capped container.]. The quality of sputum and endotracheal tube samples will be assessed based on criteria laid by American Society for Microbiology (ASM). Identification of the isolates were performed by standard microbiological procedures such as study of colony morphology, Gram stain and standard biochemical tests[10]. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results will be read and interpreted as per CLSI guidelines. For quality control of disc diffusion tests, ATCC control strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* 27853 were used. **Results:** Out of the 400 cases of lower respiratory tract infections, we have isolated 216 (54%) pathogenic organisms, mostly bacteria and few yeast. Among these positive cases, 121 (56%) were males and remaining 95 (44%) were females, (Table-1). Among the GNB isolates, like *Klebsiella pneumoniae*, *E. coli* and *Acinetobacter* sp, ampicillin, amoxiclav and cefuroxime were the most resistant antibiotics. There were 26 MDR strains of *Klebsiella pneumoniae*. Among *Staphylococcus aureus* strains, all were resistant to penicillin, erythromycin and clindamycin. *Streptococcus pneumoniae* isolates were all resistant to erythromycin and clindamycin. **Conclusion:** Overall, we could observe decrease in susceptibility of GNB isolates to carbapenem drugs (restricted drugs), when compared to amikacin and piperacillin/tazobactam (2nd line), against which more strains were sensitive. This might be due to frequent, disproportionate use of higher antibiotics. Drugs like amikacin, piperacillin/tazobactam, and Cefoperazone/sulbactam could be the drugs of choice for empirical treatment of LRT infections to start with and can be tailored based on the sensitivity report once come in. Rationale and judicial use of antibiotics have to be adhered to.

Key words: LRTIs, South Trivandrum district, sputum, Bartlett score, MRSA, ESBL, MDR, disc diffusion method, *Klebsiella pneumoniae*, *Escherichia coli*

*Address for Correspondence:

Dr L. Suresh Babu, Professor, Department of Microbiology, Dr. Somervell Memorial CSI Medical College and Hospital Karakonam, Trivandrum district, Kerala, INDIA.

Email: sureshbabu6831@yahoo.in

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INTRODUCTION

The LRTIs are among the most common infectious diseases of human, worldwide ^[1], causing significant morbidity and mortality in all age groups. They account for 3.5% deaths in adults ^[2]. Acute manifestations of LRTIs that may or may not involve lungs include acute bronchitis, bronchiolitis, influenza, community-acquired pneumonia either with or without radiological evidence and acute exacerbation of Chronic Obstructive Pulmonary Disease (COPD) ^[3]. As per World Health Organisation (WHO), among the infectious disease deaths in India, LRTIs have been attributed to almost 20% mortality ^[4].

Antibiotics used for treating lower respiratory tract infections in children grew by 46% globally during 2000–2018 (Browne et al., 2021) ^[5]. The extended spectrum-β lactamase (ESBL) Enterobacteriaceae, methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus, and drug-resistant Mycobacterium tuberculosis (MDR TB) are considered severe threats to human lives (CDC, 2019) ^[6]. High antibiotic use, fixed-dose combinations, self-medication, access to antibiotics without a prescription from a doctor, poor management of industrial effluent treatment plants, lack of hygienic condition, and inefficient infection control procedures in healthcare, are some of the factors contributing to India's high AMR proportions (Gandra et al., 2017)^[7]. There were only very few reports available on the antibiotic resistance pattern of bacterial pathogens isolated from LRTIs in Kerala ^[2], that too none in south Trivandrum district.

As per our experience in our diagnostic microbiology laboratory in recent times, we are encountering MDR strains of Klebsiella, more often, apart from other Enterobacteriaceae organisms, Pseudomonas and Acinetobacter, resistant to even carbapenem drugs and also MRSA, from various hospital acquired pneumonia cases. This is due to inappropriate, inadvertent and prolonged use of a particular antibiotic, leaving behind only the life saving drugs like colistin or tigecycline to treat such patients. By keeping all these in mind, we have undertaken this work to elucidate the antimicrobial resistance pattern of various pathogenic bacteria isolated from LRTIs,

particularly in this part of Kerala, so as to institute empirical treatment in severe unstable cases like COPD, till the susceptibility report comes in. The implication of the study will be to arrive at a common antibiogram for the prevalent pathogenic bacteria in this locality.

MATERIALS AND METHODS

The work was a retrospective study done between November 2019 and April 2021, after getting clearance from the ethics committee. 400 cases of LRTIs were included in the study. One sample from each case, of either, sputum or endotracheal aspirate/tube specimen were collected in a sterile wide mouth screw capped container. Patient were instructed to expectorate 5-10 ml sputum through a deep intense cough directly in to the container. ET specimens were collected through aspiration of secretions from the endotracheal tubes using sterile mucus trap container^[8]. The quality of sputum and endotracheal tube samples will be assessed based on criteria laid by American Society for Microbiology (ASM) ^[9]. A reliable specimen after gram staining would have more than 25 leucocytes and fewer than 10 epithelial cells per low power field of microscope (Bartlett score). Samples not fulfilling these criteria were rejected. The undiluted sputum samples were inoculated on the culture media using a Nichrome wire loop. The culture media used for inoculation were blood agar, chocolate agar and MacConkey agar. The inoculated plates were incubated at 37°C for up to 48 hours. The predominant bacterial growth obtained from samples were recorded.

Identification of the isolates were performed by standard microbiological procedures such as study of colony morphology, Gram stain and standard biochemical tests^[10]. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results will be read and interpreted as per CLSI guidelines ^[11]. The antibiotic discs used were, Ampicillin (10µg), Amoxycillin-clavulanate (20/10 µg), Piperacillin (100 µg), Piperacillin–Tazobactam (100/10 µg), Gentamicin (10 µg), Netilmicin(10 µg), Amikacin (30 µg), Cefazolin(30 µg), Ceftriaxone(30 µg), Cefuroxime (30 µg), Cefoxitin (30 µg), Cefixime(5 µg), Cefepime (30 µg), Ceftazidime (30 µg), Cotrimoxazole (1.25/23.75 µg), Cefoperazone-sulbactam (75/30 µg) , Tobramycin (10 µg), Ciprofloxacin(5 µg), Meropenem (10 µg), Penicillin(10U), Erythromycin(15 µg), Azithromycin (30 µg), Chloramphenicol (10 µg), Cloxacillin (5µg), Clindamycin (2 µg), Linezolid(30 µg), and Vancomycin(30 µg), Rifampicin(5µg).

For quality control of disc diffusion tests, ATCC control strains of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa 27853

were used. The collected data was entered in MS-Excel and statistical analysis was done using SPSS 17 software and were expressed as percentages.

RESULTS

Out of the 400 cases of lower respiratory tract infections, we have isolated 216 (54%) pathogenic organisms, mostly bacteria and few yeast. Among these positive cases, 121 (56%) were males and remaining 95 (44%) were females, (Table-1). Maximum number of isolates were from the age group 61-70 (43.5%), followed by >70 and 51-60 (20.8% each), with least number from 11-20, 1-10 and 21-30 groups (Table-2). Most of the positive cases were inpatients, 193 (89.4%), with only 10.6% as outpatients (Table-3).

Table 1: Sex wise distribution of cases (N=216)

Male	Female
121 (56%)	95 (44%)

Table 2: Age wise prevalence of cases (N=216)

≤10	11-20	21-30	31-40	41-50	51-60	61-70	>70
3 (1.38%)	1 (0.46%)	3 (1.38%)	9 (4.1%)	16 (7.4%)	45 (20.8%)	94 (43.5%)	45 (20.8%)

Table 3: Inpatient and outpatient distribution of cases. (N=216)

Inpatient	Outpatient
193 (89.4%)	23 (10.6%)

Among the organisms isolated, *Klebsiella pneumoniae* was the most predominant isolate, 101 (46.8%), followed by, *Pseudomonas aeruginosa*, 26 (12%), *Escherichia coli*, 22 (10.2%), *Acinetobacter sp.*, 18 (8.3%), *Pseudomonas sp.*, 13 (6%), *Citrobacter sp.*, 7 (3.2%), *Candida albicans*, 6 cases (2.7%), non-albicans *Candida sp.*, 5 cases (2.3%), *Moraxella catarrhalis* and *Staphylococcus aureus*, 4 each (1.9%), *Klebsiella oxytoca*, *Enterobacter sp.* and *Streptococcus pneumoniae*, 3 each (1.4%) and *Serratia marcescens* 1 (0.5%) (Table-4, 5).

Table 4: Prevalence of gram positive organisms (N=216)

<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>	Non-albicans <i>Candida sp.</i>
4 (1.9%)	3 (1.4%)	6 (2.7%)	5 (2.3%)

Table 5: Prevalence of gram negative organisms (N=216)

<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Citrobacter sp.</i>	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas sp.</i>
22 (10.2%)	101 (46.8%)	7 (3.2%)	26 (12%)	13 (6%)
<i>Acinetobacter sp.</i>	<i>Enterobacter sp.</i>	<i>Serratia marcescens</i>	<i>Moraxella catarrhalis</i>	<i>Klebsiella oxytoca</i>
18 (8.3%)	3 (1.4%)	1 (0.5%)	4 (1.9%)	3 (1.4%)

On submission of the various gram positive bacterial isolates to antibiotic susceptibility testing, among the 4 *Staphylococcus aureus* strains, all were resistant to penicillin, erythromycin and clindamycin. On the other hand, all were sensitive to, ciprofloxacin, gentamicin, cloxacillin, cefazolin, vancomycin, linezolid and rifampicin. Against cotrimoxazole, 3 of the isolates were susceptible and the remaining one was resistant (Table-6, Figure-1).

Table 6: Antibiotic susceptibility pattern of *Staphylococcus aureus* (n=4)

Antibiotic	Sensitive/resistant	Number of isolates	%
Penicillin	S	0	0
	R	4	100
Ciprofloxacin	S	4	100
	R	0	0
Cotrimoxazole	S	3	75
	R	1	25
Clindamycin	S	0	0
	R	4	100
Erythromycin	S	0	0
	R	4	100

Gentamicin	S	4	100
	R	0	0
Cloxacillin	S	4	100
	R	0	0
Cefazolin	S	4	100
	R	0	0
Vancomycin	S	4	100
	R	0	0
Linezolid	S	4	100
	R	0	0
Rifampicin	S	4	100
	R	0	0

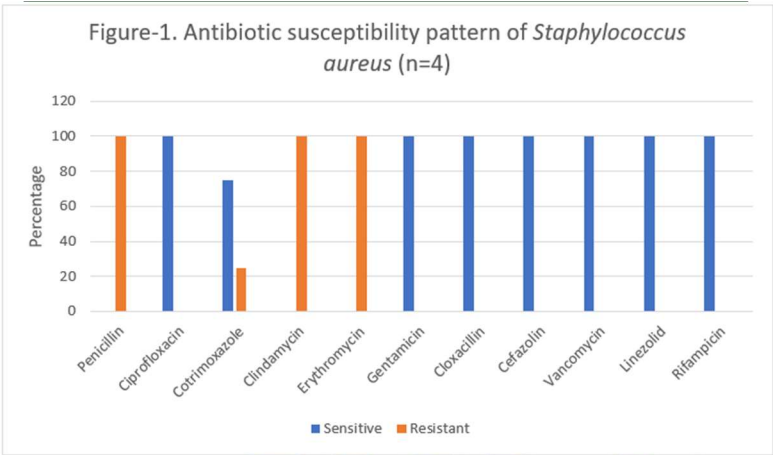


Figure 1: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Of the three isolates of *Streptococcus pneumoniae*, all were susceptible to penicillin, ampicillin, gentamicin , chloramphenicol, ceftriaxone, vancomycin, linezolid and high level gentamicin. They were all resistant to erythromycin and clindamycin. Two of the isolates were sensitive and the other one was resistant to cotrimoxazole (Table-7, Figure-2).

Table 7: Antibiotic susceptibility pattern of *Streptococcus pneumoniae* (n=3)

Antibiotic	Sensitive/resistant	Number of isolates	%
Penicillin	S	3	100
	R	0	0
Ampicillin	S	3	100
	R	0	0
Gentamicin	S	3	100
	R	0	0
Chloramphenicol	S	3	100
	R	0	0
Cotrimoxazole	S	2	66.6
	R	1	33.4
Erythromycin	S	0	0
	R	3	100
Clindamycin	S	0	0
	R	3	100
Ceftriaxone	S	3	100
	R	0	0
High level gentamicin	S	3	100
	R	0	0
Vancomycin	S	3	100
	R	0	0
Linezolid	S	3	100
	R	0	0

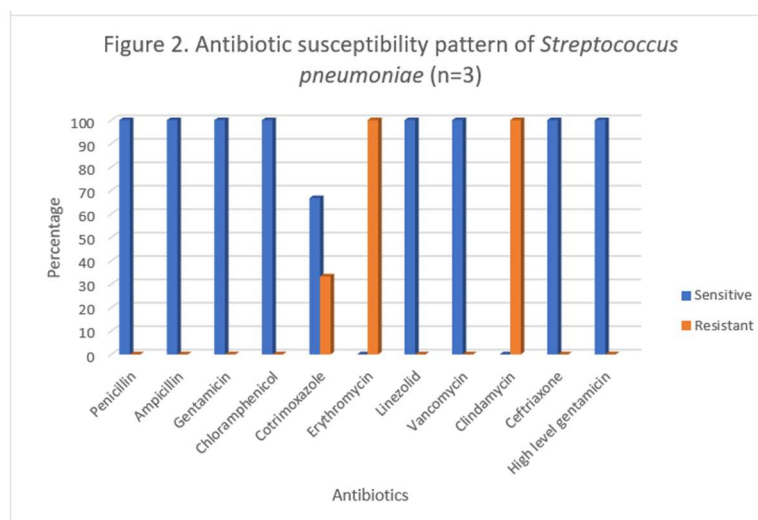


Figure-2 Antibiotic susceptibility pattern of *Streptococcus pneumoniae*

Among the gram negative bacilli subjected to AST, *Escherichia coli* strains showed highest level of resistance to ampicillin (100%), followed by amoxiclav and cefuroxime (72.7% each) and cefixime (68.2%). All the isolates were sensitive to meropenem (100%), followed by piperacillin/tazobactam (95.5%), amikacin (90.9%), cefoperazone/sulbactam (72.7%), ciprofloxacin, gentamicin, cotrimoxazole (59% each), etc., in that order (Table-8, Figure-3).

Table 8: Antibiotic susceptibility pattern of *Escherichia coli* (n=22)

Antibiotic	Sensitive/resistant	No. of isolates	%
Ciprofloxacin	S	13	59
	R	9	41
Gentamicin	S	13	59
	R	9	41
Cotrimoxazole	S	13	59
	R	9	41
Ampicillin	S	0	0
	R	22	100
Amoxiclav	S	6	27.3
	R	16	72.7
Cefuroxime	S	6	27.3
	R	16	72.7
Ceftriaxone	S	8	36.3
	R	14	63.7
Cefoperazone	S	16	72.7
	R	6	27.3
Cefixime	S	7	31.8
	R	15	68.2
Amikacin	S	20	90.9
	R	2	9.1
Piperacillin/tazobactam	S	21	95.5
	R	1	4.5
Cefoperazone/sulbactam	S	16	72.7
	R	6	27.3
Meropenem	S	22	100
	R	0	0

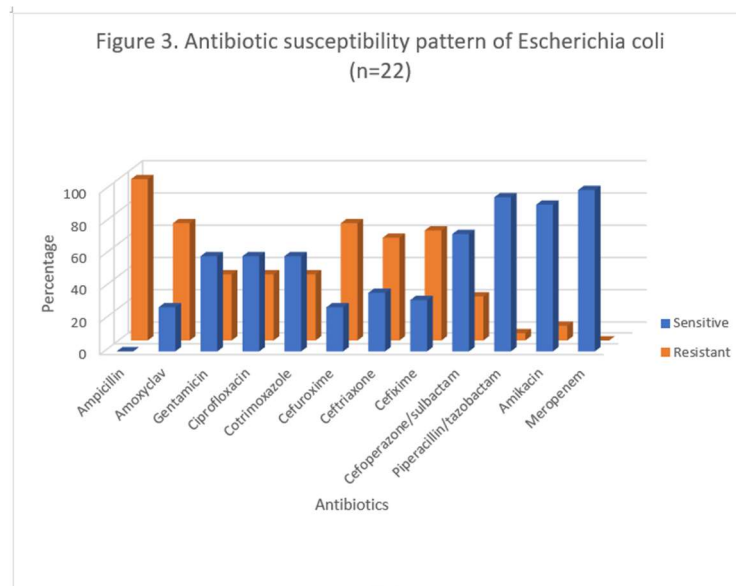


Figure 3: Antibiotic susceptibility pattern of *Escherichia coli*

Out of the 101 strains of *Klebsiella pneumoniae*, highest degree of resistance was recorded against ampicillin (100%, intrinsic resistance), followed by amoxyclav (55.5%), cefuroxime (49.5%), ceftriaxone (44.5%), etc. in that order. Most number of isolates susceptible were against amikacin (83.2%), followed by piperacillin/tazobactam (82.2%), Cefoperazone/sulbactam (76.2%), gentamicin, ciprofloxacin (73.3% each), meropenem (71.3%), etc., in that order (Table-9, Figure-4).

Table 9: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* (n=101)

Antibiotic	Sensitive/resistant	No. of isolates	%
Ampicillin	S	0	0
	R	100	100
Amoxyclav	S	45	44.5
	R	56	55.5
Gentamicin	S	74	73.3
	R	27	26.7
Ciprofloxacin	S	74	73.3
	R	27	26.7
Cotrimoxazole	S	62	61.4
	R	39	38.6
Cefuroxime	S	51	50.5
	R	50	49.5
Ceftriaxone	S	56	55.5
	R	45	44.5
Cefixime	S	58	57.4
	R	43	42.6
Cefoperazone/sulbactam	S	77	76.2
	R	24	23.8
Piperacillin/tazobactam	S	83	82.2
	R	18	17.8
Amikacin	S	84	83.2
	R	17	16.8
Meropenem	S	72	71.3
	R	29	28.7

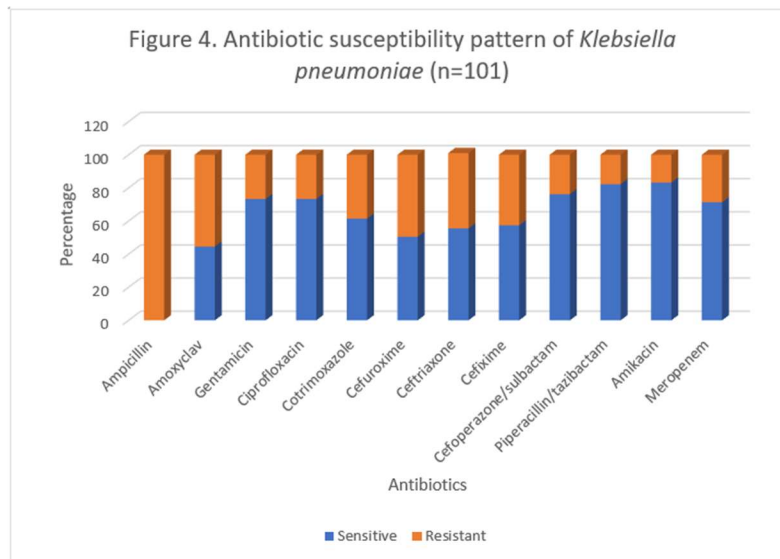


Figure-4 Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Of the 26 isolates of *Pseudomonas aeruginosa*, expectedly, all were resistant to cotrimoxazole (100%), being intrinsically resistant. Percentage of resistance to other antibiotics was relatively less, when compared to other gram negative pathogenic bacteria isolated, with highest being against netilmicin (30.8%), followed by ceftazidime (27%), tobramycin, piperacillin/tazobactam (19.2% each), etc., in that order. On the other hand, the highest susceptibility was recorded against piperacillin, Cefoperazone/sulbactam and meropenem (92.3% each), followed by amikacin (88.5%), gentamicin and ciprofloxacin (84.6% each), tobramycin and piperacillin/tazobactam (80.8% each), etc., in that order (Table-10, Figure-5).

Table 10: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=26)

Antibiotic	Sensitive / resistant	Number of isolates	%
Gentamicin	S	22	84.6
	R	4	15.4
Ciprofloxacin	S	22	84.6
	R	4	15.4
Cotrimoxazole	S	0	0
	R	26	100
Netilmicin	S	18	69.2
	R	8	30.8
Tobramycin	S	21	80.8
	R	5	19.2
Ceftazidime	S	19	73
	R	7	27
Piperacillin	S	24	92.3
	R	2	7.7
Piperacillin/tazobactam	S	21	80.8
	R	5	19.2
Cefoperazone/sulbactam	S	24	92.3
	R	2	7.7%
Amikacin	S	23	88.5
	R	3	11.5
Meropenem	S	24	92.3
	R	2	7.7

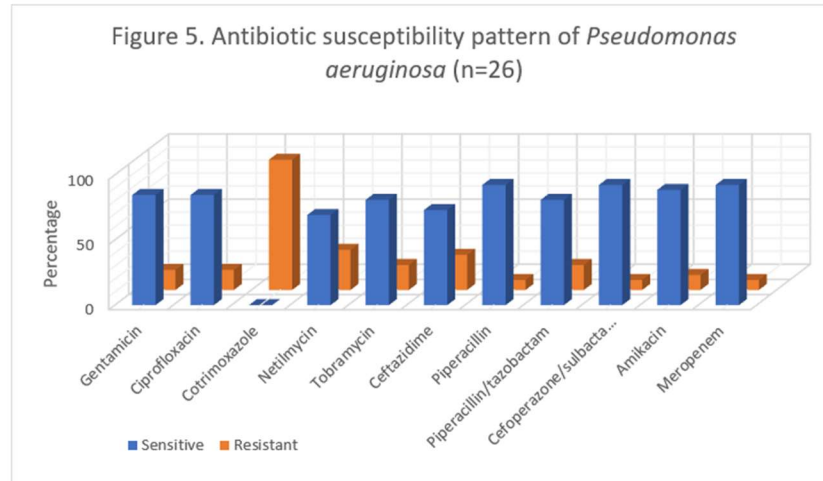


Figure 5: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Among the 13 isolates of other *Pseudomonas* sp, the resistance was less, as in the decreasing order of piperacillin (23.1%), ciprofloxacin, netilmicin, ceftazidime, amikacin and meropenem (15.4% each), etc., in that order, with 100% of the strains resistant to cotrimoxazole (intrinsic resistance). The highest number of strains sensitive were to gentamicin, tobramycin, piperacillin/tazobactam and Cefoperazone/sulbactam (92.3% each), followed by ciprofloxacin, netilmicin, ceftazidime, amikacin and meropenem (84.6% each), etc., in that order (Table-11, Figure-6).

Table 11: Antibiotic susceptibility pattern of *Pseudomonas* sp (n=13)

Antibiotic	Sensitive / resistant	Number of isolates	%
Gentamicin	S	12	92.3
	R	1	7.7
Ciprofloxacin	S	11	84.6
	R	2	15.4
Cotrimoxazole	S	0	0
	R	13	100
Netilmicin	S	11	84.6
	R	2	15.4
Tobramycin	S	12	92.3
	R	1	7.7
Ceftazidime	S	11	84.6
	R	2	15.4
Piperacillin	S	10	76.9
	R	3	23.1
Piperacillin/tazobactam	S	12	92.3
	R	1	7.7
Cefoperazone/sulbactam	S	12	92.3
	R	1	7.7
Amikacin	S	11	84.6
	R	2	15.4
Meropenem	S	11	84.6
	R	2	15.4

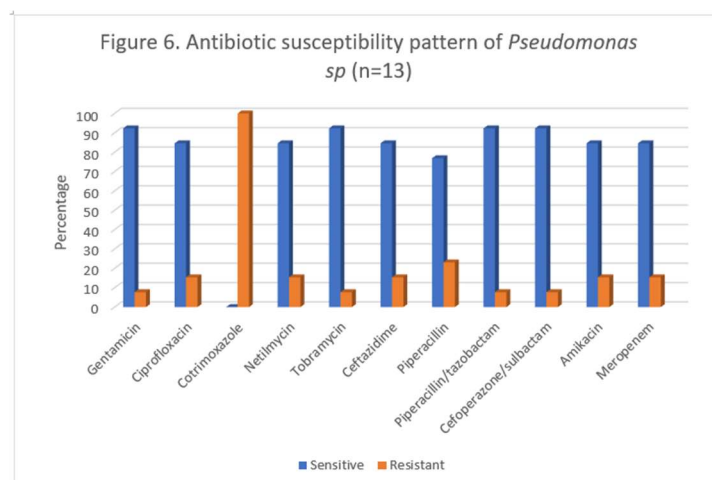


Figure-6 Antibiotic susceptibility pattern of *Pseudomonas* sp

Maximum number of isolates resistant were against ampicillin (100%), followed by cefuroxime and cefixime (77.8% each), ceftriaxone (72.2%), amoxiclav (66.7%), etc., in that order, among the 18 strains of *Acinetobacter* sp isolated. Most number of strains sensitive were to Cefoperazone/sulbactam and piperacillin/tazobactam (77.8% each), followed by ciprofloxacin (72.2%), cotrimoxazole and amikacin (61.1% each), etc., (Table-12, Figure-7).

Table 12: Antibiotic susceptibility pattern of *Acinetobacter* sp (n=18)

Antibiotic	Sensitive / resistant	Number of isolates	%
Ampicillin	S	0	0
	R	18	100
Amoxyclav	S	6	33.3
	R	12	67.7
Gentamicin	S	11	61.1
	R	7	38.9
Ciprofloxacin	S	13	72.2
	R	5	27.8
Cotrimoxazole	S	11	61.1
	R	7	38.9
Cefuroxime	S	4	22.2
	R	14	77.8
Ceftriaxone	S	5	27.8
	R	13	72.2
Cefixime	S	4	22.2
	R	14	77.8
Cefoperazone/sulbactam	S	14	77.8
	R	4	22.2
Piperacillin/tazobactam	S	14	77.8
	R	4	22.2
Amikacin	S	11	61.1
	R	7	36.9
Meropenem	S	10	55.6
	R	8	44.4

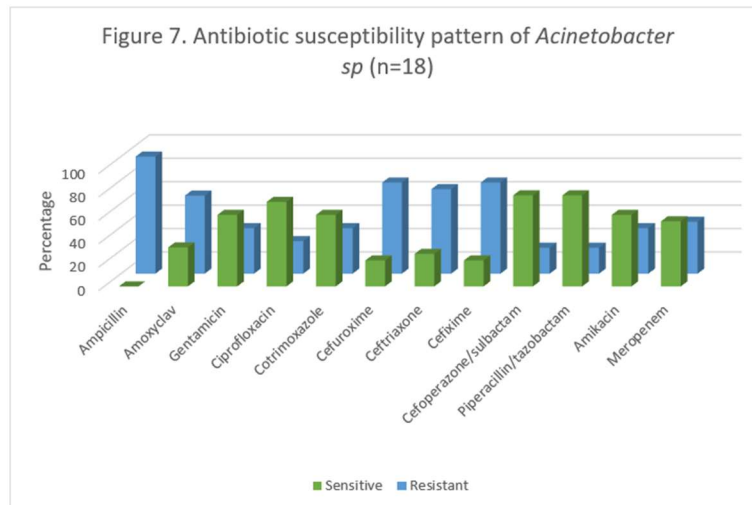


Figure 7: Antibiotic susceptibility pattern of *Acinetobacter* sp

Out of the 7 isolates of *Citrobacter* sp, all were resistant to ampicillin (100%), followed by amoxiclav and cefixime (42.9% each), gentamicin and cefuroxime (28.6% each), etc., in that order. On the other hand most of the strains were sensitive to ciprofloxacin, cotrimoxazole, ceftriaxone, Cefoperazone/sulbactam, piperacillin/tazobactam, amikacin and meropenem (85.7% each), followed by gentamicin and cefuroxime (71.4% each), etc., in that order (Table-13, Figure-8).

Table 13: Antibiotic susceptibility pattern of *Citrobacter* sp (n=7)

Antibiotic	Sensitive / resistant	Number of isolates	%
Ampicillin	S	0	0
	R	7	100
Amoxyclav	S	4	57.1
	R	3	42.9
Gentamicin	S	5	71.4
	R	2	28.6
Ciprofloxacin	S	6	85.7
	R	1	14.3
Cotrimoxazole	S	6	85.7
	R	1	14.3
Cefuroxime	S	6	85.7
	R	1	14.3
Ceftriaxone	S	6	85.7
	R	1	14.3
Cefixime	S	4	57.1
	R	3	42.9
Cefoperazone/sulbactam	S	6	85.7
	R	1	14.3
Piperacillin/tazobactam	S	6	85.7
	R	1	14.3
Amikacin	S	6	85.7
	R	1	14.3
Meropenem	S	6	85.7
	R	1	14.3

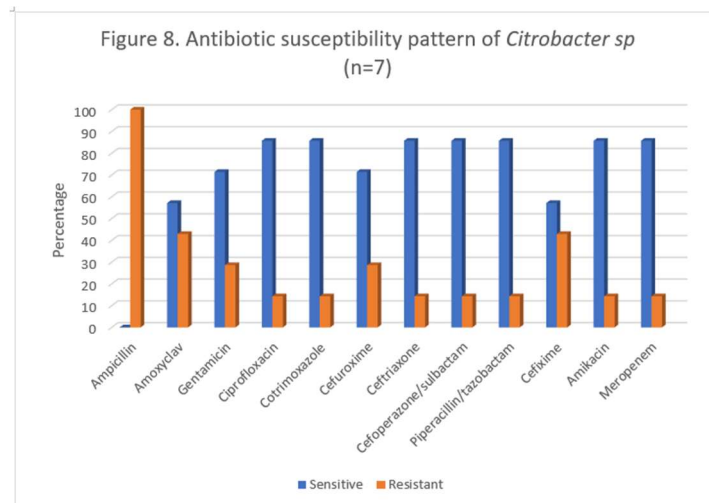


Figure 8: Antibiotic susceptibility pattern of *Citrobacter sp*

DISCUSSION

The culture positivity of 216 cases, from a total of 400 (54%) cases in our study, correlates closely with one of the studies from Rajasthan, India (45%) [12]. Among the culture positive cases, we observed 121 (56%) were males and remaining 95 (44%) females, which was similar to another study done by Kahn et. al [13]. Age-wise, 61-70 years group has shown the maximum number of positive cases, followed by >70 age group. This observation varies from a report from Bhubaneswar, India, wherein, the authors reported, 50-60 age group being the most predominant group, followed by 60-70 age category [14]. Most of the culture positive cases were inpatients (89.4%) with the remaining 10.6% only being outpatients. This finding is similar to a report from Jodhpur, India, showing 80% and 20% respectively [15]. *Klebsiella pneumoniae* was the most predominant isolate in our documentation, followed by *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter sp*, other *Pseudomonas sp* and others (Table 4,5) in that order. This observation is slightly different from Rekha *et al.* [2], though, *Klebsiella pneumoniae* is the most predominant isolate, followed by *Pseudomonas aeruginosa*, but, non-fermenting GNB and *Staphylococcus aureus* comes third and fourth, with *Escherichia coli* being 7th most isolated. In another study from Mysore, Karnataka, *Acinetobacter baumannii* was the most predominant isolate, followed by *Klebsiella spp*, *Streptococcus pneumoniae*, *P. aeruginosa* and others in that order [16].

All 4 isolates of *Staphylococcus aureus* resistant to penicillin, erythromycin and clindamycin in our study was closely in correlation with another study by Singh *S. et al.*, wherein, more than 50% of the strains were resistant to these antibiotics [15]. All the 3 isolates *Streptococcus pneumoniae* being resistant to erythromycin and

clindamycin in our study was in contrast to Rekha *et. al*, who reported a resistance of 33.3% against erythromycin and none against clindamycin [2]. On the other hand, 100% susceptibility to penicillin, vancomycin and linezolid documented in our study was in concordance with Kombade *et. al* [17].

The presence of 100% of the *Escherichia coli* strains, being resistant to ampicillin and 72.7% resistance to amoxiclav in our study varies from another study in Chennai, wherein, they reported 66% and 33% respectively (Monisha *et. al.*) [18], though, it is slightly higher of 72.7 and 63.6% by Rekha *et. al* [2].

Our study recording 100% resistance among the *Klebsiella pneumoniae* isolates to ampicillin is exemplified by being *Klebsiella spp* intrinsically resistant to it. Similar finding is seen in Rekha *et. al* article as well [2]. But, subsequently, our findings recording only moderate resistance to amoxiclav (55.5%), cefuroxime (49.5%) and ceftriaxone (44.5%), is drastically high from Rekha *et. al.* [2] findings, of 94.4%, 78.9% and 65.6% respectively. An important observation in our work is decrease in susceptibility of *K. pneumoniae* strains to carbapenem drug like meropenem (71.3%), which happened to be a restricted drug, because 2nd line drugs like, amikacin and piperacillin/tazobactam were showing higher sensitivity of 83.2 and 82.2% respectively. Singh *et. al.* [15] demonstrated more or less similar pattern of susceptibility to meropenem (78.7%). Apart from this, we observed 26 MDR strains of *Klebsiella pneumoniae*. All these may be due to the disproportionate, inappropriate and frequent use of higher (restricted) antibiotics.

Among the *Pseudomonas aeruginosa* isolates, evidently, all the strains were resistant to cotrimoxazole, being intrinsically resistant. Resistance pattern is comparatively less, when compared to that of Enterobacteriaceae organisms. The strains showing less resistance to

Netilmicin and ceftazidime in our study of 30.8 and 27% respectively was close to another study by Ashina *et al.* [12]. Higher level of susceptibility to piperacillin, piperacillin/tazobactam and meropenem in our study of 92.3, 80.8 and 92.3% respectively was in contrast to another study by Yayan *et al.* (76, 77 and 80%) [19].

Regarding the *Acinetobacter* isolates, 100% resistance was recorded against ampicillin. The higher level of resistance to cefuroxime (77.8%) and amoxiclav (66.7%) in our study was comparatively less than that of Debnath *et al.*, which recorded 95% each [20]. The sensitivity pattern of 77.8% and 61.1 % against, piperacillin/tazobactam and amikacin in our study was much higher than that of this work (52 and 46.4%).

The sensitivity pattern of *Citrobacter* sp is depicted in Table- 13 and Figure -8. The other organisms were very less in number to make an impact, while analyzing their sensitivity pattern.

CONCLUSION

Overall, we could observe decrease in susceptibility of GNB isolates to carbapenem drugs (restricted drugs), when compared to amikacin and piperacillin/tazobactam (2nd line), against which more strains were sensitive. This might be due to frequent, disproportionate use of higher antibiotics. Drugs like amikacin, piperacillin/tazobactam, and Cefoperazone/sulbactam could be the drugs of choice for empirical treatment of LRT infections to start with and can be tailored based on the sensitivity report once come in. Rationale and judicious use of antibiotics have to be adhered to.

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