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Microbial Isolates and their Resistance Patterns at an Indian Teaching Hosptial - A Retrospective Study

Poojitha A,[1] Sultana G,[2] Bindu Madhavi V,[1] N.Hanisha Gupta,[1]
Qamar U.B Siddiqui,[1] Mustafa Afzal,[3] Mohammed A. Ghufran[4]

[1]Pulla G. Reddy College of Pharmacy, Hyderabad.
Mehdipatnam, Telangana, India
[2]Department of Pharmacy Practice, G. Pulla Reddy College of Pharmacy.
Mehdipatnam, Telangana, India
[3]Clinical Microbiology and Infection Control, Care Hospital, Nampally.
Mehdipatnam, Telangana, India
[4]Department of Clinical Pharmacy, Care Hospital, Nampally.
Mehdipatnam, Telangana, India

Abstract

Rapidly rising antibiotic resistance is a challenge to comprehensive patient care in all branches of medical science. A retrospective observational study was conducted to determine the antimicrobial resistance patterns in a tertiary care hospital for six months. The data was collected from laboratory test reports and patient case files using a structured data collection form. A total of 200 cases having a positive culture report were included in the study. Of these, 186 cases were identified to have gram-negative infections, and 14 had gram-positive infections. *Escherichia coli* (*E. Coli*) (47%) was the predominant isolate among gram-negative organisms, followed by *Klebsiella* (24.5%), *Pseudomonas* (7.55%), *Enterobacter* (6.5%), and *Citrobacter* (4.5%). All Gram-positive organisms *E.coli, Klebsiella, Pseudomonas, Enterobacter* have shown maximum resistance of 92.55%, 95.91%, 93.33%, and 76.92%, respectively, Ampicillin. *E.coli* has shown the least resistance to carbapenems (7.44%). *Klebsiella* demonstrated minimum reistance of 10.2% to cefotaxime, cefazolin and levofloxacin. Pseudomonas was completely sensitive to piperacillin-tazobactam. *Enterobacter* showed least resistance to gentamicin (3.07%) and piperacillin-tazobactam (3.07%). The primary gram-positive isolate, *Enterococcus spp.* (5.5%) mainly was resistant to ciprofloxacin (90.90%), followed by levofloxacin (81.81%), tetracycline (81.81%), and gentamicin (72.72%). *E. coli* was the most resistant organism in the present study. The study results would contribute to the development of an antibiogram at the study site.

Keywords: antimicrobial resistance, antibiotics, *escherichia coli, klebsiella, pseudomonas, enterobacter*

Corresponding author: Gouhar Sultana. Department of Pharmacy Practice G.Pulla Reddy College of Pharmacy, Mehdipatnam. Telangana, India. Email: sultanagouhar@gmail.com
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Introduction

Antibiotic resistance is a global crisis and one of the greatest threats to the population's health. It is accelerated by the irrational use of antibiotics in humans and animals, high consumption of broad-spectrum antibiotics, and poor infection prevention and control. Antibiotic resistance leads to higher medical costs, prolonged hospitalization, and increased morbidity and mortality.[1]

Antimicrobial stewardship will prevent or slow the emergence of resistance among microorganisms. The development of antibiotic resistance patterns is directly proportional to the volume of antibiotics consumed,[2] and India are considered the highest consumer of antibiotics. Geographical variations in resistance to antibiotics were also reported.[2,3] Therefore, the present study was designed to determine the antimicrobial resistance patterns of microbial isolates from in-patients in a tertiary care hospital in India and contribute to the institutional data. The study results would support identifying the problematic pathogens and the development of an institutional antibiogram at the study site. The study site was a teaching hospital, and the study was carried out as a part of the curriculum for the Pharm. D course.

Methods

A retrospective, observational study was carried out at Care Hospital, Nampally, Hyderabad, India. The study duration was six months (August 2019 to January 2020), and the data were collected retrospectively from patient case files and laboratory test reports.

All infectious in-patients for whom the antimicrobial susceptibility tests have been performed and the reports were found to be positive were included in the study. Any case having negative culture results was excluded even though clinical signs and symptoms indicated an infection. The study was approved by Institutional Ethics Committee, Care Hospitals (Reference number: IEC/CARE/20353/2020/DNB).

A well-structured data collection form was used to collect the data. Information related to patients' demographics, co-morbidities, primary diagnosis or presumed indication for antibiotic therapy, and antimicrobial susceptibility test results. The data was collected

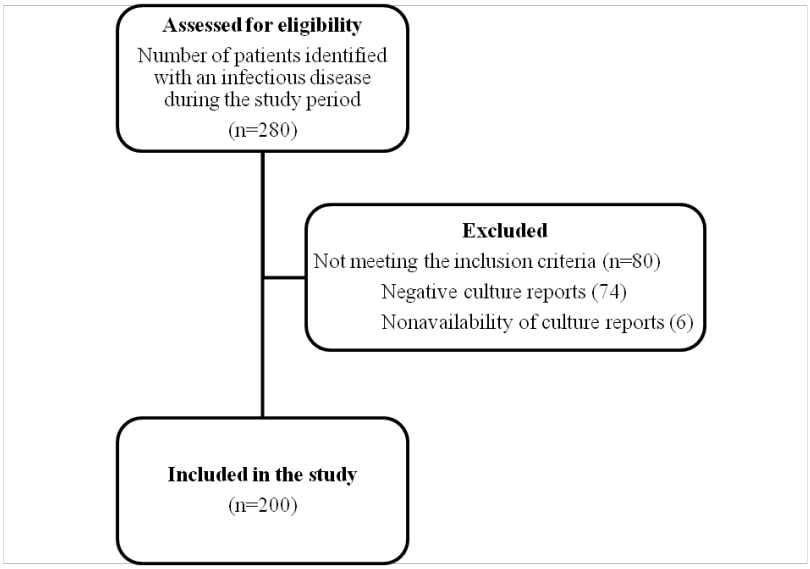

Figure 1. Flow Chart of Study Participants

from the medical records department, Care Hospital. Antimicrobial susceptibility reports were analyzed for the specimen used, the pathogen identified, sensitivity, and resistance pattern, whereas patient case files were used for the information on antibiotics prescribed. Kirby-Bauer disc diffusion method was used to test the antimicrobial sensitivity of organisms. The data obtained were analyzed using Microsoft excel.

Results and Discussion

A total of 280 in-patients were identified to have various infections during the study period. Of these, 200 cases were included in the study as the culture test result was positive (Figure.1). The exclusion of the remaining cases was either due to adverse culture reports (26.42%) or non-availability of the culture reports (2.14%). Out of 200 positive culture isolates, gram-negative (186) isolates were predominant in the present study compared to gram-positive (14) isolates. This was consistent with studiies conducted by Nazneen et al. (2016)[4], Rudrajit Paul et al. (2017)[5], and Viral Vadwai et al. (2015)[6]. Still, in another study conducted by R.S Parihar et al. (2018)[7] gram-positive isolates have taken over gram-negative isolates.

In the present study, it was observed that infections were more common in the male (118) compared to female (82), which is quite similar to the studies conducted by Ramanath K V et al. (2019),[8] Rudrajit Paul et al. (2017)[5] and R.S Parihar et al. (2018).[7]

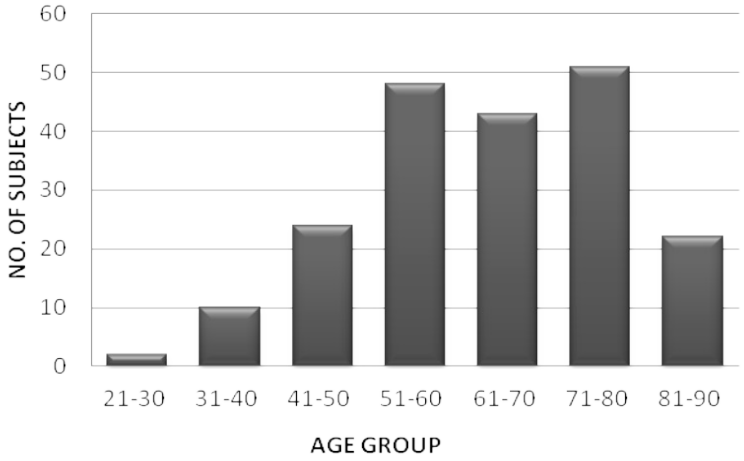

Figure 2. Age Distribution

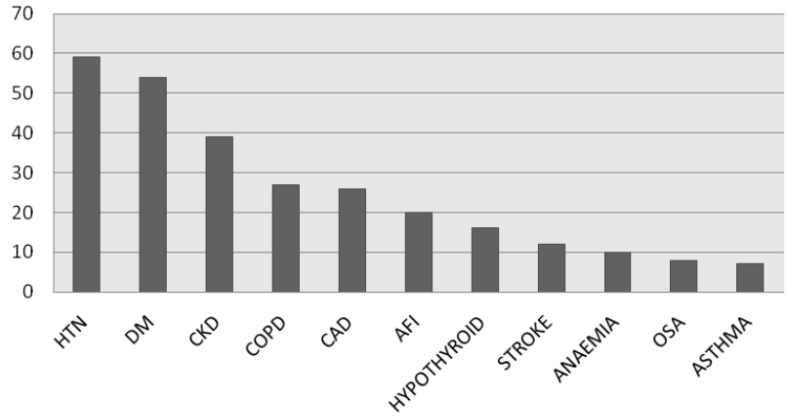

Figure 3. Graphical Representation of Subjects based on Co-morbidities

Table 1. Distribution of Subjects Based On Type of Infections

Infection	Number of Subjects	Percentage (%)
Urinary Tract Infection	76	38
Pneumonia	52	26
Sepsis	31	15.5
Urosepsis	27	13.5
Sepsis with septic shock	6	3
Fasciitis	3	1.5
Meningitis	2	1
Surgical site infection	2	1
Vaginitis	1	0.5
Total	200	100

The mean age was 63.55 ± 14.13 years. The majority of the patients were 71-80 years (51) and 51-60 years (48) (Figure 2). This was different from a study conducted in India,[8] where most patients belonged to the age group of 61-70 years (28.1%) and 41-50 years. The most common co-morbidity was hypertension (59; 29.5% of the total subjects) followed by diabetes mellitus (54) and chronic kidney disease (39)(Figure 3).

The most common infection in the present study was urinary tract infection accounting for 38% of the total subjects, followed by pneumonia and sepsis (Table 1). The studies conducted by Rudrajit Paul et al. (2017)[5] and Moremi N et al. (2016) found the most common infection was blood infections and urinary tract infections. *E. coli* was the most frequently isolated pathogen from urine, and this finding was consistent with the results of Moremi N et al. (2016).[9]

The specimens collected were urine (106), sputum (44), blood (22), pus (7), and others (21) which include ascites fluid, bronchoalveolar lavage (BAL) fluid, endotracheal secretions, swab, pleural fluid, tissue/tracheal culture as shown in Table 2. *E. coli* (94) was predominant organism in the present study which is consistent with previous studies[4,5]. In one of the earlier studies, *Acinetobacter baumannii* was observed as predominant gram-negative organism by Nidhi Bhardwaj et al. (2018).[10]

Escherichia coli

E.coli belonging to the family *Enterobacteriaceae*, is an important hospital and community acquired organism and one of the commensals of the human intestinal tract.[11] The pathogenic strains of *E.coli* can cause infections of gastrointertinal tract, urinary tract, central nervous system, circulatory system, and respiratory system.[12] They have acquired resistance to multiple antibiotics and extended spectrum β-lactamase (ESBL) producing *E.coli* pose a threat to the management of infections.[13]

E.coli was the major gram-negative isolate in urine and blood sample. Maximum resistance was observed against ampicillin (92.5%), similar results were reported by Asima Banu et al. (2011).[14] While in the findings by Nazneen et al. (2016)[4] maximum resistance was observed against third-generation cephalosporins and Rudrajit Paul et al. (2017)[5] reported penicillin and its congeners when compared to other drugs.

Table 2. Distribution of Isolated Organisms based on Specimen Tested

Organisms	Urine	Sputum	Blood	Pus	Others	Total	Percentage
Escherichia coli	71	3	11	3	6	94	47
Klebsiella	12	24	4	1	8	49	24.5
Pseudomonas	2	7	1	1	4	15	7.5
Enterobacter	5	6	2	-	-	13	6.5
Citrobacter	4	4	-	-	1	9	4.5
Proteus vulgaris	1	-	-	1	-	2	1
Acinetobacter	-	-	1	-	1	2	1
Alcaligenes	-	-	1	-	-	1	0.5
Burkholderia	-	-	1	-	-	1	0.5
Enterococcus (Gram positive)	11	-	-	-	-	11	5.5
Staphylococcus (Gram positive)	-	-	1	1	1	3	1.5

High resistance to ciprofloxacin (92.86%) and cotrimoxazole (92.86%) were reported by Omololu-Aso et al. (2017).[15] Ampicillin resistance in *E.coli* was predominant in all the samples i.e., 90% among urine isolates and 100% in other samples.

Within the urine sample, maximum resistance was observed for ampicillin (90%), cefopodoxime (88.7%), ciprofloxacin (84.5%), etc. as shown in Table 3. It was reported as ampicillin (88.4%), amoxicillin-clavulanic acid (74.4%), norfloxacin (74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and co-trimoxazole (64.2%) by Niranjan V et al. (2014)[16] in patients with urinary tract infection.

The *E.coli* isolated from all eleven blood samples showed resistance to ampicillin. The other drugs to which *E.coli* isolated from blood samples was resistant were cefotaxime followed by cefepime, co-amoxiclav, cefazoline, levofloxacin, ciprofloxacin etc.

The *E.coli* was isolated from three pus samples during the study. All three isolates were resistant to cefepime, cefpodoxime, ceftriaxone, ampicillin, co-amoxiclav, ciprofloxacin, and ofloxacin, whereas two isolates were resistant to ceftazidime and one was resistant to cefoxitin and ceftazidime/clavulanate. Similarly, *E.coli* isolated from all the three sputum samples during the study period were resistant to cefepime, ceftazidime, ceftriaxone, ampicillin, co-amoxiclav, and aztreonam whereas two were resistant to cefpodoxime, ciprofloxacin and ofloxacin and one was resistant to cefoxitin.

E.coli was also isolated from tissue samples (3), swab (2), and BAL fluid (1) and was resistant to ampicillin. *E.coli* has shown resistance to cefepime, the fourth generation cephalosporin (77.4% in urine, 81.8% in blood, 100% in pus, sputum, and swab).

Klebsiella spp.

Klebsiella was the second predominant gram-negative organism isolated in the present study and the leading organism in sputum followed by urine, blood, swab, and others (Table 4). Gill M. K, et al. (2019)[17] reported maximum isolates from pus followed by urine, blood and sputum.

Table 3. Resistance Pattern of E.coli

Antibiotics Tested	Urine (71)	Blood (11)	Pus (3)	Sputum (3)	Swab (2)	Tissue (3)	BAL fluid (1)	Total No. of resistant isolates (94)	Percentage of resistant isolates
Ampicillin	64	11	3	3	2	3	1	87	92.55%
Cefpodoxime	63	3	3	2	2	3	1	77	81.91%
Ciprofloxacin	60	6	3	2	2	3	1	77	81.91%
Ceftriaxone	60	4	3	3	2	3	1	76	80.85%
Cefepime	55	9	3	3	2	2	1	75	79.78%
Ofloxacin	55	3	3	2	2	2	1	68	72.34%
Ceftazidime	50	3	2	3	1	3	1	63	67.02%
Aztreonam	39	1	1	3	1	1	1	47	50%
Cotrimoxazole	43	2	-	-	-	-	-	45	47.87%
Cefoxitin	33	3	1	1	1	2	1	42	44.68%
Ceftazidime/clavulinic acid	26	3	1	1	1	2	1	35	37.23%
Amoxicillin / clavulinic acid (co-amoxiclav)	8	8	3	3	2	2	1	27	28.72%
Pipercillin/tazobactam	22	1	-	-	1	1	1	26	27.65%
Gentamicin	20	3	-	1	-	1	-	25	26.59%
Cefotaxime	11	9	-	-	-	-	-	20	21.27%
Cefaperazone/sulbactam	15	1	-	1	-	-	1	18	19.14%
Amikacin	10	2	-	-	1	1	-	14	14.89%
Levofloxacin	7	6	-	-	-	-	-	13	13.82%
Cefazolin	5	8	-	-	-	-	-	13	13.82%
Oxacillin	6	1	-	-	-	-	-	7	7.44%
Meropenem	7	-	-	-	-	-	-	7	7.44%
Imipenem	7	-	-	-	-	-	-	7	7.44%
Linezolid	3	-	-	-	-	-	-	3	3.19%

Table 4. Resistance Pattern of Klebsiella

Antibiotics Tested	Sputum (24)	Urine (12)	Blood (4)	Swab (2)	Pus (1)	BAL fluid (2)	Others				Total No. of resistant isolates (49)	Percentage of resistant isolates (%)
							Ascites fluid	Bronchoscopy fluid	ET secretion	Tracheal culture		
Ampicillin	23	11	4	2	1	2	1	1	1	1	47	95.91%
Ciprofloxacin	11	8	2	2	1	2	-	-	-	1	27	55.10%
Amoxicillin/Clavlanic Acid	12	5	2	1	1	2	-	1	1	1	26	53.06%
Cefoxitin	8	8	2	2	1	2	-	1	1	1	26	53.06%
Cefepime	8	8	3	2	1	2	-	-	1	1	26	53.06%
Cefpodoxime	12	6	-	2	1	-	-	-	-	1	22	44.89%
Ceftriaxone	11	5	-	2	1	1	-	-	1	1	22	44.89%
Ceftazidime	7	7	-	2	1	2	-	-	1	1	21	42.87%
Aztreonam	10	6	-	-	1	1	-	-	1	1	20	40.81%
Ofloxacin	8	7	-	2	-	1	-	-	-	1	19	38.77%
Piperacillin+ Tazobactum	2	5	2	2	1	2	-	-	-	1	15	30.61%
Ceftazidime Clavulanate	2	7	-	2	1	2	-	-	-	1	15	30.61%
Cefaperazone/Sulbactum	2	7	-	2	-	2	-	-	-	1	14	28.57%
Amikacin	2	5	2	1	-	1	-	-	-	-	11	22.44%
Gentamicin	1	4	2	-	-	1	-	-	1	-	9	18.36%
Imipenem	2	1	2	1	-	-	1	-	-	-	7	14.28%
Meropenem	2	1	2	1	-	-	-	-	-	-	6	12.24%
Cotrimoxazole	-	4	2	-	-	-	-	-	-	-	6	12.24%
Cefotaxime	1	2	2	-	-	-	-	-	-	-	5	10.20%
Cefazolin	-	2	3	-	-	-	-	-	-	-	5	10.20%
Levofloxacin	-	2	2	1	-	-	-	-	-	-	5	10.20%
Nitrofurantoin	-	3	-	-	-	-	-	-	-	-	3	6.12%
Linezolid	-	1	-	-	-		-	-	-	-	1	2.04%

Table 5. Resistance Pattern of *Pseudomonas Spp*

Antibiotics Tested	Sputum (7)	Urine (2)	Blood (1)	Pus (1)	BAL fluid (2)	Tissue (1)	Bronchoscopy fluid (1)	Total (15)	Percentage of resistant isolates (%)
Ampicillin	7	2	-	1	2	1	1	14	93.33%
Cefpodoxime	6	2	-	1	2	-	-	11	73.33%
Amoxicillin+Clavlanic Acid	6	-	-	1	2	-	-	9	60.00%
Cefoxitin	4	-	-	1	1	1	1	8	53.33%
Ceftriaxone	3	1	-	1	2	-	-	7	46.66%
Ceftazidime	2	2	1	-	-	-	1	6	40.00%
Cefepime	1	2	1	-	-	-	1	5	33.33%
Ciprofloxacin	2	1	-	-	-	-	1	4	26.66%
Ceftazidime Clavulanate	2	1	-	-	-	-	1	4	26.66%
Ofloxacin	-	1	1	-	-	-	1	3	20.00%
Levofloxacin	-	1	1	-	-	-	1	3	20.00%
Aztreonam	-	2	-	-	-	-	-	2	13.33%
Cefotaxime	-	2	-	-	-	-	-	2	13.33%
Cotrimoxazole	1	1	-	-	-	-	-	2	13.33%
Nitrofurantoin	-	2	-	-	-	-	-	2	13.33%
Gentamicin	1	1	-	-	-	-	-	2	13.33%
Cefaperazone/Sulbactum	1	-	-	-	-	-	-	1	6.66%
Cefazolin	-	1	-	-	-	-	-	1	6.66%
Amikacin	-	1	-	-	-	-	-	1	6.66%
Meropenem	-	-	1	-	-	-	-	1	6.66%
Imipenem	-	-	1	-	-		-	1	6.66%

Table 6. Resistance Pattern of *Enterobacter*

Antibiotics Tested	Urine (5)	Sputum (6)	Blood (2)	Total (13)	Percentage of resistant isolates (%)
Ampicillin	2	6	2	10	76.92%
Cefpodoxime	4	6	-	10	76.92%
Ceftriaxone	4	6	-	10	76.92%
Cefoxitin	3	4	2	9	69.23%
Cefepime	4	4	1	9	69.23%
Amoxicillin /clavulinic acid	1	5	2	8	61.53%
Ciprofloxacin	4	4	-	8	61.53%
Ofloxacin	4	4	-	8	61.53%
Ceftazidime	4	3	-	7	53.54%
Ceftazidime / clavulinic acid	4	2	-	6	46.15%
Cefaperazone/sulbactam	4	1	-	5	15.38%
Aztreonam	4	1	-	5	15.38%
Piperacillin/tazobactam	2	2	-	4	3.07%
Gentamicin	4	-	-	4	3.07%
Amikacin	3	-	-	3	23.07%
Meropenem	3	-	-	3	23.07%
Cefazolin	-	-	2	2	15.38%
Levofloxacin	1	1	-	2	15.38%
Imepenem	2	-	-	2	15.38%
Cefotaxime	-	-	1	1	7.69%
Cotrimoxazole	-	1	-	1	7.69%

Maximum resistance was observed for ampicillin in sputum (95.8%) and urine (91.66%). *Klebsiella* isolated in other samples showed 100% resistance to Ampicillin. The other predominant antibiotics to which *Klebsiella* has shown resistance in sputum include co-amoxiclav (50%), cefpodoxime (50%), ceftriaxone, ciprofloxacin, aztreonam, etc. They isolated from one ascites fluid sample has shown resistance to ampicillin and imipenem, whereas it showed resistance to ampicillin, co-amoxiclav, and cefoxitin in one bronchoscopy fluid sample.

Klebsiella has also shown resistance to the fourth generation cephalosporin, cefepime (33.3% in sputum, 66.6% in urine). The study conducted by Gill M. K (2019)[14] reported 100% resistance to ampicillin followed by ciprofloxacin, cefepime, ceftriaxone and co-amoxiclav and Li B, et al. 2012)[18] also reported maximum resistance to ampicillin (99.6%) followed by resistance to furadantin, cefotetan, trimethoprim-sulfamethoxazole, and ampicillin/sulbactam.

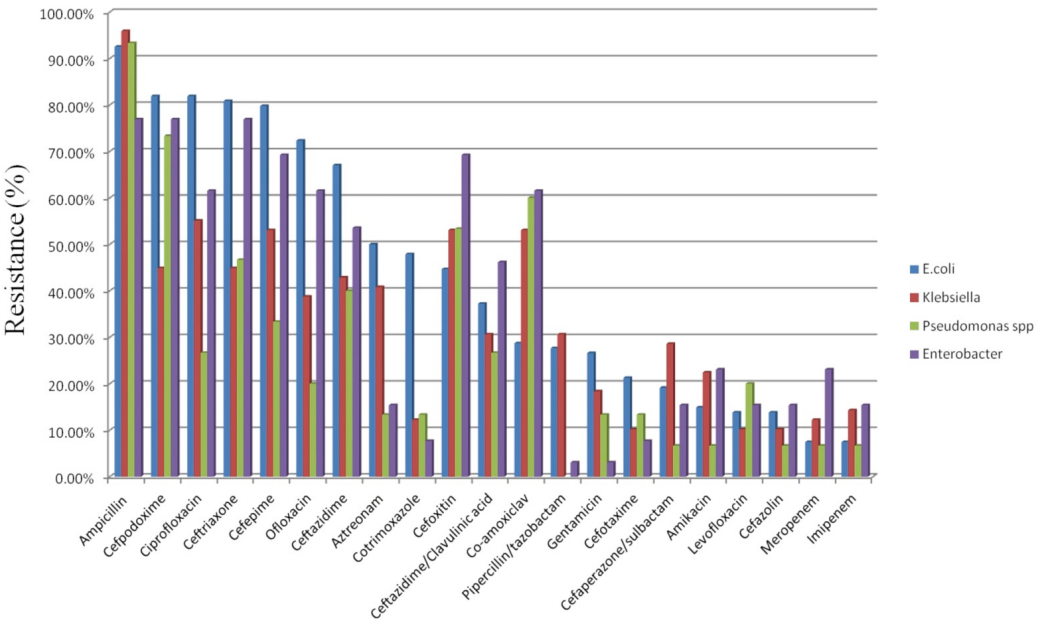

Figure 4. Summary of Antimicrobial Resistence Level of Gram Negative Organism

Maximum resistance was observed for ampicillin in sputum (95.8%) and urine (91.66%). *Klebsiella* isolated in other samples showed 100% resistance to Ampicillin. The other predominant antibiotics to which *Klebsiella* has shown resistance in sputum include co-amoxiclav (50%), cefpodoxime (50%), ceftriaxone, ciprofloxacin, aztreonam, etc. They isolated from one ascites fluid sample has shown resistance to ampicillin and imipenem, whereas it showed resistance to ampicillin, co-amoxiclav, and cefoxitin in one bronchoscopy fluid sample.

Klebsiella has also shown resistance to the fourth generation cephalosporin, cefepime (33.3% in sputum, 66.6% in urine). The study conducted by Gill M. K (2019)[14] reported 100% resistance to ampicillin followed by ciprofloxacin, cefepime, ceftriaxone and co-amoxiclav and Li B, et al. 2012)[18] also reported maximum resistance to ampicillin (99.6%) followed by resistance to furadantin, cefotetan, trimethoprim-sulfamethoxazole, and ampicillin/sulbactam.

Pseudomonas spp.

Pseudomonas spp. was isolated from 7 sputum samples, 2 urine and BAL fluid samples and one sample for blood, pus, tissue, and bronchoscopy fluid. Senthamarai et al (2014)[19] reported pus as the predominant sample from which Pseudomonas was isolated followed by sputum, urine and blood.

During the present study, *Pseudomonas* exhibited highest resistance against ampicillin (93.33%) followed by cefpodoxime (73.33%), co-amoxiclav (60%), and cefoxitin (53.33%) whereas Senthamarai et al (2014)[19] reported highest resistance for ceftazidime (65.38%), ciprofloxacin (61.53%), piperacillin (59.61%), ticarcillin/clavulanic acid (56.73%), and ceftriaxone (55.76%). (Table 5)

Pseudomonas isolated from sputum showed maximum resistance to ampicillin (100%), amoxicillin (85.7%), and cefpodoxime (85.7%) when compared to other samples. Isolates from sputum showed 100% resistance rate against amoxicillin in a study conducte
Three samples (tracheal fluid, pus, blood) obtained from patients suffering from sepsis

Table 7. Resistance Pattern of *Enterococcus spp*

Antibiotics Tested	Urine (11)	Percentage of resistant isolates (%)
Ciprofloxacin	10	90.90%
Levofloxacin	9	81.81%
Tetracycline	9	81.815
Gentamicin	8	72.72%
Ampicillin	6	54.54%
Penicillin	6	54.54%
Amoxicillin/ Clavulanate	6	54.54%
Nitrofurantoin	2	18.18%
Teicoplanin	2	18.18%
Oxacillin	1	9.09%
Rifampin	1	9.09%
Ofloxacin	1	9.09%
Amikacin	1	9.09%

maximum resistance to ampicillin (100%), amoxicillin (85.7%), and cefpodoxime (85.7%) when compared to other samples. Isolates from sputum showed 100% resistance rate against amoxicillin in a study conducted by Hoque MM, et al. (2015).[20] Resistance to anti-pseudomonal drugs, meropenem and imipenem was found in one blood sample during the study period.

Enterobacter spp.

A total of 13 samples (6 sputum, 5 urine, and 2 blood) showed the presence of *Enterobacter spp.* Maximum resistance was observed for ampicillin, cefpodoxime, and ceftriaxone followed by cefoxitin, cefepime, co-amoxiclav. Another gram-negative organism *Citrobacter* exhibited maximum resistance to cefpodoxime (89%) and ampicillin (89%) followed by nitrofurantoin (75%) (Table 6). A summary of antimicrobial resistance levels for Gram-negative organisms identified in the study are presented in Figure 4.

Gram-positive Organisms

11 urine samples were culture positive for *Enterococcus* (Table 7), which was the common gram-positive isolate in the present study. While in other studies by Nazneen et al. (2016)[4], Rudrajit Paul et al. (2017)[5] and Viral Vadwai et al.(2015)[6] it was observed as *Staphylococcus* species.

Enterococcus spp. has shown resistance to ciprofloxacin (91%) followed by levofloxacin (82%) and tetracycline (82%), whereas in study carried out by Nazneen et al. (2016)[4] it is erythromycin and in the findings depicted by Rudrajit Paul et al. (2017)[5], high resistance was shown to penicillin and its congeners, 4th generation cephalosporins, fluoroquinolones, and macrolides.

Three samples (tracheal fluid, pus, blood) obtained from patients suffering from sepsis showed the presence of *Staphylococcus spp.* All three samples showed resistance

showed the presence of *Staphylococcus spp.* All three samples showed resistance to penicillin, ampicillin, erythromycin, and ciprofloxacin.

The major limitation of the study is its short duration. Antibiotic disc sensitivity test results may vary with the hospital setting, while infection rate in a hospital may depend on the hospital environment, antibiotic use, and other infection control practices. This would limit the application of the findings of our study to other hospital settings.

The need of the hour is to promote the judicious use of antibiotics, and steps should be taken at all levels to minimize the impact and spread of resistance. Rational use of antibiotics and frequent surveillance are needed to curb this threat and preserve the antibiotics for the future. Framing and establishing antibiotic stewardship plans in all the tiers of healthcare settings are essential to monitor and ensure proper use of antimicrobials.

Other measures that can effectively control the antimicrobial resistance include creating awareness on rational use of antibiotics in the community, implementing rules and regulations for the use of antimicrobial agents in food animals, implementing strict regulations and vigilance to bring down the over the counter sale of antibiotics and promoting and supporting future research on the drivers of antimicrobial resistance to control it effectively.

Conclusion

Gram-negative organisms identified in this study have shown maximum resistance to ampicillin. *E. coli* remains the most commonly isolated gram-negative organism and was found to be highly resistant to ampicillin followed by third generation cephalosporins and fluoroquinolones. *Enterococcus*, the most common gram-positive organism identified in urine was highly resistant to fluoroquinolones.

Future Recommendation

The prevalence of multidrug resistance (MDR) was also identified during the study which is a serious concern. Further research is needed to identify and quantify the MDR resistance patterns to strengthen the antimicrobial stewardship program and improve judicious use of antibiotics.

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Conflict of Interest

None

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