



Original Research Article

Trends in Antibigram of Gram-Negative Clinical Isolates in Tertiary Care Hospital

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Abstract:

Gram-negative pathogens like *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E-coli*, *Klebsiella pneumoniae* are associated with multidrug resistance. These pathogens are capable of causing clinical diseases like urinary tract infections, blood stream infections, pyelonephritis, intra-abdominal infections, hospital acquired pneumonia etc. Antimicrobial resistance is associated with prolonged hospital stays, difficulty in treating patients, high morbidity and high mortality rates.

Methods: A cross-sectional study was conducted after obtaining ethical committee approval in the Department of Microbiology, JJMMC Davangere. Clinical samples like blood, pus, urine, sputum etc received at our department were processed following standard procedure. Only gram-negative bacterial isolates from the samples were included in the study. The data collected was entered into excel sheet and was analyzed using SPSS version 25.0.

Results: Out of 1386 positive cultures of gram-negative bacteria, 217 multidrug resistant gram-negative organisms were isolated. *Klebsiella pneumoniae* 105 (48%), *Escherichia-coli* 102 (47%), *Pseudomonas aeruginosa* 9 (4%) and *Acinetobacter baumannii* 1 were the common isolates. The prevalence of infection was highest among the patients with age group less than one.

Conclusion: The study shows increased prevalence of multidrug resistant strains of *Klebsiella pneumoniae* and *Escherichia coli* in our hospital. Implementation of hospital infection control practices, antimicrobial stewardship and proper surveillance will help in limiting the emergence and spread of drug resistant strains.

Keywords: Gram-negative, multidrug, antibiogram.

Introduction

The development of antimicrobial resistance is considered as one of the most critical health care problems faced by physicians in 21st century. In 2012 a terminology was developed to define antimicrobial resistance where multi drug resistance is defined as an acquired non-susceptibility to at least one agent in three or more antibiotic categories¹. In 2021, 4.71 million deaths were associated with antimicrobial resistance within developing countries¹.

Gram negative pathogens like *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E-coli*, *Klebsiella pneumoniae* are associated with multi drug resistance. These pathogens are capable of causing clinical diseases like urinary tract infections, blood stream infections, pyelonephritis, intra-abdominal infections, hospital acquired pneumonia etc¹. Antimicrobial resistance is associated with prolonged hospital stays, difficulty in treating patients, high morbidity and high mortality rates.

In developing countries like Africa study has been conducted that indicated the high antibiotic resistance among gram negative bacteria to antibiotics resulting in loss of efficacy for treatment of infections. Certain measures like surveillance study providing reliable data have been taken in Ghana hospital to solve this problem².

Antibiotic resistance crisis has emerged as the antibiotics tend to lose efficacy due to over use or irrational use of antibiotics, dissemination of resistance among bacteria, efflux pump upregulation, porin mutation, carbapenemase production, mutation in DNA gyrase genes³. Antibiotic resistance can also be intrinsic. Gram negative bacteria are intrinsically resistant to vancomycin as it cannot penetrate the outer membrane. *Klebsiella* has intrinsic resistance towards ampicillin due to beta-lactamase production. Apart from intrinsic resistance of an organism, bacteria can acquire resistance like carbapenemases belonging to ambler molecular classes A, B metallo-beta-lactamases⁴.

In developing countries antimicrobial resistance is spreading due to poor sanitation and lack of clean drinking of water as per WHO. In USA data reveals that one in five resistant infections are caused due to consumption of contaminated food and water. In Europe antimicrobial resistance is spreading due to cross border transfer of patients carrying multi drug resistant bacteria, transmission of multi drug resistant pathogens in and between health care settings³. In India, Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are most often clinical diseases causing pathogens along with increasing rate of extended spectrum beta-lactamase production and carbapenem resistance. In 2017 WHO specified carbapenem resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae as critically important organisms of importance for developing newer antibiotics⁵.

Several studies suggest that hospitals from India has a prevalence of ESBL producing gram negative bacteria ranges between 19% and 60%

and carbapenem resistant gram-negative bacteria between 5.3% and 59%. A study conducted in Mumbai, West India revealed that 18.5% of clinical samples have dual carbapenemase genes⁴.

In 2017 in India two newer initiatives have been started, one is Indian Council of Medical Research and secondly CDC-ICMR and AIIMS. ICMR established several new regional centres that worked for Antimicrobial Resistance Surveillance Network and CDC-ICMR and AIIMS supported with capacity building project on antimicrobial resistance. These initiatives will help in infection control and antimicrobial resistance surveillance across many regions in the country⁴.

There are many ways by which the multidrug resistant gram-negative organisms can be treated. Drugs like colistin, Fosfomycin, tigecycline, aminoglycosides are used. Colistin needs to be used at a dose of 2mg/L with concern of nephrotoxicity and neurotoxicity as per Clinical and Laboratory Standard Institute guidelines⁶. Fosfomycin was discovered in 1960s inhibits UDP-GlcNAc enolpyruvyl transferase effective against *Escherichia coli* as well as carbapenem resistant bacteria. Tigecycline is active invitro against carbapenem resistant enterobacteriales and carbapenem resistant *Acinetobacter baumannii* and is used in combination with other drugs. Aminoglycosides are used in the treatment of urinary tract infections and nosocomial pneumoniae. Piperacillin/tazobactam are broad spectrum antibiotic agents against multiple gram-negative pathogens especially against *Pseudomonas* species³.

Newer drugs like ceftazidime/avibactam, meropenem/vaborbactam, ceftolozane/tazobactam, imipenem/relebactam, cefoperazone/sulbactam, eravacycline, cefiderocol and plazomicin are used for treating infections caused by multidrug resistant gram-negative organisms³.

Ceftolozane/tazobactam belongs to cephalosporins used in combination with beta-lactamase inhibitor tazobactam in 2:1 ratio. The mechanism of action of ceftolozane is it inhibits

penicillin binding proteins. When ceftolozane used in combination with tazobactam broadens the spectrum including ESBL producers and anaerobic species. Plazomicin is a semisynthetic derivative of sisomicin and is effective against the strains that are amikacin or gentamicin resistant. It is bactericidal against ESBL producing pathogens, fluoroquinolone, aminoglycoside-resistant, Ampc cephalosporinases producing gram negative bacteria. Plazomicin is not effective against proteus species⁷.

Beta-lactamase inhibitor Avibactam impedes activities of several beta-lactam hydrolyzing enzymes and is used in combination with ceftazidime and is effective against ESBL, AmpC and carbapenem-resistant isolates⁷. MDR infections can be controlled by implementation of antibiotic stewardship program, limiting irrational use of antibiotics.⁸ bacteriophage therapy, combination antibiotic therapy, antimicrobial peptide therapy, photodynamic therapy are effective in counteracting drug resistance mechanism in multidrug gram-negative bacteria.⁹ AWaRe categorization by WHO classifies the antibiotics into Access, Watch, and reserve groups for avoiding irrational drug use.¹⁰

Objectives:

To know the trends of multidrug resistant gram-negative bacteria from the samples and their antibiotic susceptibility pattern.

Materials and methods:

Source of data: Clinical samples like blood, pus, urine, sputum etc received at Department of Microbiology, JJMMC Davangere from CG hospital, Bapuji hospital Davangere.

Study design: Cross sectional study

Sample size and study period: Totally 1386 gram-negative bacterial isolates obtained from non-repetitive samples of different patients with infectious diseases collected over a period of six months.

Place of study: Department of Microbiology JJMMC Davangere

Method of collection of data:

Inclusion criteria:

Different non-repetitive clinical samples collected from patients attending Bapuji hospital, CG hospital OPD and inpatient department including both the genders and all age groups that were referred to Department of Microbiology.

Exclusion criteria:

Pregnant women, cancer patients, and immunodeficient patients.

Procedure:

Under aseptic precautions samples are collected by skilled staff and postgraduate. Samples are collected in sterile containers and then sent to microbiology department laboratory for further processing. Samples included are blood, urine, stool, sputum, body fluids and others. Samples are processed in the microbiology laboratory following standard procedure.

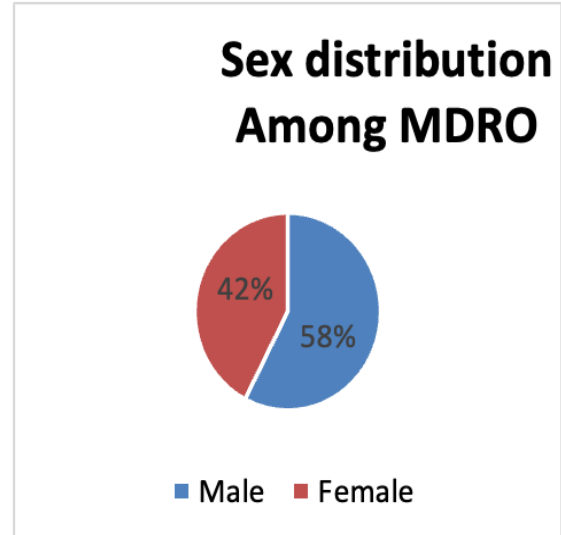
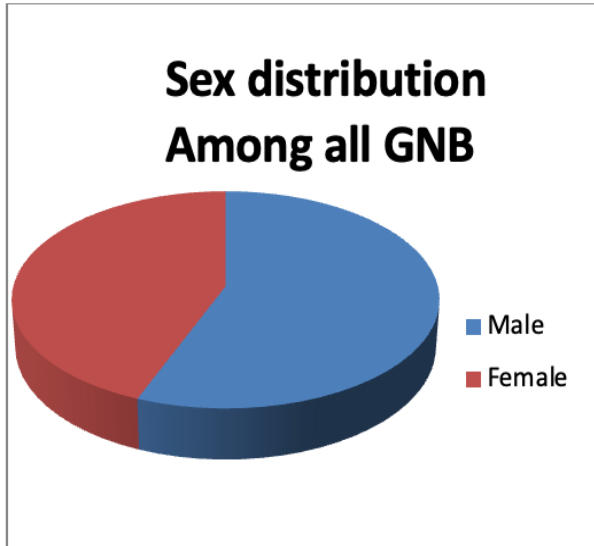
1. CLED media is used for inoculation of urine sample.
2. XLD agar used for stool samples.
3. MacConkey agar, blood agar, BHI broth used for inoculation of samples like sputum, pus, body fluids etc.
4. Blood samples are collected in Bactalert bottles following standard procedure.
5. Bactalert bottles with beep sound and green light flash are taken up for processing.
6. Further sub cultures are made to identify the morphology of the bacterial colonies.
7. Growth of pathogenic organism on MacConkey agar and blood agar and other solid media are identified using vitek-2 compact machine.
8. Only gram-negative bacteria are identified and further processed by emulsifying the colonies with saline to get 0.5-0.6 McFarland turbidity. Identification tubes as well as AST tubes will be used and processing done using standard procedure techniques and following vitek-2 kit instructions.
9. Vitek-2 compact system from BioMerieux consists of identification cards present in several wells. These identification cards are different for gram positive and gram-negative bacteria. Gram negative cards are used for further processing and AST cards

for gram negative bacteria are used. Gram positive bacterial isolates are not included.

10. These cards are placed in tubes in proper fashion that is ID cards are placed in ID tubes and AST cards placed in AST tubes.
11. Further these are placed in the machine for further processing and antimicrobial susceptibility pattern of bacterial colonies are obtained.

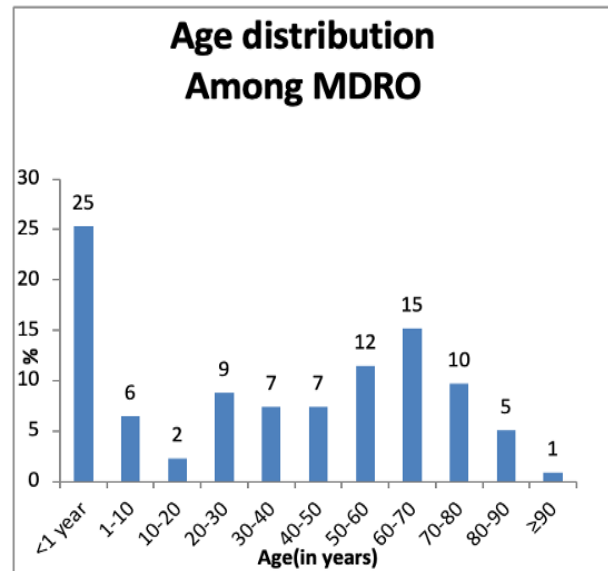
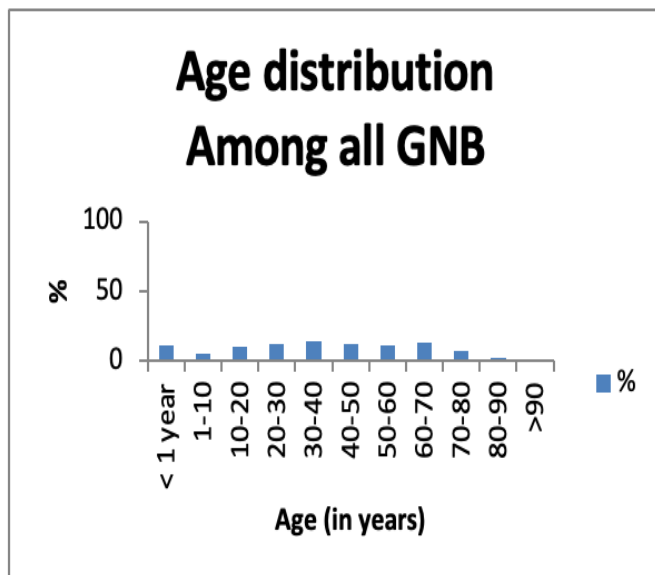
RESULTS:

In our study 1386 gram-negative bacteria were isolated. Out of which 217 were multi drug gram negative isolates. Among 1386 GNB 56% were isolated from male patients and 44% were isolated from female patients. Among multidrug strains 58% were isolated from male and 42% from females.



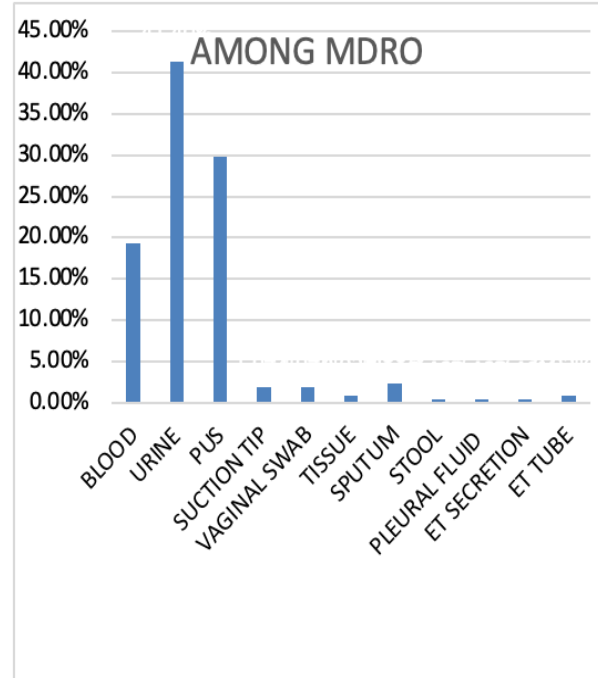
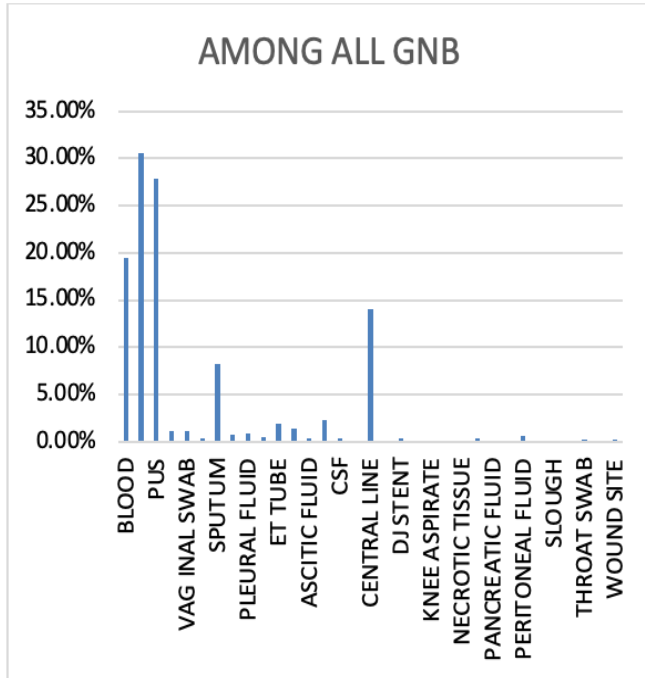
Age distribution:

In present study highest prevalence of gram-negative infections were seen among the age group 60-70 years and in multi drug resistant strains highest prevalence of infection was among age group less than one year.



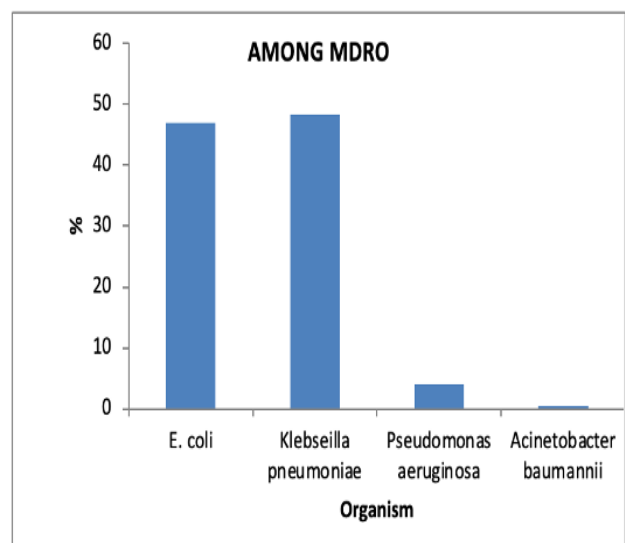
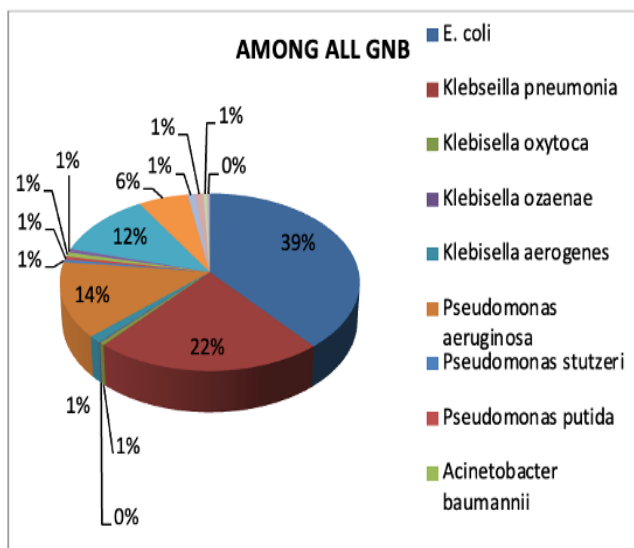
Sample distribution:

In present study urine 425 (30.6%) followed by Pus387 (27.9%) are the most frequent pathogens from 1386 gram-negative clinical isolates. In case of MDRO urine 90 (41.4%) followed by Pus 65 (29.9%) are the most frequent pathogens.



Organism distribution:

Among 1386 GNB Escherichia-coli 541(39.03%) and Klebsiella pneumoniae 308 (22.22%) were the most frequent pathogens. Among multi drug resistant strains Klebsiella pneumoniae 105 (48%) followed by Escherichia coli 102 (47%), Pseudomonas aeruginosa 9 (4%).



Sensitivity pattern of all gram-negative bacteria isolated in the study:

Table1: sensitivity pattern of klebsiella pneumoniae

	Klebsiella pneumoniae (n=203)
	Sensitive
Amikacin	87(42.8%)
Nitrofurantoin	38(18.7%)
Imipenem	79(38.9%)
Meropenem	81(39.9%)
Gentamicin	49(24.1%)
Ciprofloxacin	42(20.6%)
Tigecycline	153(75.3%)
Fosfomycin	147(72.4%)

Table 2: sensitivity pattern of Salmonella typhi

	Salmonella typhi (n=4)
	Sensitive
Amikacin	4 (40%)
Imipenem	3(75%)
Meropenem	3(75%)
Tigecycline	4(100%)
Fosfomycin	4(100%)
Cefoperazone/ Sulbactam	2 (50%)
Pipercillin tazobactam	3(75%)

Table3: sensitivity pattern of Escherichia coli

	Escherichia coli (n=439)
	Sensitive
Amikacin	283(64.4%)
Fosfomycin	403(91.7%)
Nitrofurantoin	331(75.3%)
Meropenem	346(78.8%)
Pipercillin tazobactam	213(48.5%)
Tigecycline	397(90.4%)
Trimethoprim sulfamethoxazole	248(56.4%)

Table 4: sensitivity pattern of *Acinetobacter baumannii* complex

	Acinetobacter baumannii cplx (n=166)
	Sensitive
Amikiacin	56(33.7%)
Imipenem	29(17.4%)
Meropenem	32(19.2%)
Gentamicin	48(28.9%)
Minocycline	73(43.9%)
Levofloxacin	23(13.8%)
Cefoperazone/ Sulbactam	61(36.7%)
Pipercillin tazobactam	29(17.4%)
Trimethoprim sulfamethoxazole	53(31.9%)

Table5: Sensitivity pattern of *Enterobacter cloacae* cplx

	(Enterobacter cloacae cplx n=83)
	Sensitive
Amikiacin	26(31.3%)
Imipenem	42(50.6%)
Meropenem	53(63.8%)
Tigecycline	51(61.4%)
Cefoperazone/ Sulbactam	38(45.7%)
Pipercillin tazobactam	22(26.5%)
Trimethoprim sulfamethoxazole	49(59%)

Table 6: sensitivity pattern of *pseudomonas aeruginosa*.

	Pseudomonas (n=175)
	Sensitive
Amikiacin	93(53.1%)
Imipenem	37(21.1%)
Meropenem	42(24%)
Fosfomycin	102(58.2%)
Tigecycline	27(15.4%)
Gentamicin	41(23.4%)
Cefoperazone/ Sulbactam	58(33.1)
Pipercillin tazobactam	38(21.7%)

Table 7: antibiotic susceptibility pattern of *Escherichia coli* (MDRO)

	Ecoli(n=102)		
	Sensitive	Intermediate	Resistant
Amikiacin	9(8.24)	1(0.98)	92(90.78)
Imipenem	1(0.98)	0(0)	101(99.02)
Meropenem	1(0.98)	0(0)	101(99.02)
Gentamicin	0(0)	0(0)	102(100)
Fosfomycin	89(67.25)	2(1.96)	11(30.79)
Colistin	0(0)	70(68.63)	32(31.37)
Minocycline	2(1.96)	1(0.98)	99(97.06)
Levofloxacin	1(0.98)	0(0)	101(99.02)
Cefoperazone	0(0)	0(0)	102(100)
Nitrofurantoin	12(11.76)	8(7.84)	82(80.4)
Norfloxicin	0(0)	0(0)	102(100)
Pipercillin tazobactam	0(0)	0(0)	102(100)
Cefuroxin	0(0)	0(0)	102(100)
Cefixime	0(0)	0(0)	102(100)
Cefoxitin	0(0)	0(0)	102(100)
Tigecycline	64(62.75)	0(0)	38(37.25)
Co-trimoxazole	16(15.69)	0(0)	86(84.31)

Table8: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* (MDRO)

	Klebsiella pneumonia(n=105)		
	Sensitive	Intermediate	Resistant
Amikiacin	1(0.95)	1(0.95)	103(98.1)
Imipenem	0(0)	1(0.95)	104(99.05)
Meropenem	0(0)	0(0)	105(100)
Gentamicin	0(0)	0(0)	105(100)
Fosfomycin	56(53.33)	26(24.76)	23(21.91)
Colistin	0(0)	0(0)	105(100)
Minocycline	1(0.95)	1(0.95)	103(98.1)
Levofloxacin	0(0)	0(0)	105(100)
Cefoperazone	1(0.95)	0(0)	104(99.05)
Nitrofurantoin	2(1.9)	2(1.9)	101(96.2)
Norfloxicin	1(0.95)	0(0)	104(99.05)
Pipercillin tazobactam	1(0.95)	0(0)	104(99.05)
Cefuroxin	0(0)	0(0)	105(100)
Cefixime	0(0)	0(0)	105(100)
Cefoxitin	0(0)	0(0)	105(100)
Tigecycline	53(50.48)	12(11.43)	40(38.09)
Co-trimoxazole	8(7.62)	0(0)	97(92.38)

Table 9: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (MDRO)

	<i>Pseudomonas aeruginosa</i>(n=9)		
	Sensitive	Intermediate	Resistant
Amikacin	0(0)	0(0)	9(100)
Imipenem	0(0)	0(0)	9(100)
Meropenem	0(0)	0(0)	9(100)
Gentamicin	0(0)	0(0)	9(100)
Fosfomycin	1(11.11)	2(22.22)	6(66.67)
Colistin	0(0)	7(77.78)	2(22.22)
Minocycline	0(0)	0(0)	9(100)
Levofloxacin	0(0)	0(0)	9(100)
Cefoperazone	0(0)	0(0)	9(100)
Nitrofurantoin	0(0)	0(0)	9(100)
Norfloxacin	0(0)	0(0)	9(100)
Pipercillin tazobactam	0(0)	0(0)	9(100)
Cefuroxin	0(0)	0(0)	9(100)
Cefixime	0(0)	0(0)	9(100)
Cefoxitin	0(0)	0(0)	9(100)
Tigecycline	0(0)	0(0)	9(100)
Co-trimoxazole	0(0)	0(0)	9(100)

Table10: Antibiotic susceptibility pattern of *Acinetobacter baumannii*(MDRO)

	<i>Acinetobacter baumannii</i>(n=1)		
	Sensitive	Intermediate	Resistant
Amikacin	0(0)	0(0)	1(100)
Imipenem	0(0)	0(0)	1(100)
Meropenem	0(0)	0(0)	1(100)
Gentamicin	0(0)	0(0)	1(100)
Fosfomycin	0(0)	0(0)	1(100)
Colistin	0(0)	1(100)	0(0)
Minocycline	1(100)	0(0)	0(0)
Levofloxacin	0(0)	0(0)	1(100)
Cefoperazone	0(0)	1(100)	0(0)
Nitrofurantoin	0(0)	0(0)	1(100)
Norfloxacin	0(0)	0(0)	1(100)
Pipercillin tazobactam	0(0)	0(0)	1(100)
Cefuroxin	0(0)	0(0)	1(100)
Cefixime	0(0)	0(0)	1(100)
Cefoxitin	0(0)	0(0)	1(100)
Tigecycline	0(0)	0(0)	1(100)
Co-trimoxazole	0(0)	0(0)	1(100)

Discussion:

In the present study 217 multi drug resistant gram-negative bacteria were isolated. Among these strains 58% were isolated from male and 42% from female patients. In another study conducted by Nicholas Agyepong *et al*² at Komfo Anokye Teaching Hospital in Ghana 50.5% were isolated from male and 49.5% were isolated from female patients. The present study showed highest prevalence of gram-negative infections among the age group 60-70 years and multi drug resistant strains highest prevalence of infection was among the age group less than one year. In another study by Nicholas Agyepong *et al*² highest prevalence of infections were among age group > or equal to 60 years. Sample distribution showed highest proportion of isolates from urine (41.40%), followed by pus (29.90%) followed by blood (19.30%) among all gram-negative isolates and urine (30.60%) followed by pus (27.90%) followed by blood (19.50%) among MDRO. Among pathogens isolated *Escherichia coli* 541(39.03%) followed by *Klebsiella pneumoniae* 308 (22.22%) were the frequent pathogens among all gram-negative isolates. *Klebsiella pneumoniae* 105 (48%) followed by *Escherichia coli* 102 (47%) among MDRO. In another study by Nicholas Agyepong *et al*² *Escherichia coli* was the most frequent pathogen 49 (24.5%) followed by *Pseudomonas aeruginosa* 39 (19.5%). Samples distribution showed urine samples 94 (47%) followed by wound swabs 45 (22.5%). In the present study *E. coli* (MDRO) showed highest sensitivity to Fosfomycin (67.25%) and 100% resistance to gentamicin, cefperazone, norfloxacin, piperacillin tazobactam, cefuroxin, cefixime and cefoxitin. *Klebsiella pneumoniae* (MDRO) showed highest sensitivity to Fosfomycin (53.33%) and highest resistance to Cefoxitin, cefixime, cefuroxin, levofloxacin, colistin, gentamicin and meropenem (100%). *Pseudomonas aeruginosa* (MDRO) showed highest sensitivity to Fosfomycin (11.11%) and highest resistance that is 100% resistance to all the drugs in the chart except colistin (22.22%) and fosfomycin (66.67%). In another study by

Nicholas Agyepong *et al*² isolates showed highest resistance to ampicillin (94.4%), trimethoprim/sulfamethoxazole (84.5%), cefuroxime/axetil (80.0%) cefuroxime (79.0%), cefotaxime (71.3%), cefoxitin (57.5%) and least resistant to ertapenem (1.5%).

Conclusion:

Study shows increased prevalence of multidrug resistant strains of *Klebsiella pneumoniae* and *Escherichia coli*. Infections were highest among age group less than one year. Implementation of hospital infection control practices, antimicrobial stewardship and proper surveillance will help in limiting the emergence and spread of drug-resistant strains.

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