EVALUATION OF CLINICALLY IMPORTANT GRAM-NEGATIVE ISOLATES (ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE, PSEUDOMONAS AERUGINOSA) AND THEIR SUSCEPTIBILITY PATTERN IN TERTIARY CARE HOSPITAL: 3 YR PROSPECTIVE STUDY

Ram Murgan Navaneethkrishnan¹, Sugumari Chandrasegaran²

¹Senior Assistant Professor, Department of Microbiology, Institute of Microbiology, Madurai Medical College, Madurai.

²Senior Assistant Professor, Department of Microbiology, Institute of Microbiology, Madurai Medical College, Madurai.

ABSTRACT

AIM

1. To analyse the clinically important Gram-negative isolates (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and their susceptibility pattern in a tertiary care hospital; 2. To study the epidemiology of the isolates and sensitivity pattern of the Gram negative bacteria in a tertiary care hospital, Madurai, South Tamilnadu.

METHODS

Samples collected from different wards were subjected for culture, isolation and identification of Gram negative bacterial isolates and antibacterial susceptibility testing by Vitek System and Kirby Bauer method. This study was carried out in Apollo Speciality Hospital, Lab Services, Madurai.

RESULTS

6900 non-repetitive isolates from 29,275 patients were collected from urine, blood, pus and respiratory samples from January to December 2013-2015. The Gram Positive Cocci and Gram Negative bacterial isolates were 2357 and 4543, respectively. Urine (52%), blood (7%), respiratory samples (21%) and pus (20%) accounted for the samples from which these samples were obtained. Most common isolates in urine were *E. coli* (69%) and *Klebsiella* species (16%). Most common isolates in blood were *E. coli* (38%), *Klebsiella* species (25%) and *Salmonella* (13%). Most common isolates in respiratory samples were *Klebsiella* species (44%), *Pseudomonas* (28%) and *E. coli* (16%). Most common isolates in pus samples were *E. coli* (32%), *Klebsiella* species (23%) and *Pseudomonas* (27%). *E. coli*, *Klebsiella* species, *Pseudomonas* from urine, blood, respiratory samples, pus showed high sensitivity to Carbapenems, Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides. *E. coli* showed high sensitivity to Nitrofurantoin in urine. *E. coli*, *Klebsiella* species, *Pseudomonas* from urine, blood, respiratory samples and pus showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimeth/Sulfonamides. Overall, ESBL strains increased from 64% to 66.3% in 2014, then decreased to 63.7% in 2015, while Carbapenem resistant strains increased from 2.4% to 3.8%.

CONCLUSION

In this 3 years study we found that there was a progressive increase in antimicrobial resistance in isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa*, isolated from blood, urine, pus and respiratory culture specimens. ESBL production was seen in the majority of isolates of *E. coli* and *K. pneumoniae*. These bacteria showed high sensitivity to Carbapenem, Piperacillin, Tazobactam and Cefoperazone Sulbactam.

KEYWORDS

Escherichia Coli, *Klebsiella Pneumoniae*, *Pseudomonas Aeruginosa*, Antimicrobial Resistance.

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INTRODUCTION/BACKGROUND

In the last three years, the frequency and spectrum of antimicrobial-resistant infections have increased in hospitals. Certain infections that are essentially untreatable have begun to occur as epidemics, both in the developing world and in institutional settings. This resulted in increased morbidity, mortality and health care costs. Enterobacteriaceae are common pathogens responsible for urinary tract infections, abdominal infections and hospital-acquired pneumonia.^{1,2}

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Corresponding Author:

Dr. Ram Murgan Navaneethkrishnan,

#14/2, Siva Sakthi Nagar,

K. Pudur,

Madurai-625007.

E-mail: rammdo@yahoo.co.in

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ESBLs are enzymes that mediate resistance to extended-spectrum (Third generation) Cephalosporins (e.g. Ceftazidime, Cefotaxime and Ceftriaxone) and Monobactams (e.g. Aztreonam), but do not affect Cephamycins (e.g. Cefoxitin and Cefotetan) or Carbapenems (e.g. Meropenem or Imipenem).

The prevalence of ESBL-producing bacteria have been gradually increasing in tertiary care hospitals according to a 2014 study by Joseph T. Spadafino et al.³ ESBL-producing *Escherichia coli* was 69.4% in India. Concern has arisen in recent years over increasing rates of resistance to Carbapenems, as there are few therapeutic options for treating infections caused by Carbapenem-resistant bacteria.⁴ Indian single centre studies showed a prevalence of Carbapenem resistance was 12-15% of Enterobacteriaceae.⁵

Hence, the present study was conducted in Apollo Speciality Hospitals, Madurai, South India from January to December 2013-2015. The purpose of the study was to find out the prevalence of ESBL producers and Carbapenem resistance from the clinical isolates of Gram negative bacteria and also their susceptibility to commonly used antibiotics.

Antibiotics are tested according to CLSI guidelines and our own Antibiotic testing Policy.

MATERIALS AND METHODS

Type of Study: Cross-sectional, observational, prospective study.

Settings: The study was conducted at Apollo Speciality Hospitals, Lab Services, a tertiary care hospital at Madurai.

Sample Size: 6900 isolates received from various patients' Samples (29, 275) with different Pathological condition.

Study Period: From January to December 2013-2015

Inclusion Criteria: Patients without any antibiotic treatment.

Exclusion Criteria: Patients already on antibiotic treatments.

METHODOLOGY

The antimicrobial susceptibility patterns of 6900 non-repetitive isolates from 29,275 was studied. Samples such as Blood, Urine, Pus, Respiratory samples were collected from patients attending as outpatients and in different clinical departments, respective in patient wards and intensive care units by observing standard specimen collection procedure and aseptic precautions. The Gram Positive Cocci and Gram Negative bacterial isolates were 2357 and 4543. The clinically important Gram-negative isolates (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and their susceptibility pattern was studied. Blood Samples were processed with Bactec System. Growth identified in Bactec system was streaked in MacConkey Agar and Blood Agar medium.

The other samples (Urine, pus, respiratory samples) were transported to the lab and streaked on MacConkey Agar and Blood Agar medium. After incubation for 18-24 hrs. at 37°C, the culture plates were examined and bacterial isolates were observed by Gram Staining and motility test. The identification of bacteria was performed by standard protocol by observing MacConkey plates for Lactose/Non- lactose fermenters.

VITEK 2 was used to identify the bacteria and its susceptibility pattern. Antibiotics not included in Vitek 2 were processed by the disc diffusion method (Modified Kirby-Bauer method) using Mueller-Hinton agar.

The results were interpreted as per CLSI (Clinical and Laboratory Standards Institute) guidelines corresponding to that period.^{6,7}

All antibiotic discs were obtained from Oxoid (Oxoid Ltd., Altrincham, Cheshire, United Kingdom) and BD BBL (Becton, Dickinson and Company Ltd., Franklin Lakes, New Jersey, USA).

Extended-Spectrum Beta-Lactamase (ESBL) Screening was calculated with resistance to 3rd generation group of antibiotics (Cefotaxime, Ceftriaxone and Aztreonam) for Enterobacteriaceae.

Carbapenem resistance was calculated with resistance to Carbapenems (Imipenem and Meropenem, Doripenem and Ertapenem) group of antibiotics for Enterobacteriaceae. Interpretation based on VITEK COMPACT 2 as per CLSI guidelines.⁶

RESULTS

	2013	2014	2015	Total
No. of samples processed	8955	9628	10692	29275
No. of culture positive - Gram Positive Cocci	1266	518	573	2357 (35%)
No. of culture positive - Gram Negative bacilli	1712	1535	1296	4543 (65%)
Total				6900 (24%)
Table 1: Total Number of Bacterial Isolates from Samples (2013-2015) n = 6900				

Table 1 showed, out of 29,275 samples, 6900 (24%) isolates were obtained. The Gram Positive Cocci (35%) and Gram Negative bacterial isolates (65%) were 2357 and 4543. Conventional methods were used till 2013. Vitek 2 was used from 2014 onwards.

Number of Gram Negative Bacterial Isolates - Sample-Wise n = 4543																					
Isolates	Urine					Blood					Respiratory					Pus					Total
Year	2013	2014	2015	Total	%	2013	2014	2015	Total	%	2013	2014	2015	Total	%	2013	2014	2015	Total	%	
E. coli	655	522	459	1636	69	34	34	39	107	38	67	45	43	155	16	111	88	94	293	32	2191
Klebsiella Species	172	125	95	392	16	16	33	22	71	25	97	244	84	425	44	76	59	73	208	23	1096
Pseudomonas	83	55	59	197	9	16	7	7	30	11	112	101	60	273	28	71	89	88	248	27	748
Proteus Species	27	26	7	60	2.5	1	0	0	1	0	4	3	2	9	1	17	19	23	59	7	129
Citrobacter Species	39	10	16	65	2.5	11	0	0	11	4	33	4	1	38	4	18	5	6	29	4	143
Enterobacter	0	7	7	14	0.5	0	3	7	10	4	0	5	12	17	2	0	8	17	25	2	66
Acinetobacter	3	1	2	6	0	2	2	5	9	3	7	15	27	49	4	4	10	17	31	3	95
Serratia	1	2	6	9	0.5	0	2	1	3	1	0	0	3	3	0	0	3	5	8	2	23
Salmonella	0	0	0	0	0	23	7	7	37	13	0	0	0	0	0	0	0	0	0	0	37
Burkholderia	0	0	0	0	0	1	1	1	3	1	0	0	0	0	0	0	0	0	0	0	3
Non-fermenter	4	0	0	4	0	0	0	0	0	0	5	0	0	5	1	2	0	0	2	0	12
	984	748	651	2383	100	104	89	89	282	100	325	417	232	974	100	0	281	323	903	100	4543
Table 2: Number of Gram Negative Bacterial Isolates from Samples (2013-2015) n = 4543																					

Table 2 showed, the following isolates were isolated from urine, blood, pus and respiratory samples were 52%, 7%, 20%, 21%. Most common isolates in urine were *E. coli* (69%) and *Klebsiella* species (16%). Most common isolates in blood were *E. coli* (38%), *Klebsiella* species (25%) and *Salmonella* (13%). Most common isolates in respiratory samples were *Klebsiella* species (44%), *Pseudomonas* (28%) and *E. coli* (16%). Most common isolates in pus samples were *E. coli* (32%), *Klebsiella* species (23%) and *Pseudomonas* (27%).

Antibiotics	E. coli (%)			Klebsiella Species (%)			Pseudomonas (%)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Carbapenems	94.5	98.5	98.3	84	90.1	88	98	100	86.6
Piperacillin/Tazobactam	84.5	92.7	83	74	72.5	57.6	94	67.9	77.7
Cefoperazone/Sulbactam	83	93.4	87.5	70	75	64.1	77	67.3	71.1
Aminoglycosides	86	95.4	93.1	79	77.3	72.8	81	88.8	66.6
Nitrofurantoin	73	74.1	79	43	67.8	39.1	0	0	0
3rd Generation Cephalosporins	34	30.6	46.4	39	29.5	30.4	63	65.9	55.5
Fluoroquinolones	33.7	34	31.6	20	36.8	23.9	45	47.9	53.3
Trimethoprim/Sulfamethoxazole	44.5	45	40.3	22	30.7	40.2	0	0	0

Table 3: Sensitivity Pattern of Antibiotics for Predominant Gram Negative Bacterial Isolates from Urine during 2013-2015

Table 3 showed, in urine samples, *E. coli*, *Klebsiella* and *Pseudomonas* showed high sensitivity to Carbapenems, Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides. *E. coli* showed high sensitivity to Nitrofurantoin. *E. coli*, *Klebsiella* and *Pseudomonas* showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimethoprim/Sulfamethoxazole.

Antibiotics	E. coli (%)			Klebsiella (%)		
	2013	2014	2015	2013	2014	2015
Carbapenems	100	100	100	100	89.9	95.8
Piperacillin/Tazobactam	92.6	89.5	87.5	77	59.9	58.3
Cefoperazone/Sulbactam	91.1	85.5	87.5	61	59.9	58.3
Aminoglycosides	86.9	91.6	100	62	81.2	75
3rd Generation Cephalosporins	29.5	28.6	47.5	41	32.6	50
Fluoroquinolones	29	26.1	37.5	50	33.1	41.6
Trimethoprim/Sulfamethoxazole	27	26.6	55	39	40	45.8

Table 4: Sensitivity Pattern of Antibiotics for Predominant Gram Negative Bacterial Isolates from Blood during 2013 to 2015

Table 4 showed, in blood samples, *E. coli* showed high sensitivity to Carbapenems, Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides. *Klebsiella* species showed high sensitivity to Carbapenems, Aminoglycosides. *E. coli* and *Klebsiella* showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimethoprim/Sulfamethoxazole.

Antibiotics	E. coli (%)			Klebsiella (%)			Pseudomonas (%)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Carbapenems	94.6	94.6	98.7	81.4	81.4	89.8	96.6	96.6	91.6
Piperacillin/Tazobactam	87.5	87.5	77.1	66.8	66.8	62.3	79.1	79.1	88
Cefoperazone/Sulbactam	82.1	82.1	78.3	67.1	67.1	62.3	78.6	78.6	85.7
Aminoglycosides	90.3	90.3	87.9	84.5	84.5	73.9	84.6	84.6	60
3rd Generation Cephalosporins	34.7	34.7	40.9	34.9	34.9	49.2	60.3	60.3	74.6
Fluoroquinolones	44.5	44.5	30.1	39.6	39.6	43.4	60.7	60.7	81.9
Trimethoprim/Sulfamethoxazole	35.6	35.6	32.5	43.1	43.1	52.1	-	-	-

Table 5: Sensitivity Pattern of Antibiotics for Predominant Gram Negative Bacterial Isolates from Pus during 2013 to 2015

Table 5 showed, in pus samples *E. coli*, *Klebsiella* and *Pseudomonas* showed high sensitivity to Carbapenems, Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides. *Pseudomonas* showed high sensitivity to Fluoroquinolones and 3rd generation Cephalosporins. *E. coli* and *Klebsiella* showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimethoprim/Sulfamethoxazole.

Antibiotics	E. coli (%)			Klebsiella (%)			Pseudomonas (%)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Carbapenems	100	93.7	100	96	92.8	100	100	95.5	98.3
Piperacillin/Tazobactam	100	81.8	85.7	92	68.6	67.8	85	83.6	89.2
Cefoperazone/Sulbactam	81	89.2	88	83	68.2	71.4	80	76.6	92.8
Aminoglycosides	84	83.3	95.2	83	89.3	91.6	80	81.2	87.5
3rd Generation Cephalosporins	38	34.3	40	57	34.9	45.2	60	53	71.4
Fluoroquinolones	13	40.9	30.9	48	62.5	44	60	74	75
Trimethoprim/Sulfamethoxazole	25	48.8	45.2	35	52.8	50	-	-	-

Table 6: Sensitivity Pattern of Antibiotics for Predominant Gram Negative Bacterial Isolates from Respiratory Specimen during 2013 to 2015

Table 6 Showed, in respiratory samples E. coli, Klebsiella and Pseudomonas showed high sensitivity to Carbapenems), Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides. E. coli and Klebsiella showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimethoprim/Sulfamethoxazole.

Chart 1: Resistant Strains

Overall, ESBL strains increased from 64% to 66.3% in 2014, then decreased to 63.7% in 2015. Overall, carbapenem resistant strains increased from 2.4% to 3.8%. Resistant strains are identified with Vitek 2 from 2014 onwards. Resistant strains are more prevalent in ICU, CCU and NICU.

DISCUSSION

This study revealed that Gram negative bacteria (65%) were more predominant than Gram positive bacteria (35%). Anitha et al⁸ has reported the incidence of 80.96% for Gram-negative and 18% for Gram-positives. Gram negative bacterias were more isolated, which was correlated to present study.

In this study, most common isolates in urine were E. coli (69%) and Klebsiella species (16%), Namratha W Nandihal et al⁹ showed Escherichia coli was the most commonly isolated bacteria and constituted 54.5%, which was followed by Klebsiella species of 21.5%. This study supports the findings of Namratha W Nandihal et al.

In this study, most common isolates in blood were E. coli (38%), Klebsiella species (25%) and Salmonella (13%). Shamim Mumtaz et al¹⁰ showed in blood samples the most common organism isolated was E. coli followed by Salmonella and Klebsiella. This observation was similar to this study.

In this study, the most common isolates in respiratory samples were Klebsiella species (44%), Pseudomonas (28%) and E. coli (16%). Manikandan et al¹¹ showed the prevalence of bacteria spp. isolated were as follows: Klebsiella pneumoniae (38.4%), Pseudomonas aeruginosa (21%) and Escherichia coli (10%) which correlates with the present study.

In this study E. coli, Klebsiella species, Pseudomonas from urine, blood, respiratory samples, pus showed high sensitivity to Carbapenems, Piperacillin/Tazobactam, Cefoperazone/Sulbactam and Aminoglycosides.

This is in concordance with the study of Dinesh Kumar et al¹² which showed analysis of antimicrobial susceptibility pattern of ESBL producing E. coli isolates from urine and blood demonstrated high susceptibility rates to Imipenem (100%, 100%).

Piperacillin/tazobactam (80.48, 70%), Cefoperazone/sulbactam (70.73, 80%) and Aminoglycosides such as Amikacin (73.17, 60%) and Gentamicin (68.29, 60%), respectively. Al-Zarouni et al¹³ also demonstrated higher susceptibility rates to Carbapenems and Amikacin. Kutbettin Demirda et al¹⁴ showed 100% sensitive to Imipenem and Meropenem among E. coli and Klebsiella spp. was detected. These observations were highly correlated with us.

In this study, in all samples E. coli isolates showed resistance to 3rd generation Cephalosporins, Fluoroquinolones and Trimeth/Sulfonamides. Marwa E.A. Aly et al¹⁵ showed 81-88% of MDR E. coli were resistant to Tetracycline, Fluoroquinolones and Sulfonamide/ Trimethoprim. This observation is similar to our study.

In this study, in all samples Klebsiella isolates showed resistance to 3rd generation Cephalosporins, Trimeth/Sulfonamides and Fluoroquinolones. Philippon A and Babini GS et al¹⁶ showed that the ESBL Klebsiella was resistant to third generation Cephalosporins as well as the Aztreonam and Fluoroquinolones. This finding correlates with our study.

In this study in all samples Pseudomonas isolates showed resistance to Aminoglycosides, 3rd generation Cephalosporins and Fluoroquinolones. Satyajeet K. Pawar et al¹⁷ reported that around 70% of Pseudomonas strains were resistant to Aminoglycosides, Fluoroquinolones and 3rd generation Cephalosporins. This study highly correlates with our study.

In this study overall ESBL strains increased from 64% to 66.3% in 2014, then decreased to 63.7% in 2015. Mathai D et al¹⁸ noted that the prevalence of ESBLs in India has now reached epidemic proportions, ranging from 62% to 100% in E. coli and Klebsiella spp. isolated from skin and soft tissue infections, blood stream infections and respiratory infections as observed in the 10 Indian medical centre SENTRY study. Our study highly correlates with Indian standards.

High percentage of ESBL producing *E. coli* has been reported with 60% in a study done at a tertiary care hospital in Chennai.¹⁹ These findings correlates with our study.

In this study, overall Carbapenem resistant strains increased from 2.4% to 3.8%. Camilla Rodrigues²⁰ reported that a recent pilot study from a tertiary care centre in central Mumbai that receives referral samples from the city and the State found a steady increase in Carbapenem resistance in Enterobacteriaceae from 0% in 2006 to 8% in 2009.

CONCLUSION

In this 3 years study, we found that there was a progressive increase in antimicrobial resistance in isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolated from blood, urine, pus and respiratory culture specimens. ESBL production was seen in the majority of isolates of *E. coli* and *K. pneumoniae*. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed high sensitivity to Carbapenem and Piperacillin Tazobactam and Cefoperazone Tazobactam. Hence, for rational and judicious use of existing antibiotics, the Clinicians and Paramedical have to be sensitised with suitable awareness programmes and the present scenario to be brought to the notice of policy makers to formulate the policy and legislation for rational use of Antibiotics.

LIMITATIONS

This is a single centred study.

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