WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 2411-2425. Research Article ISSN 2277– 7105

EVALUATION OF RESISTANCE TO CARBAPENEM BY DISC DIFFUSION METHOD AND SUSCEPTIBILITY TO POLYMYXIN-B USING E-STRIP IN ENTEROBACTERIACEAE

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Article Received on
20 June 2017,

Revised on 10 July 2017,
Accepted on 30 July 2017

DOI: 10.20959/wjpr20178-9189

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ABSTRACT

Although this family of enterobacteriaceae includes more than 70 genera, the health-care associate, most commonly found in this current study were Escherichia coli, Klebsiella species, Enterobacter, Salmonella, Enterobacter and Proteus species. Isolates were inoculated in Muller Hinton Agar, antibiotic and polymyxin-B E-strip were applied and incubated at 37 degrees Celsius the inhibition zone size were read after overnight of incubation and compared to antimicrobial chart of NCCLs. The present study showed that in 119 isolates samples 10(8%) of them showed Expended Spectrum Beta lactamase production which cause resistance to 3rd generation cepharosporin antibiotics, and all 10 isolates were from Escherichia. Coli strain, none of Klebsiella, Proteus, Citrobacter, Salmonella or Enterobacter species showed ESBLs production. The present study also showed that in 119 isolated Enterobacteriaceae samples 25(21%) of them showed resistance to carbapenem antibiotics, within 25 carbapenem resistant Enterobacteriaceae isolates, 9(36%) were E. coli whereas 16(64%) were Klebsiella. The present study also showed that all carbapebem resistant Enterobacteriaceae isolates were susceptible to polymyxin-B antibiotic. Given the fact that the prevalence of CRE was increasing during the past decades, it is urgent for us to establish regional surveillance worldwide, carry out more effective antibiotic stewardship and infection control measures to prevent further spread of carbapenem resistance in Enterobacteriaceae. Health departments

should be well positioned to play a leading role in prevention, efforts by assisting with surveillance, situational awareness, and coordinating prevention efforts.

KEYWORD: *Evaluation, Resistance, Carbapenem, Disc Diffusion Method, Susceptibility, Polymyxin-B, E-Strip, Enterobactericiae.*

1. INTRODUCTION

The *Enterobacteriaceae* is a heterogeneous group of gram negative rods, non-spore, non-acid fast which do not retain crystal violet dye in the gram staining method, *Enterobacteriaceae* family is an important cause of urinary tract infections (UTIs), bloodstream infections, and hospital acquired pneumonias and various intra-abdominal infections. They exhibit general morphological and biochemical similarities. This group more than 70 genera such as *Escherichia Coli, Salmonella, Shigella, Klebsiella, Proteus, Enterobacter* (**Ananthanaraya and Paniker, 2013).**

Jayoung *et al*, 2010 found that among 347 Enterobacteriaceae, 28.2% were ESBL producers and 1/347 was carbapenem resistant, **Claudia *et al.*, 2012** studied that among 120 isolates, 42% *K. pneumoniae* 40% *E. coli* resistant to polymyxin-B.

There is a registered increase in ESBLS-producing *E-coli*, from 1.1% to 10.4% in the European Center for Disease Control **(ECDC**), which showed eight and two fold increases in the percentage of ESBL-producing *E. coli* and *K. pneumoniae* isolates respectively **(*Bochichio et al., 2006*).**

The current study was conducted to isolate Enterobacteriaceae and to evaluate the prevalence of their resistance and pathogenicity in inpatient and outpatient, the study showed that among 119 isolates (10)8% was ESBL producers which made them to resist 3rd group of cephalosporins drug, (25)21% was carbapenem resistant Enterobacteriaceae which allowed them to resist carbapenem drugs, in addition *Klebsiella* showed high resistance to carbapenem drugs 64% whereas *E. coli* showed resistance of 36% to carbapenem, it is quiet surprise to found that carbapenem resistant Enterobacteriaceae are still increasing day by day.

Although antimicrobial development efforts remain a cornerstone of CRE response efforts, interventions aimed at preventing the transmission of infections with these organisms are also important. Delaying the emergence of carbapenem resistance, particularly in areas where this

resistance is still uncommon, can decrease the impact of these organisms as we await additional treatment options.

Preventing the spread is important before CRE gains a foothold in more hospitals or in the community. This requires active case detection and contact precautions for colonized or infected patients as well as cohosting of patients and staff; appropriate antibiotic use in all settings; and communication about infections when patients transfer. Since, Enterobacteriaceae are a large family of gram-negative bacilli that are normal inhabitants of the gastrointestinal tract of humans and other animals and these organisms are a common cause of community-acquired and health-care–acquired infections greater care should be made.

As per **World Health Organisation (WHO), 2010,** despite some serious efforts, mainly from industrialized countries, a definite solution to the problem still seems to be far off. Several parts of the world are already endemic for carbapenamese encoding genes while the situation in others including the central Balkans and many African and Asian countries is not well documented. Until a reliable alternative to carbapenem drugs is found or the presence of carbapenamese effectively overcome, the rationalization of antibiotic use in humans, the application of strict infection control measures whenever carbapenem resistance is detected and the active surveillance for the presence of carbapenamese encoding genes are of the outmost importance.

AIM

To evaluate the resistance to carbapenem by disk diffusion method and susceptibility to polymyxin -B using E-strip in enterobacteriaceae.

OBJECTIVES

- To determine resistance of Enterobacteriaceae to 3rd generation cephalosporins (ESBL).
- To determine the percentage of Enterobacteriaceae resistant to carbapenem (CRE).
- To determine the susceptibility of Carbapenem Resistant Enterobacteriaceae (CRE) to Polymyxin –B.

2. MATERIALS AND METHODS

Place of Research

The study was conducted in the Department of Microbiology, Nims Hospital, Rajasthan a 300 bedded tertiary care hospital.

SOURCE OF SAMPLES AND STUDY GROUP

- In this project isolates belonging to Enterobacteriaceae were collected.
- All samples were taken from inpatients and outpatient of both sex and of any ages.
- The samples include all exudative samples, respiratory samples, urine, blood, endotracheal fluid, sputum, wound swab and pus.
- All the samples yielding *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Citrobacter*, *Salmonella*, *Proteus* samples were taken into consideration.

METHODOLOGY

Antibiotic Sensitivity test

Penicillin and third generation of cephalosporin antibiotics were used for long time to kill or to treat Enterobacteriaceae, but now days some bacteria get resistant to these groups of antibiotics, for this reason carbapenems are used to treat infectious caused by these multidrug resistant bacteria.

This bacterium gets resistant to beta lactam antibiotics due to expression of beta lactamase enzyme (ESBL).

The big issue is that now days some microorganisms can produce extended spectrum beta lactamase (ESBL) and carbapenamese enzymes which help them to resist carbapenems antibiotics which was considered as senior killer for this multidrug resistant bacteria.

Muller Hinton Agar

Muller Hinton agar is considered to be the best for routine susceptibility testing of bacteria for the following raisons.

1. It is non-differential or non-selective medium; this means most of all microorganisms can grow on it.
2. It has low sulphonamide, trimethoprim and tetracycline inhibitors.
3. It has starch which is known to absorb toxin released from bacteria, so that they cannot interfere with the antibiotics.

4. It has loose agar, this allows for better diffusion which will leads to a true zone of inhibition.

PROCEDURE

1. The E-strip package was removed from the freezer (-20°C) at least 30 minutes before use.
2. 4 individual colonies were transferred to a tube of saline.
3. Compared turbidity to that in the 0.5 McFarland standards. Turbidity was adjusted to match the standard.
4. Sterile cotton swab was dipped into the inoculum and pulled out slightly, the swab was rotated several times against the inside of the tube above the fluid level to remove excess liquid. The entire surface of the agar plate was streaked over by rotating the plate approximately 60 degrees.
5. Left the lid of the plate ajar for 5 minutes to allow any excess moisture to be absorbed before applying strips.
6. E-test package was cut along the broken line. Strip was applied to agar surface using forceps.
7. The strip with the 'E end' was placed at the edge of the plate and with the scale visible (i.e. facing upward).
8. Incubate Plates at 37°C for 18-24 hrs.

NOTE

1. Read MIC at the point where the ellipse intersects the scale. If a MIC value between twofold dilutions is seen, read the MIC value at completed inhibition of all growth.

2. If the intersect differs on either side of the strip, read the MIC as the greater value, Ignore any growth at the edge of the strip.

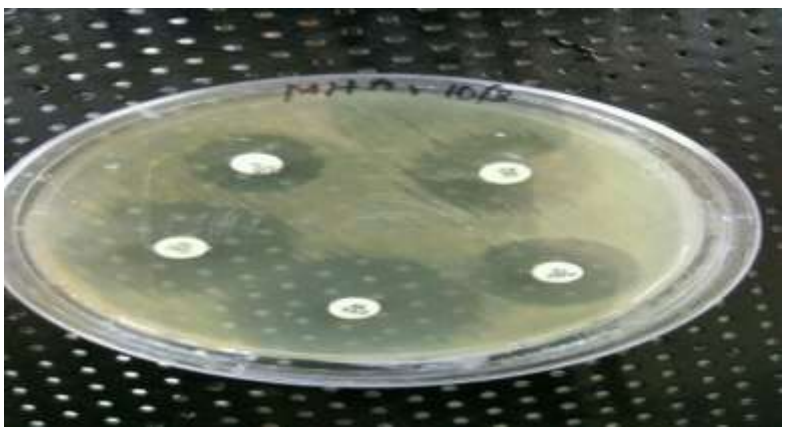

Fig 1: Mha Plates Showing Sensitivity.

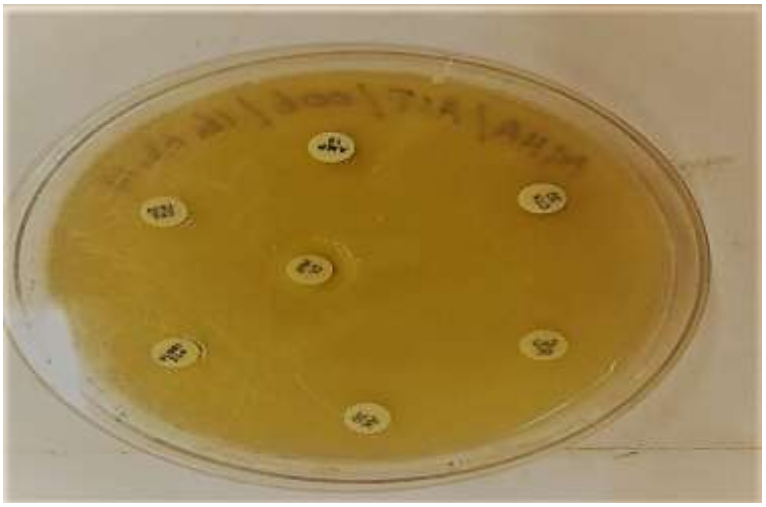

Fig 2: Mha Plates Showing Resistance.

Fig 3: Sensitive plate to Polymyxin -B, Colistin and Tigecycline.

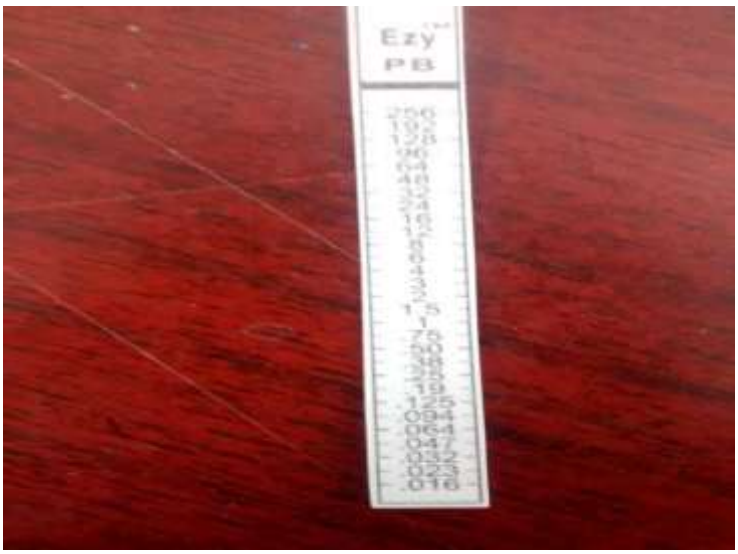

Figure 4: Polymyxin-B E-strip.

Table 1: Shows Readings of Zone of Inhibition.

S. No.	Organism	Samples	Zone Size
1	*E. coli*	Urine	14
2	*K. pneumoniae*	Urine	16
3	*E. coli*	Wound	14
4	*K. oxytoca*	Wound	12
5	*E. coli*	Urine	14
6	*K. oxytoca*	Wound	14
7	*K. pneumoniae*	Wound	14
8	*E. coli*	Urine	12
9	*E. coli*	Urine	14
10	*K. pneumoniae*	Wound	15
11	*K. pneumoniae*	Urine	14
12	*K. pneumoniae*	Urine	13
13	*E. coli*	Urine	15
14	*K.pneumoniae*	urine	13
15	*K.pneumoniae*	sputum	14
16	*E.coli*	urine	13
17	*K.pneumoniae*	urine	13
18	*K.pneumoniae*	Endotracheal fluid	14
19	*K.pneumoniae*	Endotracheal fluid	14
20	*K.pneumoniae*	wound	13
21	*E.coli*	wound	12
22	*E.coli*	Urine	14
23	*K.oxytoca*	wound	13
24	*K.pneumoniae*	Urine	12
25	*K.pneumoniae*	wound	13

Fig 5: MHA showing minimum inhibitory concentration.

3. RESULTS

Among of 119 isolated samples 73 (61%) were urine samples, 32(27%) samples were wound swab samples, 5(4%) were endotracheal fluid samples, 7(6%) were blood samples and 2(2%) were sputum samples.

Among of 119 isolated samples 57(48%) was found to be *E. coli*, *37*(31%) were *Klebsiella*, 8(7%) *Enterobacter*, *4*(3%) *Salmonella*, 7(6%) *Citrobacter*, 6(5%) *Proteus.*

According to NCCLs guidelines isolates resistant to third generation cephalosporins and are susceptible to the respective drug combination with clavulanic acid are considered as ESBLs producers Enterobacteriaceae.

During the study, among of 119 isolates 10(8%) of them showed ESBLs production. All 110 isolates were E-coli (ESBLs), and they were also 100% resistant to ampicillin, 99% resistant to cotrimaxazole, ciprofloxacin, cephotaxime and cefuroxime.

Among of 119 isolated samples 25(21%) of them showed resistance to carbapebem antibiotics. Within 25 isolates of carbapebem resistant Enterobacteriaceae 9(36%) were *E. coli* whereas 16(64%) were *Klebsiella.*

Resistance of *Klebsiella species* in this project showed that they were resistant to Imipenem 25%, Meropenem 100% and 99% to Ertapenem**.** Among of 57 *E-coli* isolates, 9(16%) of them showed resistance to carbapebem, 15% resistance to Imipenem, 26% Ertapenem and 30% to Meropenem. 4(80%) of them were resistant 100% to Ertapenem and Meropenem and 99% resistant.

SENSITIVITY TEST OF CRE TO POLYMYXIN-B RESULT

Antibiotic sensitivity test was done for the microorganisms which showed resistance to carbapenem drugs by use of disc diffusion method. According to NCCL guideline all carbapenem resistant Enterobacteriaceae in this study showed susceptibility to polymyxin-B.

According to NCCLs guideline MIC less than 2.0µg/ml is interpreted as sensitive whereas MIC greater than 2.0µg/ml is interpreted as resistant. Minimum inhibitory concentration among 25CRE isolates in this study showed that 22 CRE isolates were sensitive to Polymyxin -B and only 3 isolated CRE resisted to Polymyxin-B, where all resisted isolates were *Klebsiella* (*2 Klebsiella pneumoniae* and one *Klebsiella oxytoca*) organisms.

Table 1: Numbers of Isolated Samples

S.No	SAMPLES	NUMBERS
1	Urine	73
2	Wound	32
3	Blood	7
4	Endotracheal fluid	5
5	Sputum	2

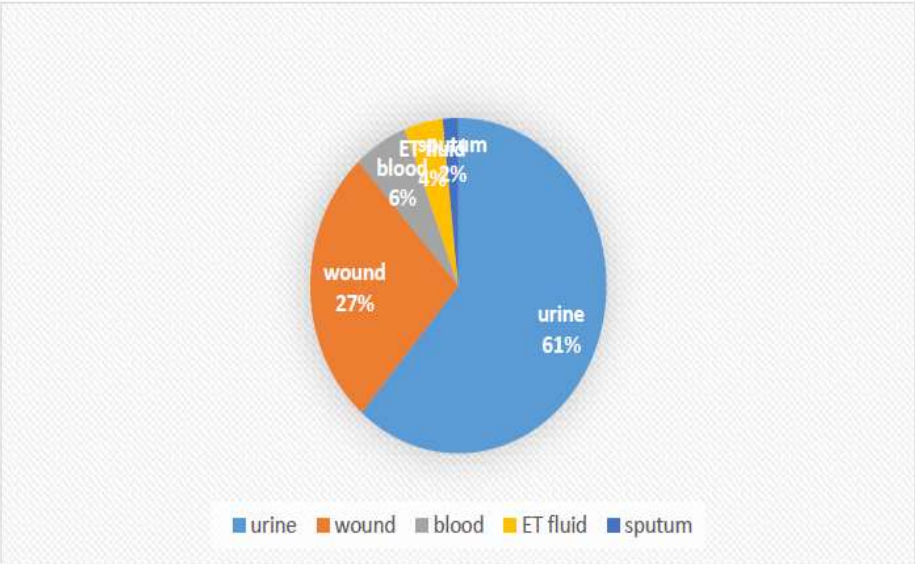

Figure 1: Percentages of isolates samples

Table 2: Number of Isolated Organism.

S.No	ISOLATES	NUMBERS
1	*E. coli*	57
2	*Klebsiella*	37
3	*Enterobacter*	8
4	*Salmonella*	4
5	*Citrobacter*	7
6	*Proteus*	6

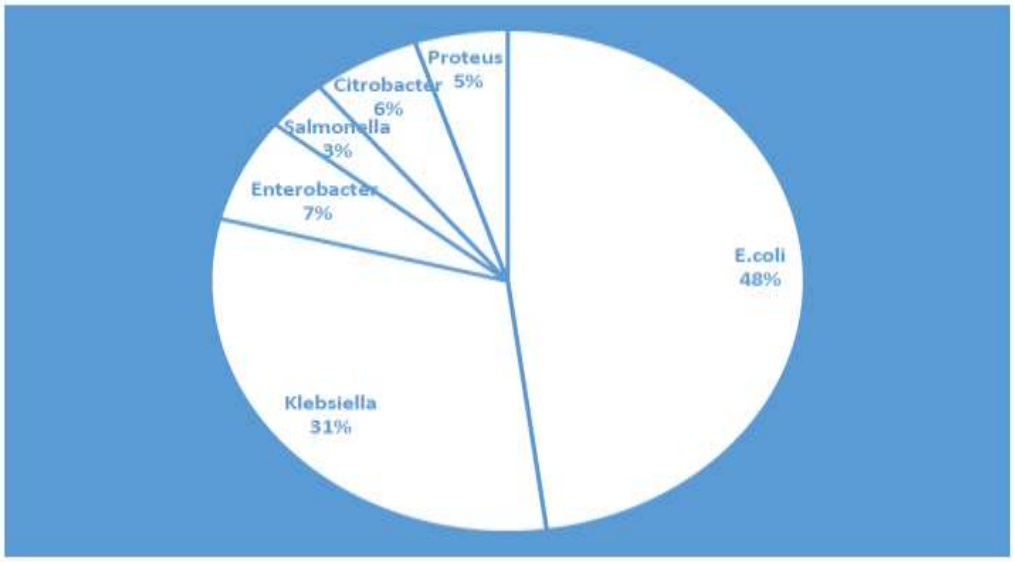

Figure 2: Percentage of isolated organisms.

Table 3: Spectrum Beta Lactamase Producers.

SNO	SAMLE	ORGANISM	AMP	CTX	CXM	PTZ	CFS	AK	CIP	NIT	COT	IMP	ETP	MRP
1	urine	*E.coli*	R	R	R	22	22	18	R	24	R	27	R	14
2	urine	*E.coli*	R	R	R	16	14	16	R	20	R	27	28	29
3	urine	*E.coli*	R	13	R	20	21	22	R	17	R	26	27	28
4	urine	*E.coli*	R	R	R	14	R	28	23	24	R	29	27	28
5	urine	*E.coli*	R	R	R	22	19	19	R	15	R	29	28	30
6	urine	*E.coli*	R	R	6	21	18	20	R	17	R	29	27	26
7	urine	*E.coli*	R	15	22	17	23	25	13	14	23	25	17	26
8	urine	*E.coli*	R	R	R	19	17	21	R	16	R	25	26	12
9	urine	*E.coli*	R	6	R	22	16	19	6	R	R	26	26	13
10	urine	*E.coli*	R	11	R	21	15	16	R	18	R	26	27	11

Table 4: Rates of Carbapenem Resistant Enterobacteriaceae Isolates.

SNO	ORGANISMS	NUMBERS
1	*E. coli*	9
2.	*Klebsiella*	16

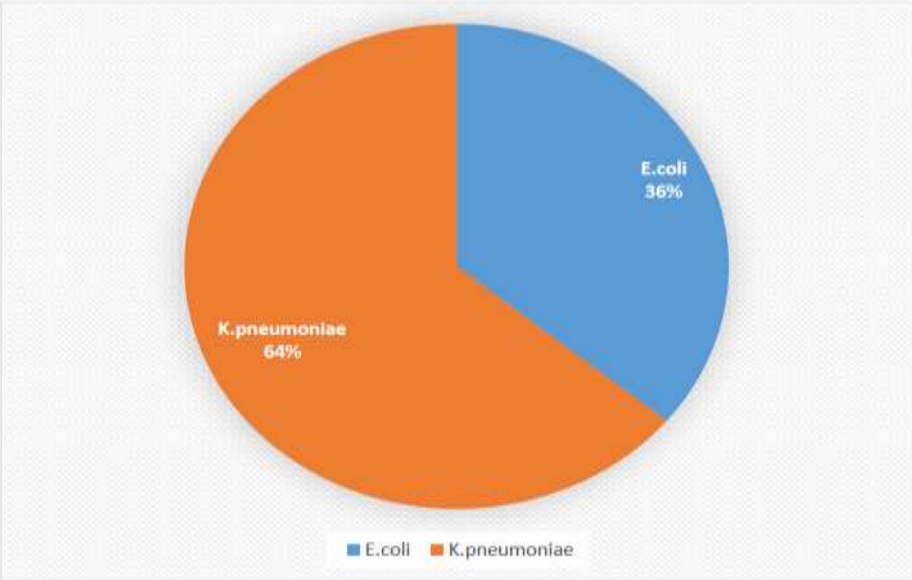

Figure 4: Carbapenem resistant organism.

Table 5: *Klebsiella* resistance to Carbapebem.

SNO	ORGANISM	IMIPENEM	ERTAPENEM	MEROPENEM
1	*Klebsiella*	R	R	R
2	*Klebsiella*	21	R	R
3	*Klebsiella*	22	R	R
4	*Klebsiella*	19	R	R
5	*Klebsiella*	16	R	R
6	*Klebsiella*	19	R	R
7	*Klebsiella*	15	R	R
8	*Klebsiella*	14	R	R
9	*Klebsiella*	R	R	R
10	*Klebsiella*	17	R	R
11	*Klebsiella*	R	R	R
12	*Klebsiella*	25	R	R
13	*Klebsiella*	R	R	R
14	*Klebsiella*	R	12	R
15	*Klebsiella*	18	R	R
16	*Klebsiella*	18	R	R

Table 6: Resistance of *E. coli* to Carbapenem

SNO	ORGANISM	IMIPENEM	ERTAPENEM	MEROPENEM
1	*E. coli*	27	R	14
2	*E. coli*	R	R	R
3	*E. coli*	26	23	R
4	*E. coli*	R	R	R
5	*E. coli*	13	R	R
6	*E. coli*	R	R	R
7	*E. coli*	14	R	R
8	*E. coli*	R	R	R
9	***E. coli***	**26**	**27**	**R**

MINIMUM INHIBITORY CONCENTRATION TEST RESULT

Table 7: Minimum Inhibitory Concentration Readings.

SNO	ORGANISMS	MIC
1	*E. coli*	1.5
2	*K. pneumoniae*	2
3	E. coli	1.5
4	*K. oxytoca*	2
5	*K. oxytoca*	1.5
6	*E. coli*	2
7	*K. pneumoniae*	1
8	*E. coli*	2
9	*E. coli*	1.5
10	*K. pneumoniae*	2
11	*K. pneumoniae*	2
12	*K. pneumoniae*	3
13	*K. pneumoniae*	1.5
14	*E. coli*	1.5
15	*K. pneumoniae*	1
16	*E. coli*	1.5
17	*K. pneumoniae*	2
18	*K. pneumoniae*	2
19	*K. oxytoca*	3
20	*K. pneumoniae*	3
21	*E.coli*	2
22	*E.coli*	1
23	*K.oxytoca*	1.5
24	*Pneumoniae*	0.75
25	*Pneumoniae*	2
23	*K.oxytoca*	1.5
24	*Pneumoniae*	0.75
25	*Pneumoniae*	2

4. DISCUSSION

Enterobacteriaceae is a family of bacteria that commonly cause infections in health-care settings as well as in the community. Among Enterobacteriaceae resistance to broad-spectrum antimicrobials is common. Over the past decade, carbapenem-resistant enterobacteriaceae

(CRE) have been recognized in health-care settings as a cause of difficult-to-treat infections associated with high mortality. **Jayoung *et al*, 2010** found that among 347 Enterobacteriaceae, 28.2% were ESBL producers and 1/347 was carbapenem resistant, **Claudia *et al.*, 2012** studied that among 120 isolates, 42% *K. pneumoniae* 40% *E. coli* resistant to polymyxin-B.

There is a registered increase in ESBLS-producing *E-coli*, from 1.1% to 10.4% in the European Center for Disease Control **(ECDC**), which showed eight and two fold increases in the percentage of ESBL-producing *E. coli* and *K. pneumoniae* isolates respectively **(*Bochichio et al., 2006*).**

The current study was conducted to isolate Enterobacteriaceae and to evaluate the prevalence of their resistance and pathogenicity in inpatient and outpatient, the study showed that among 119 isolates (10)8% was ESBL producers which made them to resist 3rd group of cephalosporins drug, (25)21% was carbapenem resistant Enterobacteriaceae which allowed them to resist carbapenem drugs, in addition *Klebsiella* showed high resistance to carbapenem drugs 64% whereas *E. coli* showed resistance of 36% to carbapenem, it is quiet surprise to found that carbapenem resistant Enterobacteriaceae are still increasing day by day.

CONCLUSION

In the present study "Evaluation of resistance to carbapenem by disk diffusion method and susceptibility to Polymyxin–B using E-strip in *Enterobactericiae*" was carried out in Nims Hospital, Rajasthan. According to the present study carbapenem can be used to treat infection caused by extended spectrum beta lactamase producers whereas polymyxin-B can be used as drug of choice for carbapenamese producers or carbapebem resistant Enterobacteriaceae.

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