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PREVALENCE OF EXTENDED SPECTRUM β-LACTAMASE (ESBL) AND METALLO β-LACTAMASE (MBL) PRODUCING E.coli AND Klebsiellapneumoniae IN PATIENT VISITING BHAROSA HOSPITAL

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Abstract

E. coli andK. pneumoniae cancause serious infections thatmay often be fatal among hospital acquired infections. Main objective of this research is to study the prevalence of ESBL and MBL producing E.coli and K. pneumoniae in hospital patient.For the detection of ESBL and MBL producing E. coli andK. pneumoniae blood, urine, pus,bloodetc. were taken as samples. Samples were processed using standard microbiological procedure followed by AST by Kirby Bauer method and results were interpreted using CLSI guidelines. ESBL and MBL were confirmed by combined disc test (CDT).Out of 1125 clinical specimens 354 showed significant growth. In this study, 286 (80.79%) E. coli and 68 (19.21%) K. pneumoniae were isolated from various clinical specimens. The percentages of the isolated organisms were comparatively higher in female (72.88%) than male (27.22%). During the screening 39.51% of E. coliand 29.41% of K. pneumonia were found to be ESBL producer whereas 0.69% E.coli and 10.29% of K. pneumonia were found to be MBL producer.The isolates showed higher resistance to Ampicillin, Cefotaxime followed by, and Ceftazidime. The effective drugs were found to be Amikacin and Imipenamin case of ESBL. Of 354 isolates, 193 (54.51%) were MDR positive and among them most of the cases were observed in female than male. Out of 286 Escherichia coli and 68K.pneumoniae, MDR was seen in 165 (57.51%) and 28 (41.17%) isolates respectively. The study showed increasing trend of MDR and ESBL production in E. coli and K. pneumoniae, so constant survey of antibiotic sensitivity should be done to control and manage spread of these isolates in different units of health institutions. Key words: Escherichia coli, Klebsiella pneumoniae, MDR, ESBL, MBL and CDT.

CHAPTER I

INTRODUCTION

1.1Background

Urinary tract infection (UTI) is one of the most common infectious diseases ranking next to upper respiratory tract infection (Ramesh et al 2008; Hryniewiczet al2001). UTIs are often associated with significant morbidity and mortality (Hryniewicz et al 2001; Raksha et al 2003; Ramesh et al 2008). Worldwide, about 150 million people are diagnosed with urinary tract infection each year, costing the

global economy in excess of 6 billion dollars (Gonzalez and Schaeffer 1999). Urinary tract infection occurs in every age and in both sexes, however it is more common in female due to short urethra, its closeness to the anus and absence of prostatic secretion. Also, sexual intercourse increases the chances of contamination of female urethra by fecal flora (Awaness et al 2000 and Adedeji and Adbulkadir 2009).

Urinary Tract Infection is the most common disease aliment among Nepalese population as well as one of the commonest nosocomial infection (Kattel et al 2008). According to the annual report published by the Department of Health Services, morbidity of UTI among outpatients were 311,944. For the appropriate treatment of UTI, culture and sensitivity test is essential though for most of the part of Nepal this facility in not available. In Nepal, there is limited information available on the prevalence and antibiotic susceptibility pattern of pathogens associated with urinary tract infection.

The irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major threat that has been observed over the last decade. Enterobacteriaceae have become one of the most important cause of hospital acquired and community acquired infection. Infection Caused by multidrug resistant Enterobacteriaceae are among the most serious threat to human health both in hospital and the community. Infection due to Gram negative bacilli are on rise world over. The rampant use of broad spectrum antibiotics can lead to colonization with resistant strains with an increase morbidity, mortality and significant economic loss. βlactum antibiotics are commonly used to treat bacterial infection due to their low toxicity and high efficacy. βlactum bind to the penicillin- binding protein of the bacterial cell wall, causing peptidoglycan disruption and cell lysis. The continuous exposure of bacteria to variety of β lactams has leads the production β lactamase enzyme [AmpC, extended – spectrum β lactamase (ESBL), and metallo – β-lactamase enzyme (MBL)], the most common mechanism of β lactam resistance among Enterobacteriaceae.

ESBLs are β-lactamase capable of conferring bacterial resistance to the penicillins, first, second, and third generation cephalosporins and aztreonam but not the cephamycins and carbapenems by hydrolysis of these antibiotics and which are inhibited by β- lactamase inhibitors such as clavulanic acid. There are different types of ESBLs for example, SHV type, TEM type, CTX-M and Toho β-lactamases, TEM type, OXA types, PER types and VEB-1 etc.

Metallo- β lactamase (MBL) is an enzyme that makes bacteria resistant to a broad range of β-lactam antibiotics. These include the antibiotics of the carbanpenaem family, which are a mainstay for the treatment of antibiotics resistant bacterial infections. The MBL enzymes efficiently hydrolyze all β-lactams, such as penicillins,cephalosporins and carapenems, except azetronam in vitro (Bushet

al1995).These enzymes belong to Ambler class B and Bush 3 group and require divalent cations, usually Zinc, as cofactor for enzyme activity. They are inhibitred by metal chelators such as EDTA but are not affected by therapeutic β-lactamase inhibitors like sublactam, tazobactam or clavulanic acid (Livermore and Woodford 2000).

Enterobacteriaceae exhibiting one or more of the following β-lactam resistance mechanisms: carbapenemases, ESBL, Plasmid –mediated AmpC β-lactamases The emergence of ESBL and MBL in Enterobacteriaceae is becoming a therapeutic challenge that leads to global, AmpC hyperproduction, and decreased permiability(Genel Nathalle et al2012).

World is facing serious problem with antibiotic resistant bacteria and have created great problem in health sectors. There is an urgent need to investigate alternative treatment options while there are still a few antibiotics left. New resistance mechanisms emerge and spread globally threatening our ability to treat common infectious diseases, resulting in death and disability of individuals who until recently could continue a normal course of life. Antibiotic resistance is become a Pressing issue today, both globally and in Nepal.

The bacteria carrying these enzymes can cause infections in hospitalized patients(nosocomial infection), with common sites including the urinary tract, blood, wounds etc. In developing countries like Nepal , due to easy availability of antibiotics, self medication is a common practice which further promote for the development of antibiotic resitance among bacteria.The constantly increasing ESBL and MBL resistant enterobacteriaceae, seem to create the major health problem in hospital. This study will help to know the current situation of AST and MDR pattern.

Every time someone takes antibiotics , the weak bacteria are killed but some strong ones survive. The surviving bacteria then unfortunately mutate and develop a resistance to the same antibiotics . This in turn means that the same medicine will have little to no effect on ythe future. Such development of antibiotics going on today has accelerated its pace and is starting to emerge as a frightening public health hazard. Because such antibiotics rsistant bacteria doesnot just limit themselves to their host individual. They get transmitted from one person to another person untill we have a whole population infected with superbug that cannot be cured by the medicines at our disposal.However , their convience of administration and ease of availability brought about their abuse -not only by the general population but also by msdical personnel. And now, following years of misuse and abuse of the antibiotics , the world is facing a grave new crisis of antribiotics resistance.

Hence , this study was carried out on topic "**Prevelance Of ESBL and MBL in *Klebsiella pneumoniae*and *E.coli* in patients visiting Bharosa Hospital"**. This study help to tackle the challege by regulating theprescription and admistration of antiboiotics.

1.4 Objectives

1.4.1 General objective

To study the prevalence of ESBL and MBL producing *E.coli* and *Klebsiellapneumoniae* in patient

1.4.2 Specific Objectives

- To isolates and identify *Escherichia coli* and *Klebsiellapneumoniae* from urine sample.
- To determine the antibiotic susceptibility pattern of the isolates.
- To access ESBL producing *E.coli* and *Klebsiellapneumoniae.*
- To detect MBL producing *E.coli* and *Klebsiellapneumoniae.*
- To determine the co-production of ESBL and MBL among *E.coli*and *Klebsiellapneumoniae.*

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

The materials, equipment and various reagents used in different stages of this study .

3.2 Methods

3.2.1 Study site and period

Cross sectional study was carried out in the Microbiology Laboratory of Bharosa hospital, Old Baneshwor, Kathmandu, Nepal. It is hospital based study. And total duration of this study was six months from February 2018-September 2018.

3.2.2 Study population

All indoor patients visiting hospital and admitted to the hospital were enrolled in this study. Single sample from the individual patient was used and repetition was avoided with taking the notice of the selection bias. This was the quantitative and cross-sectional study.

3.2.3 Sample size and types

In this study, a total of 1125 samples that were sent for routine laboratory investigation were processed. Different samples like urine, sputum, blood, pus, and wound swab were examined in laboratory.

3.2.4 Inclusion and exclusion criteria

Only samples collected aseptically in a clean, sterile, leak proof container with no visible signs of contamination and labeled and transported properly withdemographic information of the patients were accepted for the study else rejected and requested for repetition.

3.2.5 Sample collection and processing

Urine was collected using sterile bottle and plastic caps. The collection of specimens like pus, wound swab, catheter tip was performed. All the specimens collected from patients were brought

immediately to the laboratory. Specimens collected in a clean, leak proof container with no visible signs of contamination and labeled properly with demographic information of patients were accepted otherwise a second sample was requested. The laboratory request form was filled thereafter. The samples were processed using standard Microbiological procedure (Cheesbrough 2000). AST was done and interpreted using CLSI guideline (CLSI 2014).

3.2.5.1 Urine samples

Patients were given a sterile, dry, wide necked, leak proof container and requested to collect 10-20ml of clean voided mid-stream urine. The patients were instructed to clean the area around the urethral opening with clean water and then begin to void and collect the mid-stream urine sample. The container was then labeled properly and immediately delivered to the laboratory as soon as possible for further processing. In case of delay, the sample was refrigerated at 4-6°C, and when a delay in delivery of more than two hours was anticipated, boric acid (1.8% w/v) was added as preservative.

Urine culture

The urine sample was cultured onto the Cysteine Lactose Electrolyte Deficient Agar (CLED) medium by the semi-quantitative culture technique using a standard loop. A loop full of urine was then streaked on the plate to make straight line inoculums down the center of the plates and without flaming or reentering urine; then streaked by making series of passes at 90^{o} angle. The plates were then incubated aerobically at 37^{o}C overnight (Forbes et al 2007).

The approximate number of colonies was counted i.e. colony forming unit (CFU) per ml of urine estimated in accordance to the volume of urine inoculatedpreviously. For example, 100 colonies on inoculation 0.001 ml of urine would correspond to 10^{5} CFU/ ml (Cheesbrough, 2000).

The bacterial count was reported as:

- Less than 10^4 CFU/ml organisms: not significant
- 10^4-10^5 CFU/mlorganisms: doubtful significance (suggest repeat specimen).
- More than 10^5 CFU/mlorganisms: significant bacteriuria (Cheesbrough, 2000)

3.2.5.2 Blood

About 1-2 ml of blood was withdrawn from patients and dispensed into the sterile screw capped culture bottle with Brain Heart Infusion (BHI) broth. Aerobic incubation was done for the blood culture to isolate aerobes only. The blood samples showing the contaminant growth were excluded in the study.

Blood culture

The inoculated BHI broth was incubated at 37°C overnight and on the next day, it was cultured in Mac Conkey agar and Blood agar and incubated overnight at 37°C.

3.2.5.3 Pus, wound swab and catheter tip

All the samples were collected aseptically from patients. Skin surface was disinfected prior to specimen collection. For pus sample, the aspirates werecollected in sterile syringe. Wound swabs were collected from the deepest part of the wound, avoiding the superficial microflora. For the catheter sample, the skin was cleansed around insertion site with 70% alcohol to reduce contaminating skin flora and any residual antimicrobial ointment was removed. It was allowed to dry. Then, the catheter was removed aseptically and 2 inches of the distal tip of the catheter was clipped

directly into a sterile container. The specimen was transported directly to the Microbiology Laboratory to prevent drying.

Pus, wound swab and catheter tip culture

These specimens were cultured onto Mac Conkey agar and Blood agar respectively and were incubated at 37°C for overnight and were observed for significant bacterial growth.

3.2.6 Specimen processing

3.2.6.1 Macroscopic examination

Specimens were observed carefully for color, consistency, turbidity and presence and absence of blood depending on the type of specimen.

3.2.6.2 Microscopic examination

The microscopic examination was done by preparing smears of suitable sample in a clean grease free glass slide. The Gram staining was done and observed under microscope for presence of bacteria.

3.2.7 Identification of *Escherichia coli* and *Klebsiella pneumoniae*

The identification of *Escherichia coli* and *Klebsiella pneumoniae* were done by using standard microbiological techniques which comprises colony morphology, Gram staining, motility and various biochemical reactions **(Appendix C)**.

3.2.8 Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility testing (AST) was performed using disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI) using Kirby-Bauer method (CLSI, 2014). Inoculums were prepared by suspending the single isolated colony on the nutrient broth to the final turbidity of a 0.5 McFarland standard. The bacterial suspension was spread over the Muellar-Hinton agar homogeneously and anti-microbial disks were dispensed onto the centre and periphery of the agar plates and incubated for 18 hours at 37°C. The diameter of zone of inhibition was measured for all and interpreted as recommended by CLSI guideline and reported the organism as "Resistant", "Intermediate" and "Sensitive" **(Appendix-D)**.

Antibiotics such as Ceftriaxone (30 µg), Ceftazidime (30 µg), Nalidixic acid (30 µg), Amoxy-clav (30 µg), Ampicillin (25 µg), Co-trimoxazole (25 µg), Cefotoxime (30 µg), Imipenem (30 µg), Gentamicin (30 µg), Amikacin(30 µg), Cefepime (30 µg), Aztreonam (30 µg), Nitrofurantoin (300 µg) and Piperacillin/Tazobactam (100/10 µg) were used (Cheesbrough, 2000).

3.2.9 Screening and confirmation for ESBL producers

Screening of ESBL production and combined disc test (CDT) were performed based on Kirby-Bauer disc diffusion method as recommended by CLSI guidelines (2014). For the screening of ESBL, 3rd generationCeftazidime 30 µg was used. The confirmation was done by the use of Ceftazidime combine with Clavulanic acid (30 /10 µg). A≥ 5-mm increase in a zone diameter for Ceftazidime , each tested in combination with Clavulanate and the zone diameter of Ceftazidime when tested alone were confirmed as ESBL producers.

3.2.10 Screening and confirmation for MBL producers

This test was performed by imipenem EDTA combined disc test. Two (10µg) imipenem discs were placed on plate inoculated with the test organism and 10µl of 0.5M EDTA solution was added to one disc. A zone of diameter difference between the imipenem and imipenem +EDTAof greater than or equal 7mm interpreted as a positive result for MBL production.

3.3 Quality control

Quality control is considered as one of the important factors for the correct result interpretation (Cheesebrough 2000). Strict quality was maintained to obtain reliable microbiological results. The quality of each agar plate prepared was maintained by incubating one plate of each batch in the incubator. Control strains of ATCC like *E. coli* and *Klebsiella spp* were used for the standardization of Kirby Bauer test. Strict aseptic condition was maintained while carrying whole procedures. The purity plate was used to ensure that the inoculum used was pure culture as well as to assess that the biochemical tests were taken in an aseptic condition.

3.3.1 Monitoring and regular evaluation of laboratory equipment reagents and media.

Laboratory equipment like incubator, refrigerator, autoclave and hot air oven were regularly monitored for their efficiency. The temperature of the incubator and refrigerator was monitored every day.

Reagents and media were regularly monitored for their manufacture and expiry date and proper storage. After preparation, they were properly labeled with preparation date, expiry date. For stains and reagents, wherever a new batch of them was prepared, a control smear was stained to ensure correct staining reaction.

While using readymade dehydrated media, the manufacturer's instructions for preparation, sterilization and storage were followed to prevent the alteration of the nutritional, selective, inhibitory and biochemical properties of the media. The quality of media prepared was checked by incubating one late of each lot for sterility and using standard control strains for performance testing.

3.3.2 Purity Plate

The purity plate was used to ensure that the inoculation used for the biochemical tests was pure culture and to see whether the biochemical tests were performed inan aseptic condition or not. Thus, while performing biochemical tests, the same inoculum was sub cultured in respective medium and incubated. The media were then checked for the appearance of pure growth of organisms.

3.3.3 Quality control during sample collection and processing

During sample collection, aseptic technique was followed for collecting samples from objects in order to avoid contamination. During sample processing, all the tests were carried out appropriately in aseptic condition.

3.3.4 Quality control during antimicrobial susceptibility testing

Mueller Hinton agar and the antibiotic discs were checked for their lot number, manufacture and expiry date, and proper storage. For the standardization of Kirby- Bauer test and for performance testing of antibiotics and MHA, control strains of *E coli* (ATCC 25922) and *K. pneumoniae* ATCC 700603 were tested primarily. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4 mm and the pH at 7.2-7.4.

3.4 Statistical Analysis

All the results were entered in the Statistical Package for Social Science (SPSS) version 16 software package. The statistical analysis was performed for the variables (organisms, samples, age and sex of patients, multidrug resistance ESBL and MBL production) using Chi-square test. The P-value <0.05 was assumed to be significant for the analyses.

3.5 Ethical consideration

Written informed consent was taken from the participant during the study. NHRC approval was taken prior to the work. Permission from the BharosaHospital was taken to conduct the study in its microbiological laboratory. Also, the proposal of the study was approved from the Department of Microbiology of St. Xavier's College as well asfromtheMicrobiologicalDepartmentofBharosaHospital.

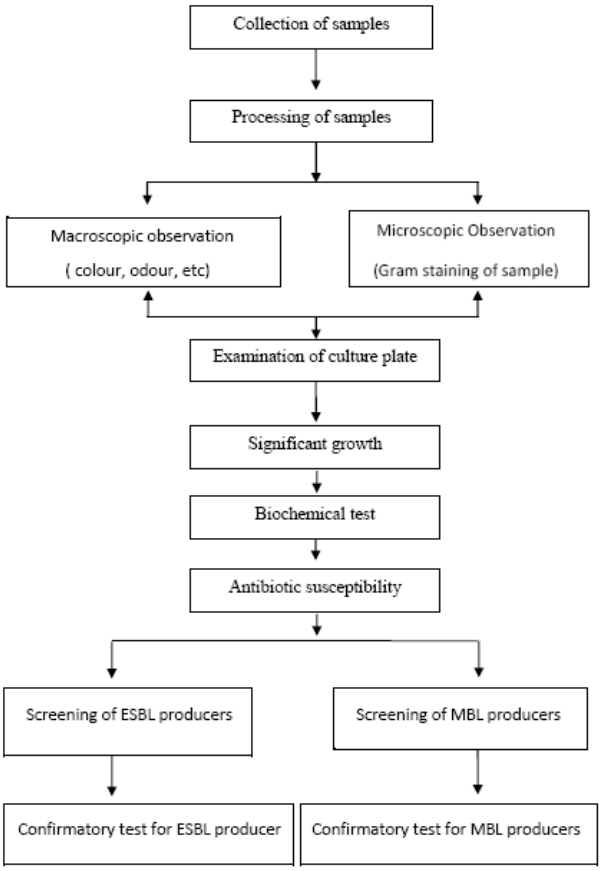
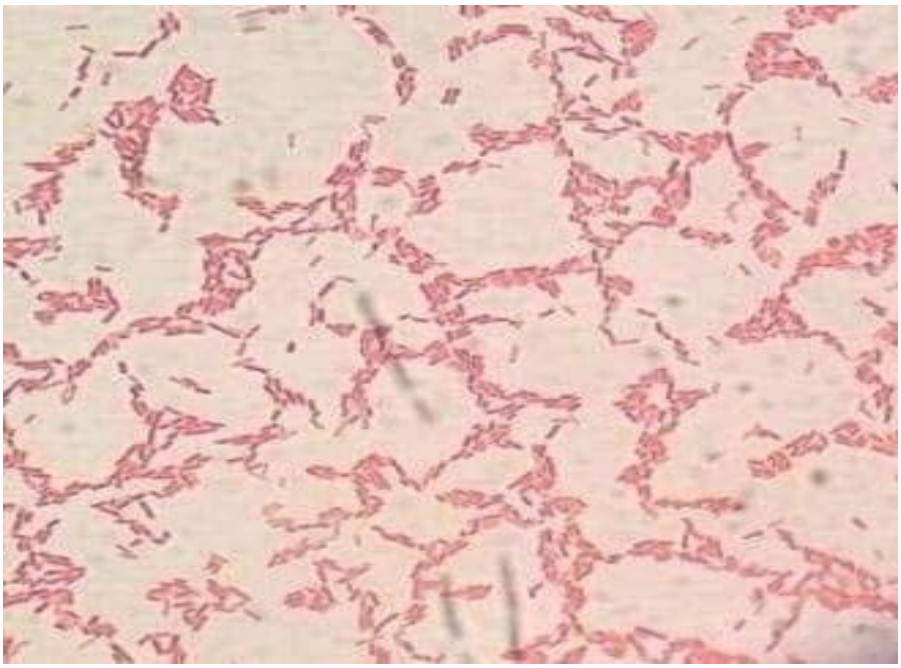


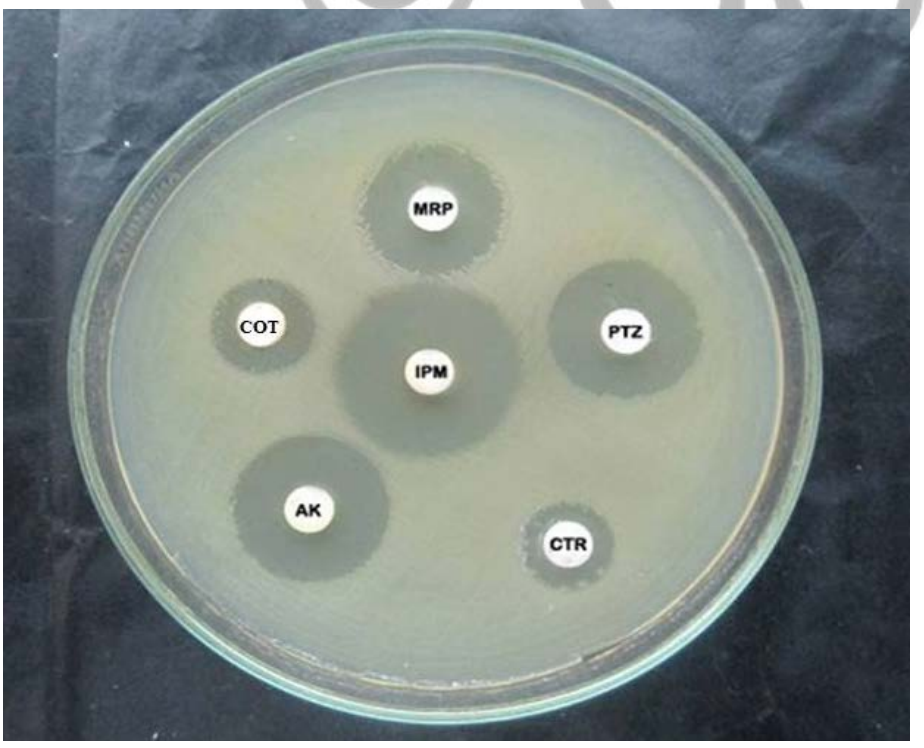
Fig1: Flow chart for the processing of clinical samples



Photograph 1: Microscopic observation of Gram negative rod shaped bacteria

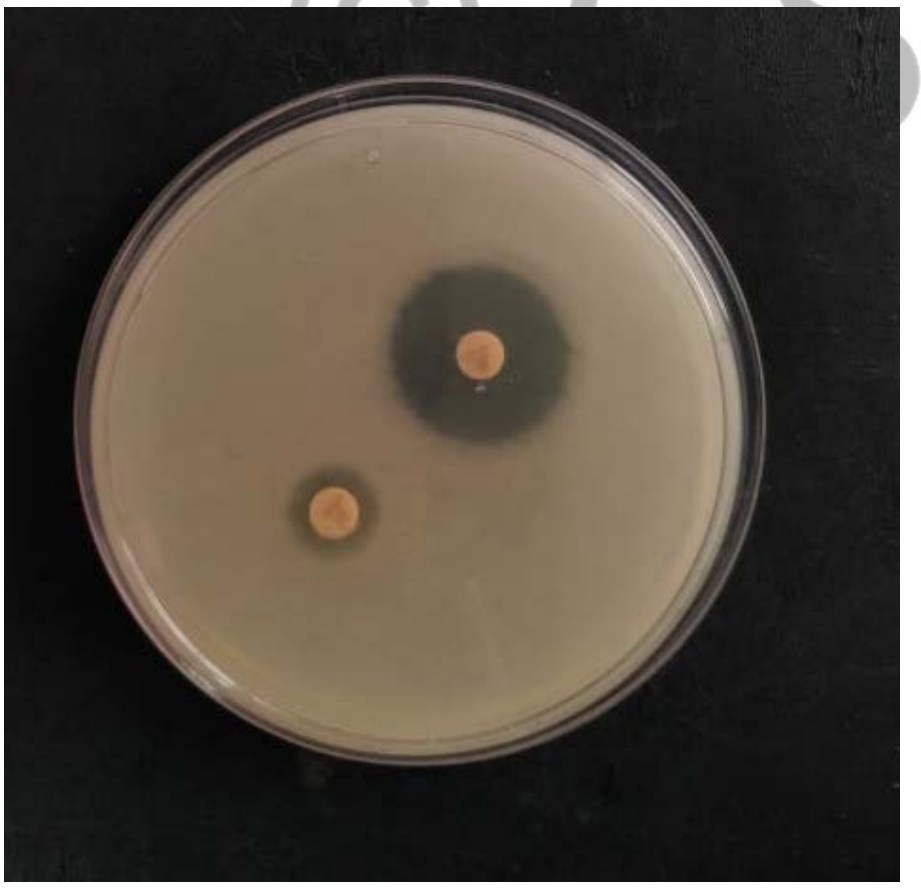
Photograph 2: Significant growth of lactose fermenting pink colored colonies of *Escherichia coli* on Mac Conkey Agar

Photograph 3: Biochemical test of *E.coli* (Test positive to MR and SIM:VP, Citrate and Ureasenegative ; TSIA: Y/Y G+)



Photograph 4: Antibiotic susceptibility tests of E. *coli*

Photograph 5: ESBL confirmation test on Mueller Hinton Agar



Photograph 6: MBL confirmation test on Mueller Hinton Agar

CHAPTER IV

RESULTS

In this study, 1125 samples were collected out of which 498 samples were collected from male and 627 from female from hospital. Out of 354 growth samples, 286 samples of *E. coli*and 68 samples of *Klebsiella* spp., organisms were selected for the study.

4.1 Culture positivity of samples

Out of 1125 samples, 354 (31.46%) samples showed growth while 771 (68.53%) showed negative culture results.

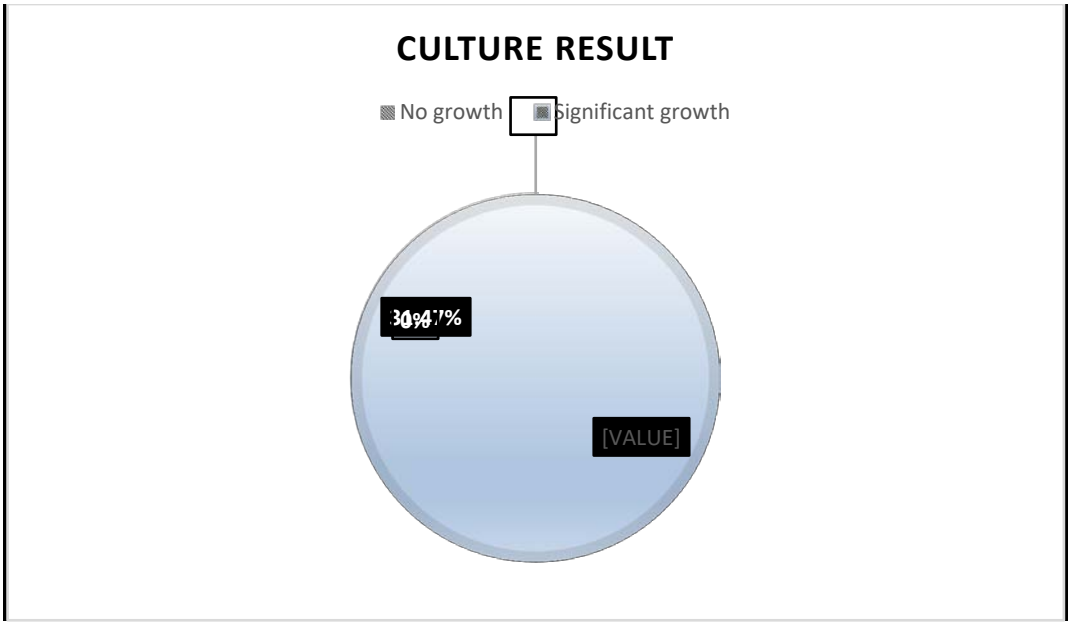


Figure 1: Culture positivity of samples

4.2 Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates in various clinical specimens

Out of 354 clinical specimens, 286(80.79%)*Escherichia coli*and68(19.21%)*Klebsiella pneumoniae* were isolated. Prevalence of *E. coli* was mostly found in urine which was 278(86.33%) followed by*K. pneumonia* which was only 54(13.67%). *K. pneumoniae* was isolated mostly in sputum sample 8(100%).

Table 1: Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates in various clinical specimens

Types of specimens	Total (n)	Bacterial isolates	
		***E. coli* (%)**	***K. pneumoniae* (%)**
Urine	322	278(86.33)	54(16.77)
Pus	12	8(66.66)	4(33.34)
Sputum	8	0(0)	8(100)
Catheter/Foley's tips	0	0(0)	0(0)
Blood	0	0(0)	0(0)
Wound swab	1	0(0)	1(100)
ET/suction tube	0	0(0)	0(0)
Total	**354(100)**	**286(80.79)**	**68(19.21)**

4.3 Growth positivity according to gender of patients

Growth positivity was higher in female 258(72.89%) in comparison to male96(27.11%). In male *E. coli*was found 74(77.08%) and *K. pneumonia*22(29.92%) while in case of female*E. coli*212(82.18%) and *K. pneumonia* 46(17.82%).

Table 2: Growth positivity according to gender of patients

Gender of patients *(n)*	Total Growth (%)	Organisms (%)	
		E. coli	***K. pneumonia***
Male (498)	96 (27.11)	74(77.08)	22(29.92)

Female (627)	258(72.89)	212(82.18)	46(17.82)
Total (1125)	**354 (100)**	**286(80.81)**	**68(19.18)**

n= number of patients according to gender

4.4 Antimicrobial susceptibility pattern of the isolates

Of 354 isolates tested for antimicrobial susceptibility,45(66.17%) of *K. pneumoniae*and 166(58.04%) of *E. coli* showed highest resistance to ampicillin. It was found that, 278(96.51%) of *E. coli*and 55(80.88%) of*K. pneumoniae* were more sensitive to Imipenamfollowed by nitrofurantoin260(90.91%) and 52(74.48%) respectively.

Table 3.1: Antimicrobial susceptibility pattern of the *K. pneumoniae*

Antibiotics used	Number of resistant (%)	Number of sensitive (%)
Cotrimoxazole	30 (44.11)	38(55.89)
Ampicillin	45 (66.17)	23(33.83)
Nitrofurantoin	16 (25.52)	52(74.48)
Amikacin	19(27.94)	49(72.06)
Ciprofloxacin	26 (38.23)	42(61.77)
Ofloxacin	25(36.76)	43(63.24)
Ceftriaxone	37(54.41)	31(45.59)
Cefotaxime	38 (55.88)	30(44.12)
Ceftazidime	34(50.00)	34(50.00)
Gentamycin	24 (35.29)	44(64.71)
Imipenam	13(19.12)	55(80.88)

Table 3.2: Antimicrobial susceptibility pattern of the *E. coli*

Antibiotics used	Number of resistant (%)	Number of sensitive (%)
Cotrimoxazole	141(49.30)	145(50.70)
Ampicillin	166 (58.04)	120(41.91)
Nitrofurantoin	26 (9.09)	260(90.91)
Amikacin	38(12.59)	248(87.41)
Ciprofloxacin	141(49.30)	145(50.70)
Ofloxacin	146(51.05)	140(48.95)
Ceftriaxone	127 (44.41)	159(55.59)
Cefotaxime	137(47.90)	149(52.10)
Ceftazidime	136(47.55)	150(52.45)
Gentamycin	38 (13.29)	248(86.71)
Imipenam	10(3.49)	278(96.51)

4.5 ESBLproducer among the isolates

Out of 354 isolates, 133(37.57) were confirmed as ESBL producer. Among them 113(39.51%) of *E. coli*and 20(29.57%)of *K. pneumoniae*were confirmed.

Table 4: ESBL producer among the isolates

Organisms	No. of isolates	ESBL producer	
		Screening (%)	Confirmed (%)
E. coli	286	137(47.90)	113 (39.51)
K. pneumoniae	68	38(55.88)	20 (29.41)
Total	**354**	**175 (49.43)**	**133 (37.57)**

4.6 Distribution of ESBL producersin male and female patient.

ESBL producing organisms were more prevalent in females than those in males. Our study showed that among 133 of the total confirmed ESBL producers, 88(66.16%) of female patients and the remaining 45(33.84%) of male counterparts.

Table5: Distribution of ESBL producers according sex of patients.

ESBL	Sex				Total	P Value
	Female	%	Male	%		
Positive	88	66.16	45	33.84	133	0.037 (<0.05)
Negative	170	76.92	51	23.08	221	
Total	**258**		**96**		**354**	

4.7 Antibiotic susceptibility pattern between ESBL producers and non-producers

According to table 6, ESBL producer is highly sensitive towards gentamycin (n= 150) followed by nitrofurantoin (n=126). In the case of non-ESBL producer, organism show more sensitive towards amikacin 218(98.19%) followed by nitrofurantoin 215(96.84%). ESBL producers were found to be more resistant towards antibiotics in comparison to non-ESBL producer.

Table 6.Summary of the comparison of antibiotic susceptibility pattern between ESBL producers and non-producers

Antibiotics	ESBL Producers		ESBL non-producers	
	Sen	Res	Sen	Res
Ciprofloxacin	56(42.42)	76(57.58)	193(86.93)	29(13.07)
Ofloxacin	52(39.39)	80(60.61)	190(85.85)	32(14.42)
Nitrofurantoin	126(95.45)	6(4.55)	215(96.84)	7(3.6)
Amoxycilin	25(18.93)	107(81.07)	165(74.32)	57(25.68)
Nalidixic acid	13(9.84)	119(90.16)	136(61.26)	86(38.74)
Gentamycin	150(86.36)	18(13.64)	209(94.14)	13(5.86)
Amikacin	123(93.18)	9(6.82)	218(98.19)	4(1.81)
Cefotaxime	1(0.75)	131(99.25)	173(77.92)	49(22.08)
Ceftriaxone	1(0.75)	131(99.25)	191(86.03)	31(13.97)

4.8 MBL producer among the isolates

Of 354 isolates of *E. coli* and *K. pneumoniae*, screening positive of MBL present in10(3.49%) and 13(19.11%)cases respectively. And it was confirmed in 2(0.69%)of*E. coli*and 7(10.29%)*K. pneumoniae*.

Table 7: MBL producer among the isolates

Organisms	No. of isolates	MBL producer	
		Screening (%)	Confirmed (%)
E. coli	286	10(3.49)	2 (0.69)
K. pneumoniae	68	13(19.11)	7 (10.29)
Total	**354**	**23(6.49)**	**9(2.54)**

4.9Distribution of MBL producers in male and female patient

Similarly, MBL producing organisms were found more in females than those in males. Out of 354 samples, 7(77.77%) of female and 2(22.23%)of male were confirmed as MBL producers.

Table 8. Distribution of MBL producers among male and female

MBL	Sex				Total	P Value
	Female	%	Male	%		
Positive	7	77.77	2	22.23	9	0.964 (>0.05)
Negative	251	72.75	94	27.25	345	
Total	**258**		**96**		**354**	

4.10. Distribution of MBL producing isolates on the basis of samples

Out of 354 samples, maximum samples were taken as urine 332(93.78%) and then pus which is 12(3.39%). Maximum MBL positive was detected from urine sample 8(2.26%) which is followed by sputum samples 1(12.5%). Here the percentage of ESBL producer was found empty in blood sample. Since the sample of wound swab was one and it gives MBL negative hence its percentage was found 1(100%).

Table9. Distribution of MBL producing isolates on the basis of samples

Samples	Total (%)	MBL (%)	
		Positive(n=9)	Negative(n=345)
Urine	332(93.78)	8(2.409)	324 (97.59)
Catheter tips	0	0	0
Sputum	8(2.26)	1(12.5)	7(87.5)
Stool	1(0.28)	0	1(100)
Pus	12(3.39)	0	12(100)
Blood	0	0	0
Wound swab	1(0.28)	0	1(100)
Total	**354(100)**	**9(2.54)**	**345(97.45)**

4.11 Antibiotic susceptibility pattern of MBL producers

MBL positive isolates showed high resistance towards y Cefeperazolesulbactam 8(88.88%), Cefepime 8(88.88%), Ampicillin sulbactam 8(88.88%)and Meropenem 8(88.88%). They were found to be sensitive towards to Tobramycin 9(100%) and Colistin 9(100%).

Table 10: Antimicrobial susceptibility pattern of the MBL

Antibiotics used	Number of resistant (%)	Number of sensitive (%)
Aampicillinsubactam	8(88.88)	1(11.11)
CefeprezoleSubactam	8(88.88)	1(11.11)
Cefepime	7(77.78)	2(22.22)
Cefoxitime	8(88.88)	1(11.11)
Colistin	0(0)	9(100)
Meropenem	8(88.88)	1(11.11)
Tobramycin	0(0)	9(100)
Imepenem	9(100)	0(0)

4.12 Pattern of MDR for the isolates

Out of 354 samples, 193(54.51%) were found to be MDR. Among them 165 (57.69%) of *E. coli* and28(41.17) of *K. pneumoniae*was seen as MDR.

Table 11: Pattern of MDR for the isolates

Bacterial isolates (n)	MDR (%)	Non- MDR (%)
***E. coli* (286)**	165(57.69)	121(42.31)
***K. pneumoniae* (68)**	28(41.17)	40(58.83)
Total 354	**193(54.51)**	**161(45.48)**

n= number of bacterial isolates

4.13 Distribution of MDR in male and female patient

MDR cases were found more in females than those males. Among 193 case of MDR, 136 (70.46%) of female patients and 57 (29.54%) of male counterparts.

Table12. Distribution of MDR in male and female patient.

MDR	Sex				Total	P Value
	Female	%	Male	%		
Positive	**136**	70.46	57	29.54	**193**	
Negative	122	75.77	39	24.23	161	**0.317(>0.05)**
Total	**258**		**96**		**354**	

4.14.Distribution of MDR producing isolates on the basis of samples

Out of 354 samples, maximum samples were collected from urine 332(93.78%) followed by pus 12(3.39%). MDR positive was detected in 184 (54.42%) of urine sample followed by sputum samples 2(25%). In case of wound swab MDR was found negative, whichwas shown in table.

Table 13 Distribution of MDR producing isolates on the basis of samples

Samples	Total (%)	MDR (%)	
		Positive(n=193)	Negative(n=345)
Urine	332(93.78)	**184(54.42)**	148 (44.58)
Catheter tips	0	0	0
Sputum	8(2.26)	2(25)	6(75)
Stool	1(0.28)	1(100)	0
Pus	12(3.39)	**6(50)**	6(50)
Blood	0	0	0
Wound swab	1(0.28)	0	1(100)
Total	**354(100)**	**193(54.5)**	**161(45.5)**

CHAPTER V

DISCUSSION

The invention and production of antibiotics was one of the greatest advances of modern science and medicine as well. But the emergence of MDR cases is threatening the advantages of many antimicrobial agents. Similarly, the prevalence rate of ESBL is major health problem has drawn attention to need for better diagnostic technique. In this study, *E. coli* and *K. pneumoniae* have been isolated from urine and sputum in large number as compared to other clinical specimens.

From the pia-chart it shows that out of 1125 sample, 354(31.46%)samples showed positive growth while 771(68.53) showed negative culture or no growth.Similar result was shown in the studied carried out by Neupane et al (2016), Giwa et al (2018), Behroozi A et al (2010) and Chauhan et al (2015) where positive growth were 33.2%, 35%, 33.15% and 36.58% respectively. Lower growth observed in this study that might be due to inclusion of all the patients requesting for culture, the types and number of specimens used, the prior use of antibiotics before collecting sample, the nature of infection, climate and topographical situation of area under study, or the possible presence of fastidious bacteria that normally don't grow on the media and the duration of the study.The other possible cause of low rate of growth positivity might be due to urine samples obtained from patients on antibiotics therapy, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine media usedAmong positive culture *E.coli*were found to be predominant microorganism 286(25.42%) followed by*K. pneumoniae* 68 (6.04%). These results resembled the outcomes of the previous studied by Karn et al (2016), Kumari et al (2017), Baral (2008), Poudyal (2010). Higher frequency of *E. coli* among clinical isolates indicates that they are the major humans' pathogens.

Of 354 clinical specimens,*E. coli* were mostly isolated from urine 278(86.33%) followed by pus 8 (66.66%).From blood, sputum catheter and wound swab the isolated number was found nil. In the same way *K. pneumoniae* were mostly isolated from sputum 8 (100%) followed by urine 54(16.77%). Isolation of *K.pneumoniae* fromcatheter and blood was found to be nil. Here, 80.79% of E. *coli* and 19.21% of *K. pneumonia* were isolated from clinical sample.These were nearly similar to Thapa et al (2017),Chhetri et al (2001), and Sharma (2004) who reported highest prevalence in urine. However, findings from this study appear higher than those reported by Nepal et al (2017) (*E. coli*51.5%and *K. pneumoniae* 14.6%). Though Enterobacteriaceae are human gut flora can cause various infection, possess high prevalence in urine sample indicates its predilection mostly for the UTI.

Of 498(44.26%)male patient, 96(27.11%) showed positive growth and out of 627 (55.74%)female patient, 258(72.89%) showed positive growth.This indicate that a greater number of females get effected than male one. In male 74(77.08%) of*E. coli* were found to be predominant followed by 22(29.92%) of *K. pneumoniae*. Similarly, in case of female 212(82.18%) of *E. coli* were found to be predominant followed by 46(17.82%) of *K. pneumonia*. The variation in prevalence rate might be due to local use of antibiotics, the number of specimens processed, sanitary conditions and the rates of bacterial isolation and the study period involved. In this study, the higher prevalence of *E. coli* than *K. pneumoniae* resembled the study carried out by various other researches including Shrestha (2009), Chhetri et al (2001), Sharma (2004), Baral et al (2012), Paudel (2013) and Thakur (2013) in Nepal, Wilson and Gaido (2004), Obiogbolu et al (2009) and Kumar et al (2014) in the international context.

Among 354 isolates tested for antimicrobial susceptibility, 45(66.17%) of *K. pneumonia* showed highest resistance to ampicillin followed by cefotaxime 38(55.88%) and Cefotazidime 34(50%) but 55(80.88%) of *K. pneumoniae* were more sensitive to Imipenam followed by nitrofurantoin 52(74.48%) and amikacin 49(72.06%).Similarly, 166(58.04%) of *E. coli* showed highest resistance to ampicillin followed by Ofloxacin 146(51.05%) and Ciprofloxacin 141(49.30%). It was found that, 278(96.51%) of *E. coli* and were more sensitive to Imipenam followed by nitrofurantoin 260(90.91%) and Amikacin 248(87.41%). In this study, Imipenam was found to be the most effective antibiotic against the bacterial isolates which showed only few percentagesof resistance. In contrast, the result of the study conducted by Babakhani et al (2015) showed higher resistance to Imipenam (67.0%) and Ofloxacin (75.0%) while the study of Sarathbabu et al (2012) showed Amikacin 755(34.3%),Gentamycin 808(40%) and Imipenem 315(14.3%) were the most effective antibiotics. This

variation in AST may be due to different geographical locations and use as well as misuse of antibiotics. The other reason behind thismay be due to routine exposure of bacteria and discontinued of antibiotics for a while. Thus, present bacterial strains may show resistance to these drugs.

Of 354 isolates, ESBLs were predominantly present among *E. coli*137(47.90%)compared to *K.pneumoniae* 38(55.88%). In the same way 113(39.51%) of *E. coli* and 20(29.41%) of *K. pneumonia* were confirmed ESBL producer.Our study showed high prevalence of ESBL that is 37.57%. Our finding was similar to that of Ramesh et al (2008) and Kumar et al (2006) who reported a high prevalence of ESBLs in *E. coli.*According toAltinkum et al (2013), 7.4% of *E. coli* and 33.9% of *K. pneumoniae* were ESBL producers. Similarly Chander et al (2013) showed 13.51% of *E.coli* and 16.55% of*K. pneumoniae* as the ESBL producers which was lower in compared to this study. Inthe same way, the study conducted by Wyawahare et al (2016) showed 76.19% of *E. coli* and 33.81% of *K. pneumoniae* as the ESBL producers, which showed higher prevalence of ESBL in *E. coli* than in *K.pneumoniae*. But Paterson DL (2006) reported a high percentage of ESBL producing *K. pneumoniae*and stated that infections caused by these bacteria must be considered as risk factors for the bacterial infections due to ESBL producer in the community.It is difficult to make valid comparison of the prevalence of ESBLproducers because of variations in study designs (Friedman et al 2011).

The prevalence of ESBL producing bacteria varies from country to country and from center to center. In the United States, ESBL producing bacteria ranges from 0 to 25% with the average being around 3% (NNIS System Report 2004). In Japan, the prevalence of ESBL producing bacteria is <0.1% (Yagi et al 2000). In Asia, the percentage of ESBL producers is 4.8, 8.5 and up to 12% in Korea, Taiwan, and Hong Kong respectively (Pai et al 1999; Yan et al 2000; Ho et al 2000). In India, the percentage of ESBL producers ranges from 22 to 75% (Agrawal et al 2008, Abhilash et al 2010, Aruna and Mobashshera 2012 and Dalela 2012). The variation on ESBL positivity might be due to the number of isolates studied variation in institution to institution, geographic location and also country to country. The prevalence of ESBL production is high in the referral centers and the intensive care units where the patients are referred from the peripheral centers and where the antibiotic use is profuse (Metri et al 2011). The higher prevalence in Asian countries compared to western countries can be explained by the fact that western countries have strict infection control policies and practices, efficient and effective antibiotic audit systems, shorter average hospital stays, better nursing barriers, and other important health care measures which substantially decrease the chances of acquisition and spreads of ESBLs

strains (Chowdhury et al 2015). In the context of Nepal also, the prevalence of ESBL producers have been reported ranging from as low as 7.3% by Baral et alin 2012 to as high as 31.8% by Pant et alin 2016. In this study, prevalence rate of ESBL was found 25.422% which is higher than the study of Baral and lessen than Pant et al.

In our study it was found that ESBL incidence rate washigher infemale 88(66.10%)than in male45(33.84%).This might be due to over use of drugs by female than male.Female easily get suffered from diseases as they have week immunity than man. Misuse of antibiotics is responsible for higher incidence of ESBL case. Not only this but also due to their ignorance during minor diseases and consumption of medicine without prescription from doctor. Statistical analysis showed that there is significant relationship between gender and ESBL production ($P<0.05$). In contrast, the study of Abujnah et al 2015 and Yadav et al 2017 showed higher prevalence in female than male in case of ESBL.

The ESBL positive isolates showed high resistance towards Ceftriaxone131 (99.25%),Amoxycilin 107(81.07%), and Nalidixic acid 119(90.16%).They were found to be sensitive towards Gentamycin 150(86.36%), Amikacin 123(93.18%) and Nitrofurantoin 126(95.45%). This indicates that Gentamycin ,Amikacinand Nitrofurantoinmight be the drug of choice for treating infection caused by ESBL producing strains.Some are resistance to β-lactams in Enterobacteriaceae is mainly due to the production of β-lactamases, which may be encoded either chromosomally or on plasmids. In contrast, the result of the study conducted by Babakhani et al (2015) showed higher resistance to Imipenam (67.0%) and Ofloxacin (75.0%) and the study of Sarathbabu et al (2012) showed Amikacin, Gentamycin as the most effective antibiotics. This variation in AST may be due to different geographical locations and use as well as misuse of antibiotics. Interestingly, this study also showed low resistance to drugs like Gentamycin. This might be due to routine exposure of bacteria only to newly developed antibiotics thus eliminating resistance against antibiotics previously used but have been discontinued for a while. Hence, present bacterial strains might have shown sensitive to these drugs.

The increasing and rapid spread of MBL producing Enterobacteriaceae particularly *E. coli* and *K.pneumonia* constitutes a serious threat to public health worldwide. In this study the average

production of MBL was found 9(2.54%) ofGram-negative isolates. The prevalence of MBL production in *E. coli* was reported as 2(0.69%) and in *K.pneumonia* it was reported as 7(10.29%).This indicates that MBL rate was found more in *K.pneumonia* than *E. coli.*In a study conducted by Khanal et al 2013 in Nepal, 18.98% of the *E. coli* and 21.08% of the *K. pneumoniae* were found to be MBL producer. Likewise, another study conducted by Pandey et al (2013), MBL production was 8% and 3% for *K. pneumoniae* and *E.coli* respectively.Similarly, study from tertiary care hospital in Nepal reported lower incidence of MBL producing gram negative bacteria 1.3% (Mishra SK et al 2012).The increasing and rapid spread of these MBLs in gram negative bacilli is becoming a therapeutic challenge. Alternatives treatment for this is unavailable or expensive and toxic with poor outcome. Proper detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination (Dahiya et al 2015).

In this study, 2 (22.23%) of male and 7 (77.77%) of female were found to be MBL producers. In contrary to our findings in a study conducted by Marra et al (2006), 57% of MBL producing isolates were from male patients. Our findings were also not in harmony with study conducted by Tsakris et al (2008) and Chkraborty et al (2010) in which male predominance in MBL producing isolates were found to be 93.3% and 66.8% respectively. This might be due to over use of drugs by female than male. Misuse of antibiotics is responsible for higher incidence of MBL case. Not only this but also due to their ignorance during minor diseases and consumption of medicine without prescription from doctor.Since, P value is 0.964 (>0.05) so null hypothesis is true and accepted. Thisstatistical analysis showed that there is no significant relationship between gender and MBL production.

Among MBL producers, the highest MBL was shown by Urine sample with 8(2.40%) followed by sputum with 1(12.50%) whereas, pus, blood, catheter and stool samples showed nil MBL producer. This observation is of serious concern because of the severity of urine tract infections. Majority (2.40%) of the isolates obtained in this study were from urinary sample. This can be partially attributed to large number of urinary samples in comparison with other specimens. Moreover, urinary tract infections (UTIs) are one of the most common infectious diseases (Daoud and Afif 2011). This might be the reason behind the more collection of urine sample and more MBL case observed from urine case. But this study was different with the study conducted by Shashwati et al (2014) which also

showed maximum MBL production from isolates in blood samples (42.28%) and in the same way similar to the finding of Kumar et al (2014) which also showed maximum MBL production from blood samples (66.67%).

MBL positive isolates showed high resistance towards Cefeperazole sulbactam 8(88.88%), Cefepime 8(88.88%), Ampicillin sulbactam 8(88.88%) and Meropenem 8(88.88%) but were found to sensitive towards Tobramycin 9(100%) and Colistin 9(100%). This indicated that Tobramycin and Colistin might be the drug of choice for treating serious infection caused by MBL producing strains.Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance gene and the only viable option remains the potentially toxic drug Polymyxin B and Colistin (Gupta et al 2013). The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge. Plasmid mediated MBL genes detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination (Dahiya et al 2015).

Multidrug resistance is defined as the resistance to at least two classes of first line agents including Ampicillin, Chloramphenicol, Fluoroquinolones (Ciprofloxacin and Ofloxacin) and cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime). MDR was seen in*E. coli* 165 (57.61%) and in*K. pneumonia* 28 (41.18%) isolates respectively. By this study, it showed that there is less distribution of MDR among *Klebsiella pneumoniae* than *E.coli.*The reason behind this variation might be due to the different resistance mechanism for MDR varies according to microorganisms. This study showed less isolates of *E. coli* and *K. pneumoniae* found to be MDR in comparison to the study of Moini et al (2015) which showed MDR of 50% and 46.6% respectively but showed more isolates than the study of Nepal et al (2017) which showed MDR *E. coli* and MDR *K. pneumoniae* of 62.9% and 59% respectively. In the same way, a study done by Rimal et al (2017) showed the prevalence of *E. coli* and *K. pneumoniae* showing MDR to be 34% and 24% respectively. The MDR could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein loss and target mutations (Hirsh and Tam 2010).

Of 193 MDR case*,* 136 (70.46%) were isolated from different specimens of female patient and 57(29.54%) were isolated from male patient. Similar case was observed in the study of Abujnah et al 2015 and Yadav et al 2017 showed higher prevalence of MDR in female than in male.Misuse of antibiotics by female is responsible for higher incidence of MDR case. Not only this but also due to

their ignorance during minor diseases and consumption of medicine without prescription from doctor. Statistical analysis showed that there is no significant relationship between gender and MDR case (P=0.317). No proper reason could be adduced for this but it might be due to large number of females admitted to the hospital than the males.

The study showed that, 184 (54.42%) of MDR isolates were from urine sample followed by pus 6(50%) and sputum 2(25%). No microbial growth was observed from blood so its value was nil. Here, all theMBL producers were found to be ESBL producers and all ESBL producers were found to be MDR. The MDR isolates may have greater chances of limiting the choices of antibiotic treatment. Many studies carried out by Heffernan and Woodhouse (2011), Abujnah et al (2015), Shrestha et al (2017) have reported *E. coli* and *K. pneumoniae* as the major ESBL producing organisms. However,Pitautand Laupland (2012) showed *K. pneumoniae* as the major ESBL and MBL producing organism but Poudyal et al (2011) reported *K. pneumoniae* as the second common organism producing ESBL.

The knowledge on the extend of the ESBL mediated resistance appears to be limited due to the inability of the standardized methods of susceptibility testing or the commercially available systems to detect this resistance (Bonnet 2013). The emergence and the spread of the ESBL producing strains have led to questions regarding the optimal therapy for infections, which are caused by the ESBL producing strains (Parajuli et al 2016). The confirmation of the ESBL production by clavulanic acid inhibition can be difficult in some strains, not only because the activity of the beta-lactamase varies with different substrates, but also because the organism may contain additional resistance mechanisms that can mask the presence of the ESBL activity (Hanson 2013). Proper antibiotic guidelines and its effective implementations could be milestone for revolution in the field of antibiotic resistance control in Nepal.

The increasing prevalence of antibiotic resistance is a seriousconcern. The multidrug resistance among important human pathogens is increasing. In a case of developing countries like Nepal, a high degree of resistance could due to consumption of drugswithout doctor's prescription from pharmacy. Self-medication is a common practice in our country, which might be the reason for major cause of antibiotic resistance in clinical isolates. Patientsrarely visithospitals when they are unable to treat themselves. Inadequate hospital control measures, misuse of medicines, self-medication

andincomplete dosage of antibiotics are the factors to promote the antibiotic resistance in clinical isolates in developing country like Nepal. The limitation of this study is that molecular epidemiologic and characterization of ESBLs, and MBL were not carried out. In order to control and minimize such cases, strict antibiotic policies and measures to limit the self-administration of antibiotics should be implemented.

CHAPTER V

DISCUSSION

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of the study conducted by Babakhani et al (2015) showed higher resistance to Imipenam (67.0%) and Ofloxacin (75.0%) while the study of Sarathbabu et al (2012) showed Amikacin 755(34.3%),Gentamycin 808(40%) and Imipenem 315(14.3%) were the most effective antibiotics. This variation in AST may be due to different geographical locations and use as well as misuse of antibiotics. The other reason behind thismay be due to routine exposure of bacteria and discontinued of antibiotics for a while. Thus, present bacterial strains may show resistance to these drugs.

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fact that western countries have strict infection control policies and practices, efficient and effective antibiotic audit systems, shorter average hospital stays, better nursing barriers, and other important health care measures which substantially decrease the chances of acquisition and spreads of ESBLs strains (Chowdhury et al 2015). In the context of Nepal also, the prevalence of ESBL producers have been reported ranging from as low as 7.3% by Baral et alin 2012 to as high as 31.8% by Pant et alin 2016. In this study, prevalence rate of ESBL was found 25.422% which is higher than the study of Baral and lessen than Pant et al.

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The ESBL positive isolates showed high resistance towards Ceftriaxone131 (99.25%),Amoxycilin 107(81.07%), and Nalidixic acid 119(90.16%).They were found to be sensitive towards Gentamycin 150(86.36%), Amikacin 123(93.18%) and Nitrofurantoin 126(95.45%). This indicates that Gentamycin ,Amikacinand Nitrofurantoinmight be the drug of choice for treating infection caused by ESBL producing strains.Some are resistance to β-lactams in Enterobacteriaceae is mainly due to the production of β-lactamases, which may be encoded either chromosomally or on plasmids. In contrast, the result of the study conducted by Babakhani et al (2015) showed higher resistance to Imipenam (67.0%) and Ofloxacin (75.0%) and the study of Sarathbabu et al (2012) showed Amikacin, Gentamycin as the most effective antibiotics. This variation in AST may be due to different geographical locations and use as well as misuse of antibiotics. Interestingly, this study also showed low resistance to drugs like Gentamycin. This might be due to routine exposure of bacteria only to newly developed antibiotics thus eliminating resistance against antibiotics previously used but have been discontinued for a while. Hence, present bacterial strains might have shown sensitive to these drugs.

The increasing and rapid spread of MBL producing Enterobacteriaceae particularly *E. coli* and *K.pneumonia* constitutes a serious threat to public health worldwide. In this study the average production of MBL was found 9(2.54%) ofGram-negative isolates. The prevalence of MBL production in *E. coli* was reported as 2(0.69%) and in *K.pneumonia* it was reported as 7(10.29%).This indicates that MBL rate was found more in *K.pneumonia* than *E. coli.*In a study conducted by Khanal et al 2013 in Nepal, 18.98% of the *E. coli* and 21.08% of the *K. pneumoniae* were found to be MBL producer. Likewise, another study conducted by Pandey et al (2013), MBL production was 8% and 3% for *K. pneumoniae* and *E.coli* respectively.Similarly, study from tertiary care hospital in Nepal reported lower incidence of MBL producing gram negative bacteria 1.3% (Mishra SK et al 2012).The increasing and rapid spread of these MBLs in gram negative bacilli is becoming a therapeutic challenge. Alternatives treatment for this is unavailable or expensive and toxic with poor outcome. Proper detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination (Dahiya et al 2015).

In this study, 2 (22.23%) of male and 7 (77.77%) of female were found to be MBL producers. In contrary to our findings in a study conducted by Marra et al (2006), 57% of MBL producing isolates were from male patients. Our findings were also not in harmony with study conducted by Tsakris et al (2008) and Chkraborty et al (2010) in which male predominance in MBL producing isolates were found to be 93.3% and 66.8% respectively. This might be due to over use of drugs by female than male. Misuse of antibiotics is responsible for higher incidence of MBL case. Not only this but also due to their ignorance during minor diseases and consumption of medicine without prescription from doctor.Since, P value is 0.964 (>0.05) so null hypothesis is true and accepted. Thisstatistical analysis showed that there is no significant relationship between gender and MBL production.

Among MBL producers, the highest MBL was shown by Urine sample with 8(2.40%) followed by sputum with 1(12.50%) whereas, pus, blood, catheter and stool samples showed nil MBL producer. This observation is of serious concern because of the severity of urine tract infections. Majority (2.40%) of the isolates obtained in this study were from urinary sample. This can be partially attributed to large number of urinary samples in comparison with other specimens. Moreover, urinary tract infections (UTIs) are one of the most common infectious diseases (Daoud and Afif 2011). This

might be the reason behind the more collection of urine sample and more MBL case observed from urine case. But this study was different with the study conducted by Shashwati et al (2014) which also showed maximum MBL production from isolates in blood samples (42.28%) and in the same way similar to the finding of Kumar et al (2014) which also showed maximum MBL production from blood samples (66.67%).

MBL positive isolates showed high resistance towards Cefeperazole sulbactam 8(88.88%), Cefepime 8(88.88%), Ampicillin sulbactam 8(88.88%) and Meropenem 8(88.88%) but were found to sensitive towards Tobramycin 9(100%) and Colistin 9(100%). This indicated that Tobramycin and Colistin might be the drug of choice for treating serious infection caused by MBL producing strains.Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance gene and the only viable option remains the potentially toxic drug Polymyxin B and Colistin (Gupta et al 2013). The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge. Plasmid mediated MBL genes detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination (Dahiya et al 2015).

Multidrug resistance is defined as the resistance to at least two classes of first line agents including Ampicillin, Chloramphenicol, Fluoroquinolones (Ciprofloxacin and Ofloxacin) and cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime). MDR was seen in*E. coli* 165 (57.61%) and in*K. pneumonia* 28 (41.18%) isolates respectively. By this study, it showed that there is less distribution of MDR among *Klebsiella pneumoniae* than *E.coli.*The reason behind this variation might be due to the different resistance mechanism for MDR varies according to microorganisms. This study showed less isolates of *E. coli* and *K. pneumoniae* found to be MDR in comparison to the study of Moini et al (2015) which showed MDR of 50% and 46.6% respectively but showed more isolates than the study of Nepal et al (2017) which showed MDR *E. coli* and MDR *K. pneumoniae* of 62.9% and 59% respectively. In the same way, a study done by Rimal et al (2017) showed the prevalence of *E. coli* and *K. pneumoniae* showing MDR to be 34% and 24% respectively. The MDR could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein loss and target mutations (Hirsh and Tam 2010).

Of 193 MDR case, 136 (70.46%) were isolated from different specimens of female patient and 57(29.54%) were isolated from male patient. Similar case was observed in the study of Abujnah et al

2015 and Yadav et al 2017 showed higher prevalence of MDR in female than in male.Misuse of antibiotics by female is responsible for higher incidence of MDR case. Not only this but also due to their ignorance during minor diseases and consumption of medicine without prescription from doctor. Statistical analysis showed that there is no significant relationship between gender and MDR case (P=0.317). No proper reason could be adduced for this but it might be due to large number of females admitted to the hospital than the males.

The study showed that, 184 (54.42%) of MDR isolates were from urine sample followed by pus 6(50%) and sputum 2(25%). No microbial growth was observed from blood so its value was nil. Here, all theMBL producers were found to be ESBL producers and all ESBL producers were found to be MDR. The MDR isolates may have greater chances of limiting the choices of antibiotic treatment. Many studies carried out by Heffernan and Woodhouse (2011), Abujnah et al (2015), Shrestha et al (2017) have reported *E. coli* and *K. pneumoniae* as the major ESBL producing organisms. However,Pitautand Laupland (2012) showed *K. pneumoniae* as the major ESBL and MBL producing organism but Poudyal et al (2011) reported *K. pneumoniae* as the second common organism producing ESBL.

The knowledge on the extend of the ESBL mediated resistance appears to be limited due to the inability of the standardized methods of susceptibility testing or the commercially available systems to detect this resistance (Bonnet 2013). The emergence and the spread of the ESBL producing strains have led to questions regarding the optimal therapy for infections, which are caused by the ESBL producing strains (Parajuli et al 2016). The confirmation of the ESBL production by clavulanic acid inhibition can be difficult in some strains, not only because the activity of the beta-lactamase varies with different substrates, but also because the organism may contain additional resistance mechanisms that can mask the presence of the ESBL activity (Hanson 2013). Proper antibiotic guidelines and its effective implementations could be milestone for revolution in the field of antibiotic resistance control in Nepal.

The increasing prevalence of antibiotic resistance is a seriousconcern. The multidrug resistance among important human pathogens is increasing. In a case of developing countries like Nepal, a high degree of resistance could due to consumption of drugswithout doctor’s prescription from pharmacy. Self-medication is a common practice in our country, which might be the reason for major cause of

antibiotic resistance in clinical isolates. Patientsrarely visithospitals when they are unable to treat themselves. Inadequate hospital control measures, misuse of medicines, self-medication andincomplete dosage of antibiotics are the factors to promote the antibiotic resistance in clinical isolates in developing country like Nepal. The limitation of this study is that molecular epidemiologic and characterization of ESBLs, and MBL were not carried out. In order to control and minimize such cases, strict antibiotic policies and measures to limit the self-administration of antibiotics should be implemented.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1CONCLUSION

ESBL and MBL case among bacterial infection is major health problem in Nepal. The main reason behind this is due to its high prevalence rate. And also due to MDR case.The prevalence of MDR and ESBL were found to be higher. *Escherichia coli* and *Klebsiella pneumoniae* species were one of the most frequently isolated pathogens with high level of drug resistance and production of various Beta-lactamases. *Escherichia coli*were predominant followed by *Klebsiella pneumoniae.*The highest percentage of MBL and ESBL was observed in females than in males. The drugs tested, Imipenam, Gentamycin, Amikacin and Nitrofurantoin were found to be less resisted in case of ESBL. More than half of the isolates of *Escherichia coli* and *Klebsiella pneumoniae* were found to be MDR. So, regular monitoring rate of ESBL and MBL production along with MDR among the clinical specimen is very necessary. Moreover, to control such prevalence rate strict policy to rationalize the use of antibiotics is necessary. Similarly, the early detection of β-lactamases producing isolates is important for the reduction of mortality rates and also to avoid circulation of such strains within the hospital.

6.2 RECOMMENDATION

1. The high prevalence of ESBL producing isolates were found. Therefore ESBL testing in clinical laboratories is recommended before giving drugs.

2. Imipenem, Nitrofurantoin, Amikacin and Gentamicin were found to be the effective drug for the treatment.

3. To limit the spread of resistance, stringent guidelines for antibiotic policies should be done.

4. Public awareness programme regarding the consequence of self administration of antibiotics should be implemented.

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