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Prevalence of Multidrug Resistant Extended-Spectrum ß-Lactamase-Producing Bacteria from Different Clinical Specimens in Kathmandu Model Hospital, Kathmandu, Nepal

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Abstract

The goal of the current study was to examine the prevalence of multidrug resistant (MDR) Extended spectrum ß-lactamase (ESBL) producing bacteria from different clinical specimens and their susceptibility to selected antibiotics. A total of 4898 different samples including urine, blood, pus, throat, sputum, stool, cerebrospinal fluid, perianal, tissue, eye and ear samples from patients, suspected of bacterial infections, were collected at the Kathmandu Model Hospital, Nepal. Out of 4898 samples, 932 exhibited bacterial growth among which 536 bacteria (57.51%) were multidrug resistance. 321 isolates among MDR exhibited zone of inhibition of ≤ 27 mm against Cefotaxime, the primary screening test of ESBL. Random selection of 253 from the primarily screened bacteria resulted into 97 confirmed cases of ESBL. Urine sample was the most frequent sample to be requested constituting 2702 out of 4898. Out of total 536 MDR, gram negative accounted for 67.70%; and 45.77% isolates of indoor patients were MDR whereas only 17.01% cases were MDR in outpatient. *E. coli* was found to be the most predominant isolate with 505/932 isolates among which 450 were from urine. Similarly, out of 310 isolates of gram positive bacteria, 102 isolates were *S. aureus* with 74 isolates from pus samples. Out of 74 *S. aureus* isolated from 293 pus samples, 4 isolates were MRSA. The infection rate was found to be significantly higher in female (23.37%) than in male (15.88%). Similarly, MDR pattern was found to be higher in female (60.29 %) than in male (54.55 %) but the result was found to be statistically insignificant. Imipenem was found to be the most effective antibiotic against ESBL positive isolates with 93.20% susceptibility followed by Amikacin 90.72%, Nitrofurantoin (84.54%) and Chloramphenical (80.41%). In conclusion, our data showed a high prevalence of MDR ESBL-producing bacteria in our clinical samples. It is paramount to have sound infection control measures including routine monitoring of ESBL-producing isolates.

Keywords: *Extended Spectrum Beta Lactamase (ESBL); Escherichia coli; Staphylococcus aureus*

Background

Appropriate use of antibiotics is central to limiting the development and the spread of resistant bacteria in hospitals and communities. Use of broad-spectrum antibiotics, in particular the third-generation cephalosporin in nosocomial infections have been linked to the emergence of antibiotic resistance and increase in treatment costs [1]. The hospital setting is particularly conducive to the development of

antibiotic resistance as patients who are severely ill, immuno-compromised or have devices and/or implants in them are likely to receive frequent courses of empirical or prophylactic antibiotic therapy [2]. Easier access to antibiotics leads to the inappropriate use of antibiotic and often the high cost of antibiotic results in an incomplete course being purchased, sufficient only to alleviate symptoms. Developing countries are often unable to afford costly second line antibiotics to treat infections due to resistant organisms, resulting in prolonged illness with longer periods of infectivity and further spread of resistant isolates. These factors contribute to emergence of antibiotic resistance worldwide, however condition is even worst in developing countries [3].

The major trouble causing MDR isolates that have been widely observed and studied include Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Resistant Coagulase negative Staphylococci, Glycopeptide intermediate sensitivity *S. aureus* (GISA), and Vancomycin resistant Enterococcus (VRE). In later years, however, Extended Spectrum ß-lactamase (ESBL), Metallobetalactamase (MBL) and AmpC ß-lactamase encoding organisms have been observed which not only resist ß-lactam antibiotics but also non ß-lactam antibiotics. These later organisms may even exhibit resistivity towards those antibiotics *in vivo* which they are susceptible *in vitro* [4].

Resistance to other classes of antibiotics, especially the Fluoroquinolones, is often associated with ESBL-producing organisms. Many clinical laboratories are still not aware of the importance of screening for ESBL-producing Enterobacteriaceae originating from the community [5].

Extended Spectrum β-Lactamases (ESBLs)

Over 150 different ESBLs have been described. Most ESBLs are derivatives of TEM or SHV enzymes. There are > 90 TEM derived β-lactamases and > 25 SHV derived ß-lactamases [6].

The current National Committee for Clinical Laboratory Standards (NCCLS) recommendations for detection of ESBL in *Klebsiella* spp. and *E. coli* includes an initial screening test with any two of the following β-lactam antibiotics: Cefpodoxime, Ceftazidime, Aztreonam, Cefotaxime, or Ceftriaxone. Isolates exhibiting an Minimum Inhibitory Concentration (MIC) > 1 microgram/ml should be confirmed phenotypically using Ceftazidime plus Ceftazidime/Clavulanic acid and Cefotaxime plus Cefotaxime/Clavulanic acid. A two-fold concentration decrease in a MIC for either antimicrobial agent tested in combination with Clavulanic acid versus its MIC when tested alone should be considered an ESBL producer [7].

NCCLS has developed broth micro dilution and disk diffusion screening tests using selected antimicrobial agents. Each *Klebsiella pneumoniae*, *K. oxytoca*, or *Escherichia coli* isolate should be considered a potential ESBL-producer if the disk diffusion is as follows: Cefpodoxime < 22 mm, Ceftazidime < 22 mm, Aztreonam < 27 mm, Cefotaxime < 27 mm, Ceftriaxone < 25 mm and MICs: Cefpodoxime > 2 µg/ml, Ceftazidime > 2 µg/ml, Aztreonam > 2 µg/ml, Cefotaxime > 2 µg/ml and Ceftriaxone > 2 µg/ml. The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and Ceftazidime show the highest sensitivity for ESBL detection [8].

One approach to the detection of ESBLs is to perform disk approximation testing with isolates of *E. coli* or *Klebsiella* spp. for which the MICs of Cefuroxime, Ceftazidime, or related compounds are outside of the susceptible range (intermediate or resistant by National Committee for Clinical Laboratory Standards criteria). Disk approximation testing functions via the placement of Cefuroxime and Ceftazidime disks close (20 or 30 mm) to an Amoxicillin-Clavulanate disk on a plate inoculated with the test organism. Enhancement of the zone of inhibition or a so-called ghost zone between either of the cephalosporin disks and the Clavulanate-containing disk (Amoxicillin-Clavulanic acid) indicates the presence of a Bush group 2be enzyme [9].

As a general rule, laboratories should test all isolates of *E. coli* or *Klebsiella* spp. from in-patients using both Ceftazidime (the best indicator for TEM and SHV-derived ESBLs) and Cefotaxime (the best indicator for CTX-M types). Alternative, they can test with Cefpodoxime, as a good indicator for all ESBL types. Earlier advice to screen only with Ceftazidime is no longer adequate in view of the emergence of

CTX-M types. Any organism showing reduced susceptibility to Cefotaxime, Ceftazidime or Cefpodoxime should be investigated for ESBL production [10].

Colonization and infection with ESBL producing bacteria have also been associated with indiscriminate use of antibiotics, prolonged hospitalizations, increasing numbers of immuno-compromised patients, and medical progress resulting in increased use of invasive procedures and devices [11].

Multidrug resistance trend in Nepal

The trend of microbial resistance in Nepal is remarkably high but the surveillance has not been carried out enough so as to statistically assume the actual fact, [12-13]. In the study at Tribhuvan University Teaching Hospital (TUTH), out of 161 blood borne isolates, 26 (16.14%) were found to be MDR. The incidence of MDR in *Salmonella Paratyphi* A, *S. Typhi* and *Staphylococcus aureus* was found to be 12.12%, 7.8% and 30.00% respectively [14]. In a similar study carried out at Kathmandu Model Hospital, 4 (5.19%) MDR isolates were isolated. Out of 4 isolates, 3 MDR isolates were *S. Typhi* and 1 isolate was *S. Paratyphi* A [15].

In a similar study at Kanti Children Hospital and TUTH, among 52 *S. aureus* isolates 25% was found to be MRSA [16]. In another study carried out in Bir Hospital and TUTH, 75.9% MRSA was isolated from Bir Hospital and 64.5% MRSA from TUTH [17]. In the nosocomial isolates, burden of multiple drug resistance was found to be high as compared to other settings, as in study by Banjara [18] in different wards at TUTH. Banjara [18] found wound isolates resistant to 4 or more than 4 commonly used drugs including *S. aureus* (40.0%), *Pseudomonas aeruginosa* (46.3%), *E. coli* (56.3%), *K. pneumoniae* (62.5%), *Acinetobacter* spp. (60.0%), *Citrobacter freundii* (44.4%), *P. mirabilis* (71.4%) and *K. oxytoca* (100.0%) [18].

Objectives

The objectives of this study were to determine the prevalence of multidrug Resistant Extended-spectrum ß-Lactamase-Producing Bacteria from Different Clinical Specimens in Kathmandu Model Hospital, Kathmandu, Nepal.

Methods

The study was conducted in Kathmandu Model Hospital, Kathmandu, Nepal. This study was approved by the Institutional Review Board, National College and the informed consents were obtained from the participants. A total of 4898 different samples from patients suspected of bacterial infections were collected and processed according to the standard laboratory methods.

Data collection

Each patient requesting for bacterial culture was directly interviewed for his/her clinical history during sample collection. The information of patients included name, age, sex, signs and symptoms, prior infection, duration of fever, other underlying diseases and prior antibiotic administration if any.

Sample Collection

All samples from patients requesting for bacterial culture were collected in leak-proof, dry containers, free from all traces of disinfectants and other chemicals. The processed samples include urine, blood, sputum, pus, fluid, throat swab, cerebrospinal fluid, tissue, perianal swab, stool, ear and eye swab, urethral swab, catheter tips, endotracheal tips etc. Standard procedures were followed for the collection of different samples in their specific standard ways. Each sample was clearly labeled with laboratory number, date, patient's name, sex, age, bed number (if inpatient), time of collection and a brief clinical history.

Urine sample Collection

Persons requesting urine culture were instructed proper method for mid-stream urine collection in wide mouthed sterile leak proof container. They were requested to cover the urine bottle soon after collection of 5 to 10 ml urine sample.

Blood sample collection

Using tourniquet, a suitable vein was located in arm of the blood culture requesting patient. Wearing gloves, the vein puncture site was thoroughly disinfected using 70% ethanol and allowed to dry. With 10 ml syringe, 5 -10 ml of blood was withdrawn from the patient (2 - 5 ml from children). Then the blood was dispensed to the sterile screw capped blood culture bottle containing 0.05% polyanethol sulfonate and Brain Heart Infusion (BHI) Broth.

Pus sample collection

While collecting pus from abscesses, wounds or other sites, special care was taken to avoid contaminating the specimen with commensal organism from the skin. Pus from the abscess was collected at the time the abscess was incised and drained or after it has ruptured naturally. Five milliliter (5 ml) of pus was aspirated or collected from a drain tube and transported to a leak proof sterile container. If it was not available, needle capped syringe itself was transported. Two sterile cotton wool swabs were used to collect sample from the infected site in the case of undischarged pus.

Throat swab sample collection

A plain sterile cotton swab was used to collect as much exudates as possible from the tonsils, posterior pharyngeal wall and any other area that was inflamed or bears exudates. Care was taken not to touch the tongue or buccal surfaces and duplicate swabs from the same area were taken. Then the swab was packed in its tube and delivered to the laboratory.

Sputum sample collection

People requesting for sputum culture were given with clean, wide mouthed, impermeable container with a tightly fitting cap and advised to cough deeply to produce sputum and they were requested not to mix the sputum sample with saliva. They were requested to submit morning sample just after waking up and before any mouthwash if possible.

Stool sample collection

The patient requesting for stool culture was given with two small wooden sticks and open mouthed clean container with a leak proof lid. S/he was properly instructed to collect stool sample on a piece of toilet tissue or old newspaper and to transfer about 5 mg of it to the container, using two sticks.

Fluid sample collection

Fluid samples such as pleural, peritoneal, pericardial and synovial fluids were collected by aspiration with a needle and syringe by experienced physician in presence of a microbiologist. For this, first of all site of aspiration was located and skin was disinfected with 70% ethanol. About 1 - 5 ml of sample was drawn and transported to the laboratory at once in a sterile tube or vial.

Cerebrospinal fluid collection

Approximately, 5 to 10 ml of cerebrospinal fluid sample was collected in two sterile tubes by lumbar or ventricular puncture performed by physician. In order to avoid iatrogenic infection, disinfection of the skin was performed before puncture.

Perianal swab collection

A cotton tipped swab moistened with nutrient broth and autoclaved was inserted through the perianal sphincter, rotated and withdrawn. The swab was placed in an empty sterile tube with a cotton plug and delivered to laboratory.

Tissue sample collection

The site of infection was located and superficial skin was disinfected with 70% ethanol. A piece of tissue was taken from infected site and transferred to screw capped sterile bottle. The tissue can be obtained from internal parts of body too during operation by physician. The sample was quickly transferred to enrichment broth before getting any chance to dry.

Eye and ear swab collection

A sterile cotton swab is rubbed on cornea of eye of patient for eye swab collection. Similarly, pus of ear is collected by inserting sterile cotton swab inside ear but not high enough that it reaches internal ear. The swab is immediately brought to laboratory to culture.

Sample Evaluation

The acceptability of the sample was evaluated before processing in terms of proper labeling, saliva in sputum, visible signs of contamination, improper screwing of cap, etc. The improper sample was rejected and the patient was requested to submit next sample.

Macroscopic and Microscopic examination

The sample collected was observed for its color, odor, dryness, consistency, appearance, and reported accordingly. Then sputum and pus samples were gram stained for examining the presence of pus cells and studying the morphology, arrangement and Gram's reaction.

Culture of specimen

Culture of urine samples

Culture of each uncentrifuged urine sample was done by semi quantitative method on 5% Blood Agar (BA) and MacConkey Agar (MA) plates. An inoculating loop of standard dimension was used to take up fixed and a known volume (0.001 ml) of mixed uncentrifuged urine for inoculation. After inoculating the plates aerobically at 37˚C for 24 hours, colonies were counted [19]. The bacterial count was reported as:

- Less than 10^4 cfu/ml urine- Not significant
- 10^4 - 10^5 cfu/ml urine - Doubtful significance (suggest repeat specimen).
- More than 10^5 cfu/ml urine - Significant bacteriuria [20].

Low count bacteriuria was also taken into consideration if there was any indication which could lower the concentration of bacteria in the urine, e.g. patient under treatment, patient with certain endocrine disorder such as diabetes, chronic kidney disease where concentrating power of kidney is low, obstruction in the ureter due to tumor or stone, etc [12].

Culture of Blood

Blood culture bottle was incubated for 7 days. It was routinely inspected every day after 24 hours of incubation for the evidence of bacterial growth. Evidence of growth includes uniform turbidity, haemolysis, surface pellicle, floccular deposit on top of the blood layer, coagulation of the broth and production of gas. Whenever visible growth appears, subcultures of broth were performed on BA, MA and *Salmonella-Shigella* (SS) agar. A blind subculture was performed after 7 days of incubation in case of previous negative subculture growth.

Culture of pus and fluid samples

A loopful of pus and fluid samples were inoculated in BA and MA plates. In case of pus and wound swab, they were rubbed at the side of BA as well as MA plates. The specimen rubbed o the BA and MA plates, was spread in the medium with the help of a sterile inoculating loop [21].

Culture of other samples

Solid tissue sample was homogenized in autoclaved mortar and pestle. Then the homogenized sample was inoculated in Nutrient Broth (NB). The catheter tips were also inoculated in NB and incubated at 37°C. Subculture on MA and BA was done the next day with a loopful of broth.

For stool and perianal swab, MA and SS agar were used. A light inoculums of faeces was placed in the middle of the agar plates and streaked it up and down and across the plate. If bacteria or fungi had been seen in the Gram-stained smear, the appropriate media were used for culture of Cerebrospinal Fluid (CSF). If no organisms had been seen, or if the interpretation of the Gram smear became unclear, MA, BA and CA (with Bacitracin and Optochin) were used [22].

All BA and MA plates used for all samples were inoculated at 37°C for 24 hours while CA was incubated in candle jar at 37°C for 24 hours. Urine contributing significant growth was processed for identification of isolate. Pathogenic growth was sought in stool, perianal swab, sputum, and throat swab. In the case of fluid, CSF, pus, blood and tissue growth of the isolate was examined. So, single colony was assessed as offending organism [21].

Identification of isolates

Isolates from different samples were identified by using microbiological tools and techniques as described in the Bergey's manual of systematic bacteriology which involves morphological appearance of the colonies, staining reaction and biochemical properties. Each of the organisms was isolated in pure form before performing biochemical and other tests. The biochemical tests used for the identification include Catalase test, Oxidase test, Sulfide Indole and Motility (SIM) test, Methyl red test, Voges Proskauer test, Citrate utilization test, Oxidation Fermentation test, Triple Sugar Iron (TSI) test and Nitrate reduction test [23-24].

Antibiotic Susceptibility testing

The antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by modified Kirby-Bauer M2-A9 disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton agar (MHA).

Screening and confirmation of ESBL producers

Screening of the suspected ESBL isolates was performed according to the guidelines for screening issued by NCCLS in 2005. According to these guidelines, the zone diameter for possible ESBL isolates is ≤ 27 mm for Cefotaxime (30 µg), and ≤ 22 mm for Ceftazidime (30 µg). The suspected ESBL isolates were tested for confirmation by using the Double Disc Diffusion Synergy Test (DDST) method, using Co-amoxiclav 20 + 10 µg disc and Cefotaxime and Ceftazidime 30 µg discs placed 20 - 30 mm away from it. ESBL production was confirmed when the zone of either cephalosporin was expanded by the Clavulanate.

Steps for confirmation of ESBL producers

- Suspected isolate of ESBL producing organism was inoculated in nutrient broth and incubated for 4 - 6 hours.
- The standard inoculum size was carpet cultured onto MHA plates.
- After few minutes, the plates were incorporated with Co-amoxiclav disc between ceftazidime and cefotaxime.
- After overnight incubation, the results were interpreted as stated above.

Data analysis

Data analysis was made from the statistical package of Winpepi software Version 5.6. Chi-square test was used to determine significant association of dependent variables.

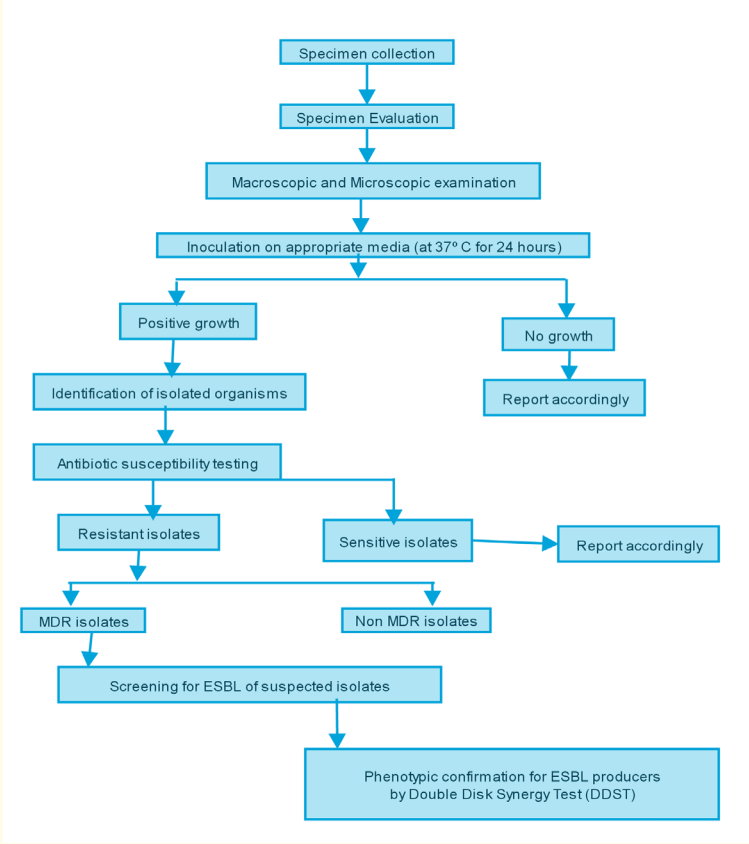

Figure 1: *Flow chart for processing of different specimens and identification of ESBL.*

Results

Distribution of samples

The study was conducted in Kathmandu Model Hospital, Kathmandu from September 2008 to April 2009. During the study period, a total of 4898 samples from 15 different specimens were collected and processed from patients requesting for bacterial culture.

Among total samples, the highest number of samples collected were urine which accounted for 55.1% (N = 2702) of total samples. This was followed by blood which comprised of 1264 samples (25.80%) (Figure 2).

The most predominant samples were from outdoor patients with 4555 (92.99%) cases and only remaining 343 (7.01%) were from inpatients. The request of culture from male was found higher than female. Male sample comprised 2840 (58%) whereas only 2058 (42%) samples were obtained from female.

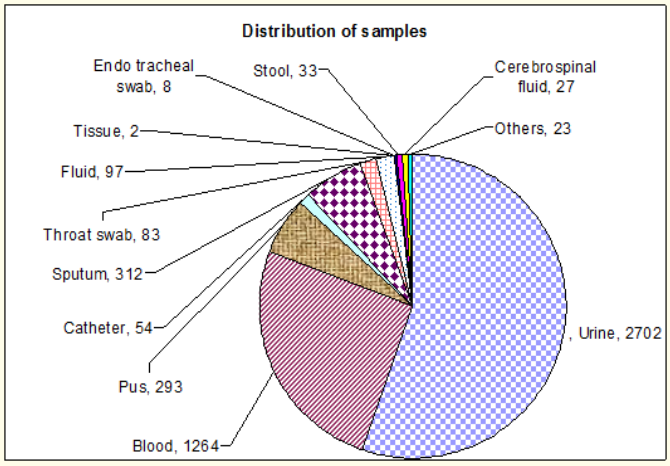

Figure 2: *Distribution of Samples.*

Bacterial Growth Pattern

Out of 4898 samples processed for culture, 932 samples showed bacterial growth. The highest growth was contributed by pus (69.28% i.e. 206 out of 293 pus samples), followed by catheter (61.11%, i.e. 33 out of 54 samples) and urine (21.13% i.e. 571 out of 2702 samples). Out of total 2702 urine samples, 698 (35.83%) urine samples showed no growth, 1433 (53.03%) showed no significant growth and 571 (21.13%) samples showed significant growth. Regarding total sample number, urine sample was followed by blood sample where bacterial growth was observed in 97 samples (7.67%) out of 264 blood samples. Out of 15 different types of samples received and processed during the research period, 3 types of samples viz. fluid, tissue and endotracheal swab showed no growth at all even though their sample size were 97, 2 and 8 respectively (Table 1).

Sample	Total	Growth seen	Growth not seen	Positivity
Urine	2702	571	2131	21.13 %
Blood	1264	97	1167	7.67 %
Pus	293	206	87	69.28 %
Catheter	54	33	21	61.11 %
Sputum	312	6	306	1.92 %
Throat swab	83	3	80	3.61 %
Fluid	97	0	97	0 %
Tissue	2	0	2	0 %
Endo tracheal swab	8	0	8	0 %
Stool	33	1	32	3.03 %
Cerebrospinal fluid	27	3	24	11.11 %
Others	23	12	11	52.17 %
Total	4898	932	3966	19.02 %

Table 1: *Distribution of Samples Along with Bacterial Growth.*

Of the total 4555 outdoor samples, 775 (17.01%) samples showed growth, whereas from 343 indoor samples, 157 (45.77 %) showed bacterial growth (Figure 3).

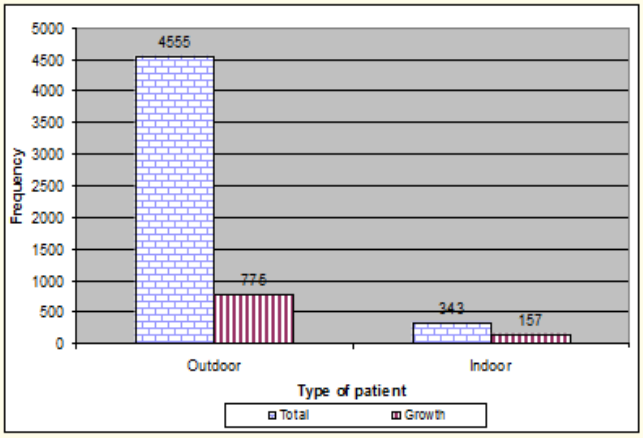

Figure 3: *Growth Pattern of In and Out Patients.*

Similarly, from 2840 male samples, 451 samples revealed growth whereas in case of female samples, out of 2058 samples, 481 samples revealed bacterial growth (Figure 4).

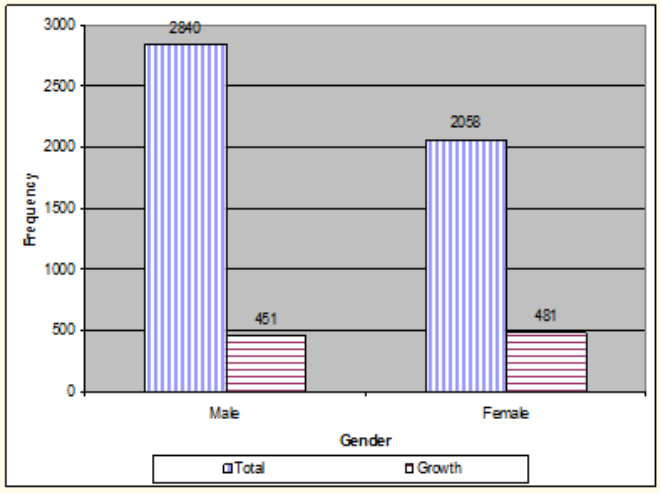

Figure 4: *Gender Wise Growth Pattern of Samples.*

Gram negative bacteria were found predominant constituting 641 out of 932 (78.1%) (Figure 4). Among the gram-negative bacteria, urine contributed 571 (69%) isolates.

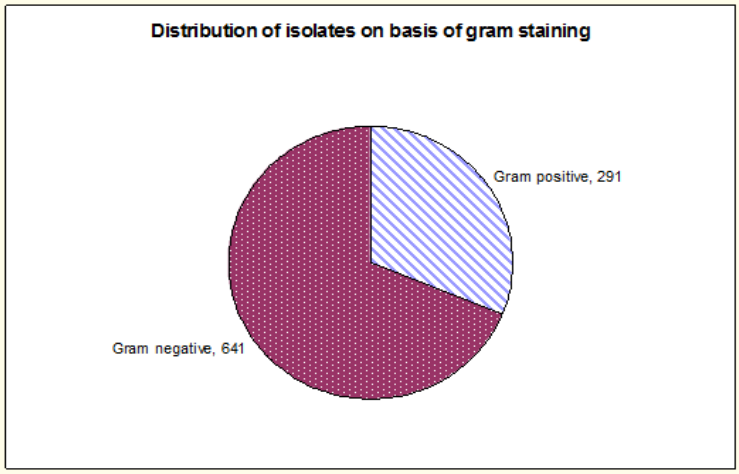

Figure 5: *Distribution of Isolates on the basis of Gram Staining.*

The distribution of organism varies in different samples, and similar was the case of their predominance in those samples. Out of total isolates, *E. coli* was found the most predominant isolates with 510/932 isolates. Among gram negative bacteria, obviously, *E. coli* was found most frequently isolated species with 510 isolates, among which, 450 were isolated from urine sample. Similarly, out of 291 isolates of gram positive bacteria, *S. aureus* was the most predominant pathogen with 102 isolates, among these 74 isolates were from pus samples. *Salmonella enterica* serovar Typhi was found to be the predominant pathogen isolated from the blood samples with 66 out of 97 (68.04 %) isolates (Table 2).

Similarly, least pathogen found from the outdoor patients was *Acinetobacter* spp. and *S. dysenteriae* growing only one out of 775 isolates. In the case of indoor patients, the most predominant pathogen was found to be *E. coli* again. Least isolated organisms from indoor samples were *Morganella* spp., *S. pneumoniae*, *Proteus* spp. and *N. gonorrheae* all of which were isolated from urine, sputum, pus and CSF samples respectively (Table 2).

Organisms	Urine		Pus		Blood		Other samples	Total
	Isolates	%	Isolates	%	Isolates	%		
E. coli	450	79	41	20			19	510
Citrobacter spp	40	7	21	10			17	78
CoNS	22	4	24	12			3	50
S. aureus	17	3	74	36			11	102
E. faecalis	16	3	1	0.5				17
Enterobacter spp	11	2	4	2			3	18
Pseudomonas spp	11	2	13	6			1	25
Morganella spp	1	0.1						1
S. paratyphi	1	0.1			31	32		32
Corynebacterium spp			2	1				2
S. typhi			2	1	66	68		68

S. viridans			24	12				24
S. pneumoniae							1	1
S. dysenteriae							1	1
Proteus spp	1	0.1						1
Acinetobacter spp							1	1
N. gonorrheae							1	1
Total	571		206		97		58	932

Table 2: *Distribution of Organisms in Major and Total Samples.*

Antibiotic susceptibility pattern of the isolates

Antibiotic susceptibility pattern of Gram negative isolates

Out of 15 common antibiotics used against Gram negative isolates, Imipenem was found the drug of choice with a susceptibility of 97.22% (193/198) which was followed by Chloramphenicol, Amikacin and Nitrofurantoin with susceptibility of 96.63% (456/475), 95.12% (452/475) and 91.07% (367/403) respectively. The least effective drugs among the tested 15 antibiotics are Cefoxitin with resistivity of 90.28% (182/202) followed by Amoxycillin 71.37% (339/475), Co-trimoxazole 46.93% (222/473) and Ciprofloxacin 42.56% (206/484) respectively (Table 3).

S.N.	Antibiotics	Resistant	Percentage	Out of
1	Amoxycillin	339	71.37	475
2	Cefixime	216	40.62	532
3	Ciprofloxacin	206	42.56	484
4	Co-trimoxazole	222	46.93	473
5	Cefotaxime	218	34.64	630
6	Nitrofuratoin	35	8.93	403
7	Norfloxacin	183	40.20	456
8	Ofloxacin	194	39.51	492
9	Ceftraixone	101	27.37	368
10	Chloramphanicol	16	3.37	475
11	Gentamycin	73	22.11	332
12	Amikacin	22	4.84	475
13	Ceftazadime	95	26.74	355
14	Imipenem	4	2.78	198
15	Cefoxitin	182	90.28	202

Table 3: *Resistivity of Gram Negative Organisms against Various Antibiotics.*

Antibiotic susceptibility pattern of Gram positive isolates

Out of 13 different common antibiotics used against Gram positive isolates, Imipenem 84.62% (22/26) was found to be the most effective. Other susceptible antibiotics include Cephalexin 80.21% (154/192). Likewise, the least effective antibiotics include Cefoxitin with 92.31% resistant (24/26) and Ofloxacin with resistivity of 80.00% (152/190). Other less effective antibiotics include Norfloxacin and Gentamycin which exhibited resistivity of 64.58% (124/192), and 55.02% (104/189) respectively (Table 4).

S.N.	Antibiotics	Resistant	Percentage	Out of
1	Amoxycillin	116	59.48	195
2	Cefixime	88	45.12	195
3	Ciprofloxacin	59	31.89	191
4	Co-trimoxazole	58	30.52	190
5	Cefotaxime	60	31.49	191
6	Norfloxacin	124	64.58	192
7	Ofloxacin	152	80.00	190
8	Erythromycin	52	27.22	191
9	Cloxacillin	46	24.46	188
10	Gentamycin	104	55.02	189
11	Cephalexin	38	19.79	192
12	Imipenem	4	15.38	26
13	Cefoxitin	24	92.31	26

Table 4: *Resistivity of Gram Positive Isolates against various Antibiotics.*

Distribution of MDR Isolates from different samples

Out of total 932 isolates, 536 (57.51 %) were found to be MDR isolates from all samples. Most of the Multi Drug Resistivity (MDR) isolates were from gram negative which accounts for 434 out of 641 (67.70 %).

The most predominant isolate was found to be *E. coli* with 510 (54.72%) isolates. In the case of indoor patients, the most predominant pathogen was found *E. coli* with 54.3% isolates; among these 72.33% were MDR isolates.

Out of 510 isolates of *E coli*, 337 isolates are MDR among which 198 are resistant to more than 4 classes of antibiotics. Similarly, 32 out of 41 *E. coli* isolates are MDR in case of pus samples with 23 isolates resistant to more than 4 group of antibiotics. In case of *S. aureus* from urine, 10 out of 17 isolates are MDR, whereas in case of pus 55 out of 74 are MDR (Table 5).

Organisms	Resistant in Urine sample				Resistant in Pus sample				Resistant in Total Samples			
	< 2	2-4	> 4	Total	< 2	2-4	> 4	Total	< 2	2-4	> 4	Total
E. coli	140	145	165	450	9	9	23	41	173	139	198	510
Citrobacter spp.	12	17	11	40	6	2	13	21	26	29	23	78
Enterobacter spp.	4	3	4	11		2	2	4	9	2	7	18
Pseudomonas	2	5	4	11	4	6	3	13	9	11	5	25
S. Typhi					2			2	68			68
S. Paratyphi	1			1					32			32
Morganella			1	1							1	1
Proteus spp.	1			1					1			1
S. aureus	7	7	3	17	19	42	13	74	30	55	17	102
CoNS	9	6	7	22	10	11	3	24	22	16	12	50

S. viridans					18	4	2	24	18	4	2	24
E. faecalis	6	6	5	17	1			1	5	5	7	17
Corynebacterium					2			2	2			2
S. pneumoniae										1		1
S. dysenteriae										1		1
Acinetobacter spp.									1			1
N. gonorrheae									1			1
Total	182	189	200	571	67	76	59	206	396	264	272	932

Table 5: *Distribution of Multidrug Resistivity Pattern of different Organisms.*

Similarly, the MDR remained more or less similar in case of gram positive and negative isolates and even in different samples, the highest MDR being found in gram negative organisms isolated from pus. The multidrug resistivity pattern of organisms in urine sample was found to be insignificant with p-value of 0.424. But, the multidrug resistivity pattern of isolates from pus samples was found to be statistically significant with $p < 0.001$.

Organisms	Urine		Pus		Total	
	MDR	Out of	MDR	Out of	MDR	Out of
Gram negative	290	514	71	83	434	641
Gram positive	29	57	52	123	102	291
Total	319	571	123	206	536	932

Table 6: *Multidrug Resistivity Pattern of Gram Positive and Negative Isolates.*

The positivity of the culture was found to be higher in female (23.37%) than in male (15.88%) with significant result ($p < 0.001$). (Table 7) The higher multidrug resistivity was found in male in-patient (73.85%) than in male out-patient (51.30%) which was found to be highly significant ($p = 0.001$). The higher multidrug resistivity was found in female in-patient (77.17%) than in female out-patient (56.30%) which was found to be highly significant ($p < 0.001$). Similarly, MDR pattern was found to be higher in female (60.29%) than in male (54.55%) but the result was found to be statistically insignificant ($p = 0.076$).

Pattern of resistance	Male			Female			Grand total
	In patient	Out patient	Total	In patient	Out patient	Total	
MDR	48	198	2840	71	219	2058	536
Non MDR	17	188		21	170		396
Total	65	386		92	389		932

Table 7: *Distribution of Multidrug Resistivity Pattern Of Isolates Among Gender.*

Antibiotic Susceptibility Pattern of *E. coli*

The antibiotic susceptibility pattern of *E. coli* showed that Amikacin was the most effective antibiotic with sensitivity of 97.82% (494/505) followed by Imipenem, Nitrofurantoin and Chloramphenicol with sensitivity of 97.65% (166/170), 95.78% (431/450) and 95.69% (444/464) respectively. Similarly, the least effective antibiotic against *E. coli* isolates was Amoxycillin with resistivity of 69.65% (342/491), followed by Co-trimoxazole and ciprofloxacin with resistivity of 47.52% (240/505) and 44.36% (224/505) respectively. The pattern of resistivity was similar in case of *E. coli* isolated from urine and pus samples too (Table 8).

Antibiotics	Urine		Pus		Total	
	R/T	%	R/T	%	R/T	%
Amoxycillin	310/450	68.89	32/41	78.05	342/491	69.65
Cefixime	185/450	41.11			194/464	41.81
Ciprofloxacin	191/450	42.44	21/41	51.22	224/505	44.36
Co-trimoxazole	212/450	47.11	19/41	46.34	240/505	47.52
Cefotaxime	146/450	32.44	29/41	70.73	175/491	35.64
Nitrofurantoin	19/450	4.22			19/ 450	4.22
Norfloxacin	186/450	41.33			200/464	43.10
Ofloxacin	184/450	40.89			198/464	42.67
Ciprofloxacin	109/450	24.22	16/41	39.02	136/505	26.93
Chloramphenicol	14/450	3.11			20/464	4.31
Gentamycin	75/450	16.67	16/41	39.02	101/505	20.00
Amikacin	4/ 450	0.89	1/41	2.44	11/ 505	2.18
Ceftazidime	108/450	24.00	16/41	39.02	133/ 491	27.08
Imipenem	3/151	1.98	1/19	5.26	4/170	2.35

Table 8: *Distribution of Resistivity Pattern of E. coli against different Antibiotics.*

Note: *R = Resistant and T = Total*

Antibiotic Susceptibility pattern of *S. aureus*

The antibiotic susceptibility pattern of *S. aureus* showed that Erythromycin is the most effective antibiotic with susceptibility of 78.95% (75/95), followed by Cephalexin and Ciprofloxacin with sensitivity of 83.96% (71/96) and 71.57% (73/102) respectively. Similarly, the least effective antibiotic was Amoxycillin with resistivity of 75.49% (77/102), followed by Ofloxacin and Co-trimoxazole with resistivity of 60.82% (59/97) and 46.08% (47/102) respectively. The pattern of *S. aureus* isolated from both urine and pus samples exhibited the highest resistivity against Amoxycillin (64.71% and 77.03% resistance in *S. aureus* isolated from urine and pus samples respectively. But *S. aureus* isolated from urine showed the least resistance against cefixime but that from pus showed the least resistance against Erythromycin (Table 9).

Antibiotics	Urine			Pus			Total		
	R	T	%	R	T	%	R	T	%
Amoxicillin	11	17	64.71	57	74	77.03	77	102	75.49
Cefixime	2	17	11.76	35	72	48.61	42	99	42.42
Ciprofloxacin	8	17	47.06	19	74	25.67	29	102	28.43
Co-trimoxazole	3	17	17.65	38	74	51.35	47	102	46.08
Cefotaxime	6	17	35.29	27	74	36.49	37	98	37.75
Ofloxacin	10	17	58.82	46	74	62.16	59	97	60.82
Erythromycin	6	17	35.29	12	70	17.14	20	95	21.05
Cephalexin	7	17	41.18	17	72	23.61	25	96	26.04

Table 9: *Antibiotic Resistivity Pattern of S. aureus isolated from different Samples.*

Note: *R = Resistant and T = Total*

Out of 15 S. aureus isolated from 2702 urine samples, 1 was Methicillin- Resistant Staphylococcus aureus (MRSA) and out of 74 S. aureus isolated from 293 pus samples, 4 isolates were MRSA. All of the MRSA isolates are found to be sensitive to Vancomycin (Table 10).

Urine			Pus			Total MRSA	MRSA resistant to Vancomycin
Total Samples	Isolation of *S. aureus*	MRSA	Total Samples	Isolation of *S. aureus*	MRSA		
2702	17	1	1264	74	4	5	0

Table 10: *MRSA from different samples.*

Pattern of ESBL on isolates from primary screening

For ESBL confirmation test by DDST technique, primary screening was done by studying sensitivity of the isolates against Cefotaxime. Those isolates that exhibited zone of inhibition ≤ 27 mm against Cefotaxime were processed for confirmation test. Among different isolates that were screened through primary screening, *E. coli* was found to exhibit the highest rate of ESBL (88 out of 174 primarily screened isolates i.e. 50.57%), followed by *Enterobacter* spp (2 out of 9 primary screened isolates i.e. 22.22%), *Citrobacter* spp. (6 out of 23 i.e. 18.75%) whereas *Pseudomonas* spp., CoNS, *S. aureus* and *Enterobacter* spp. exhibited no ESBL isolates even though some isolates were resistant to Cefotaxime. In total, 14.99% ESBL were observed (Table 11).

Organisms	Total	ESBL Susceptible isolates	ESBL confirmatory test		
			Tested isolates	Positive	%
Citrobacter spp	78	36	23	6	18.75
CoNS	50	16	12	0	0
E. coli	510	175	174	88	50.57
E. faecalis	18	12	6	1	16.66
S. aureus	102	37	12	0	0
Pseudomonas spp.	27	18	8	0	0
Enterobacter spp	18	18	9	2	22.22
S. viridians	24	9	9	0	0
Total	827	321	253	97	38.33

Table 11: *Distribution of ESBL positive isolates.*

Most ESBL positive organisms were isolated from catheter samples where 14 out of 19 (42.42%) primary screened isolates. Urine followed catheter with 67 ESBL positive out of 176 samples (16.73%). Out of 55 pus samples, 16 samples were found to be ESBL positive (Table 12).

Sample	Total samples	Cefotaxime resistant	ESBL confirmatory test		
			Samples	Positive	%
Catheter	33	19	19	14	42.42
Urine	571	251	176	67	16.73
Pus	206	55	55	16	7.76

Table 12: *Distribution of ESBL positive samples on the basis of sample.*

Among all 97 ESBL positive isolates tested against 16 antibiotics, Amoxycillin exhibited the least susceptibility (2.06%) with 2 isolates sensitive out of 97, followed by Cefixime (4.12%) with 4 isolates sensitive out of 97 isolates, and Ceftazidime as well as Ceftriaxone with susceptibility of (7.21%) with 14 isolates susceptible out of 97 isolates. Similarly, among the ESBL positive isolates, Imipenem was found to be the most effective antibiotic with 93.20% susceptibility, i.e. 88 out of 97 antibiotics was susceptible. Other susceptible antibiotic were Nitrofurantoin which was susceptible in 84.54% (i.e. 82 out of 97 isolates) and Chloramphenicol 80.41% (i.e. 78 out of 97 isolates) (Table 13).

Antibiotics	Total	Resistant		Sensitive	
		Number	%	Number	%
Amikacin	97	9	9.28	88	90.72
Amoxycillin	97	95	97.94	2	2.06
Cefixime	97	93	95.88	4	4.12
Cefoxitin	97	83	85.57	14	14.43
Ceftazidime	97	90	92.78	7	7.21
Ceftriaxone	97	90	92.78	7	7.21
Chloramphenicol	97	19	19.59	78	80.41
Ciprofloxacin	97	44	45.36	53	54.64
Cloxacillin	97	65	67.01	32	33.00
Co-trimoxazole	97	84	86.60	13	13.40
Erythromycin	97	67	69.07	30	30.93
Gentamycin	97	60	61.85	37	38.14
Imipenem	97	6	6.80	91	93.20
Nalidixic acid	97	82	84.54	15	15.46
Nitrofurantoin	97	15	15.46	82	84.54
Ofloxacin	97	84	86.60	13	13.40

Table 13: *Distribution of Antibiotic Resistivity of ESBL Positive Isolates.*

Discussion

The study was aimed to examine the status of Multiple Drug Resistance among different bacterial pathogens and underlying production of Extended Spectrum ß-lactamases. In context of Nepal, surveillance for drug resistance is very poor to estimate actual statistical data.

Out of total 2702 urine samples, 698 (35.83%) urine samples showed no growth, 1433 (53.03%) showed no significant growth and 571 (21.13%) samples showed significant growth. Dhakal [19] showed 25.16% positivity from urine samples. Similar studies carried out by Chhetri., *et al*. [26], Obi., *et al*. [27], Gautam [28], Manandhar [30], Ling., *et al*. [29] and Baral [25] showed low percentage of growth positivity. The low growth rate observed in this study might be due to inclusion of every patients requesting for urine culture regardless of their illness and symptoms. This data even signifies the health consciousness of people who promptly undergo laboratory investigation when they feel uncomfortable.

Out of total 2702 urine samples, 698 (35.83%) urine samples showed no growth, 1433 (53.03%) showed no significant growth and 571 (21.13%) samples showed significant growth. Dhakal [19] showed 25.16% positivity from urine samples. Similar studies carried out by Chhetri., *et al*. [26], Obi., *et al*. [27], Gautam [28], Manandhar [30], Ling., *et al*. [29] and Baral [25] showed low percentage of growth positivity. The low growth rate observed in this study might be due to inclusion of every patients requesting for urine culture regardless

of their illness and symptoms. This data even signifies the health consciousness of people who promptly undergo laboratory investigation when they feel uncomfortable.

Among the uropathogens, *E. col*i was found to be the most predominant organism (450 out of 571 isolates i.e. 79.00%) followed by *Citrobacter* spp. 40 i.e. 7.00%, *Enterobacter* spp. (11 i.e. 2.00 %) and *Psuedomonas* spp. (11 i.e. 2.00%). Higher prevalence of *E. coli* seen in this study also resembled the study done by various other workers viz. Shrestha [31], Chhetri., *et al*. [26], Sharma [32], Tuladhar [33], Jha and Yadav [34], Manandhar [30] and Dhakal [19] in Nepal and Steenberg., *et al*. [35], Kahlmeter [36] and Farnell., *et al*. [37] in the international context. *E. coli* has special virulent properties to cause UTI, thus being the major uropathogen throughout the world. *E. coli* can bind to the glycol-conjugate receptor (Gal α 1→4 Gal) of the uroepithelial cells of human urinary tract so it can initiate infection itself. *E. coli* is isolated in 90.0% of infections and isolates are characterized by unique virulence determinant, the p pilus (Gal-Gal receptor) [38]. *E. coli* is the most predominant organism to colonize the urethral meatus [39] and perineum [40] before ascending to the bladder. *C freundii* as second principal uropathogens were also found by Puri [41] with 27.2% of total isolates carried out at Om Hospital and Research Center, Kathmandu, Nepal [42].

Among the total isolates, 291 isolates (31.22%) were Gram positive bacteria. In a similar study performed by Blomberg., *et al*. [52-53] in Tanzania, 66.36% constituted Gram-negative isolates, Bomjan [54] reported 83.33% Gram-negative isolates in UTI at TUTH. In this study, the most predominant pathogens were *E. coli* (57.44%), followed by *Salmonella* Typhi (8.33%). *S. aureus* was the most predominant pathogen among Gram positive bacteria with 102 (out of 291 i.e. 35.05%) isolates followed by CoNS constituting 50 (17.18%) isolates. These pattern of results are compatible with findings from Manandhar [30] and Bomjan [54] where *E. coli* was the most predominant bacterial isolate whereas *S. aureus* was the predominant Gram-positive species in urine.

Among the common antibiotics used against all Gram-negative isolates, Imipenem was the drug of choice with susceptibility of 97.22% (194 out of 198) but this can only be used, if there were no alternative second line drugs of choice [8]. This was followed by Chloramphenicol, Amikacin and Nitrofurantion with susceptibility of 96.63% (459 out of 475), 95.16% (453 out of 475) and 91.07% (368 out of 403) respectively. Similarly, the most resistant drug among the tested ones were Cefoxitin with resistivity of 90.28% (182 out of 202) followed by Amoxycillin, Cotrimoxazole and Ciprofloxacin with resistivity of 71.37% (339 out of 475), 46.93% (222 out of 473) and 42.56% (206 out of 484) respectively. This finding was similar as reported by findings of Puri [41]; Bomjan [54]; Paneru [55]; Oteo., *et al*. [56] and Dhakal [19].

Among the common antibiotics used against all gram-positive isolates, Imipenem is the antibiotic of choice with susceptibility of 84.62% (22 out of 26), followed by Cephalexin and Cloxacillin with susceptibility of 79.83% (156 out of 192) and 75.32% (142 out of 188) respectively. Similarly, the antibiotics with the highest resistivity is Cefotoxin with resistivity of 92.31% (24 out of 26) followed by Ofloxacin, Norfloxacin and Amoxycillin with resistivity of 80.00% (152 out of 190), 65.00% (124 out of 192) and 59.74% (116 out of 195) respectively. Similar finding was observed by Tuladhar [33], Dhungel [43] and Baral [25].

Similarly, the MDR remained more or less similar in case of gram positive and negative isolates and even in different samples, the highest MDR being found in gram negative organisms isolated from pus. From pus sample, 135 MDR isolates were found out of 206 isolates whereas 389 isolates were MDR out of 571 in urine. Higher prevalence of MDR isolates from urine and pus were similar reported by Bomjan [54] and Banjara [18]. The multidrug resistivity pattern of organisms in urine sample was found to be insignificant with p-value of 0.424. But, the multidrug resistivity pattern of isolates from pus samples was found to be statistically highly significant with p-value of < 0.001. This finding supports that nosocomial pathogens are more virulent and more prone to cause multiple drug resistance as compared to community acquired bacterial pathogens. Generally, pus samples were obtained from hospitalized patients or patients undergone with surgery. So, in most of the cases, the pus formation is due to nosocomial infection. And in the nosocomial infections, the multidrug resistance organisms are more prone to occur since antibiotic sensitive organisms cannot adjust those patients already administering antibiotics.

Out of total 932 isolates from different samples, 534 isolates were found as MDR isolates. Among these, majority isolates were isolated from female outpatient with 219 MDR isolates. The burden of multiple drug resistance was found higher among hospital admitted patients. 119 out of 157 isolates (75.79%) from inpatients were MDR whereas 417 out of 775 isolates (53.80%) were MDR from outpatient sample. The result was statistically significant (p = 0.001). Similar findings were found by Bomjan [54] and Manandhar [58]. Antibiotics treatment and hospital infection control are intimately entwined. The prophylactic or empirical treatment of antibiotics alters the prevailing pathogens in the hospital setting and lead to establish drug resistant pathogens. So, the higher prevalence of MDR isolates found among inpatients was obvious.

The higher multidrug resistivity was found in male in-patient isolates (73.85% i.e. 48 out of 65 isolates) than in male out-patient isolates (51.30% i.e. 198 out of 386 isolates) which was found to be highly significant (p = 0.001). The higher multidrug resistivity was found in female in-patient isolates (77.17% i.e. 71 out of 92 isolates) than in female out-patient isolates (56.30% i.e. 219 out of 389 isolates) which was found to be highly significant (p < 0.001). Inpatients are highly susceptible to resistant bacteria present in hospital community and hospitalized persons where sensitive bacteria are easily killed by consumed antibiotics during stay in hospital leaving easy way for the survival of resistant isolates. Similarly, MDR pattern was found to be higher in female (60.29 %) than in male (54.55 %) but the result was found to be statistically insignificant (p = 0.076).

Antibiotic Sensitivity Pattern of *E. coli* and *S. aureus* were studied here as model organisms of Gram negative and Gram positive bacteria respectively also because of their higher frequency in causing infections. E. coli isolates from urine were found to exhibit the highest rate of resistivity with Amoxycillin i.e. 68.89% (310 out of 450) followed by Co-trimoxazole and ciprofloxacin with resistivity of 47.11% (212 out of 450) and 42.44% (191 out of 450) respectively. Similarly *E. coli* exhibited the highest rate of susceptibility against Amikacin being susceptible to 99.11% (446 out of 450) followed by Chloramphenicol and Nitrofurantoin with susceptibility of 96.89% (436 out of 450) and 95.78% (431 out of 450) respectively. Similar results were observed on *E. coli* isolated from pus with slightly different percentage of resistivity and sensitivity. The highest resistance was seen against Amoxycillin with 78.05% (32 out of 41) and highest sensitivity was observed against Amikacin with 97.56% (40 out of 41).

From 4898 samples, 932 isolates were isolated among which 321 isolates were found susceptible to ESBL, i.e. zone of inhibition against Cefotaxime ≤ 27 mm. From 321 ESBL susceptible isolates, 253 isolates were randomly selected for confirmatory test of Extended Spectrum ß-lactamase (ESBL) by Double Disk Synergy Test method. Among different isolates that were screened through primary screening, *E. coli* was found to exhibit the highest rate of ESBL (88 out of 174 primarily screened isolates i.e. 50.57%), followed by *Enterobacter* spp (2 out of 9 primary screened isolates i.e. 22.22%), *Citrobacter* spp. (6 out of 23 i.e. 18.75%) and *E. faecalis* (1 out of 6 primary screened isolates i.e. 16.66%) whereas *Pseudomonas* spp., CoNS, *S. aureus* and *Enterobacter* spp. exhibited no ESBL isolates even though some isolates were resistant to Cefotaxime. In total, 14.99% ESBL were observed from primary screened isolates for the test. A study done in Saudi Arabia showed 197 (6%) to be Multidrug Resistant (MDR) and 156 (4.8%) was positive for ESBL. Kander and Kumar [57] detected 72 (6.5%) *E. coli* isolates as ESBL producers. The similar work conducted by Baral [25] detected 28.12% cases of confirmed ESBL by DDST method.

By the early 1990s, 25 to 35% of nosocomically acquired Klebsiella pneumoniae isolates in France were ESBL producing [59]. There is considerable geographical difference in the occurrence of ESBLs. Among countries, territories, within countries, hospital-to-hospital variability in occurrence may also be marked [60]. A common environmental source of ESBL producing organisms has occasionally been discovered. These findings were analyzed to find out whether similar or dissimilar *E. coli* isolates are responsible for the ESBL production with similar drug resistance traits.

Major ESBL producing isolates were found from catheter samples (42.42 % i.e. 14 out of 19 samples susceptible to Cefotaxime) followed by urine and pus with positive cases of 16.73% and 7.76% respectively. This indicates stronger propensity of ESBL producers towards uropathogens. Bomjan [54] had found similar prevalence of ESBL producers (29.26%) among *E. coli* and *Klebsiella pneumoniae* isolates of UTI. Most of the ESBL producers were found resistant to multiple common drugs used.

Their antibiotic sensitivity test revealed that they were found to be resistant to most of commonly used antibiotics. The ESBL isolates were found to be 97.94% resistant (95/97) to Amoxycillin followed by Cefixime with resistivity of 95.88% (93/97). Ceftazidime and Ceftriaxone both with resistivity of 92.78 % (7/97). Similarly, the isolates were 93.20% sensitive (91/97) to Imipenem followed by Amikacin, Nitrofuratoin and Chloramphenicol with sensitivity of 90.72% (88/97), 84.54% (82/97), and 80.41% (78/97) respectively. Most of the commonly used and available antibiotics are found resistant to these isolates. These findings indicate stronger propensity of ESBL producers towards multiple drugs resistant limiting few therapeutic options for the treatment of ESBL producing offending bacteria.

Conclusion

E. coli was found the most predominant isolates with 510/932 (54.72%) isolates among which 450 were from urine. Similarly, out of 310 isolates of gram positive bacteria, 102 isolates were *S. aureus* with 74 isolates from pus samples. Among the tested 15 antibiotics, Imipenem was the drug of choice whereas the least effective one is cefoxitin for both gram positive and negative. For *E. coli*, Amikacin was drug of choice whereas Amoxycillin was the least effective.

For *S. aureus*, Erythromycin was the drug of choice whereas Amoxycillin was the least effective.

Out of 15 *S. aureus* isolated from 2702 urine samples, 1 was Methicillin Resistant Staphylococcus aureus (MRSA) and out of 74 *S. aureus* isolated from 293 pus samples, 4 isolates were MRSA. All of the MRSA isolates were found to be sensitive to Vancomycin. The positivity of the culture was found to be significantly higher in female (23.37%) than in male (15.88%). Similarly, MDR pattern was found to be higher in female (60.29 %) than in male (54.55 %) but the result was found to be statistically insignificant. Among 526 MDR isolates, 321 were susceptible to ESBL production, the primary screening test of ESBL. Random selection of 253 from the primarily screened bacteria resulted into 97 confirmed cases of ESBL production. Imipenem was found to be the most effective antibiotic against ESBL positive isolates whereas Amoxycillin exhibited the least susceptibility.

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