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Prevalence and antimicrobial susceptibility Pattern of ESBL producing Gram Negative Bacilli

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Abstract : The production of extended-spectrum- β lactamases (ESBLs) is an important mechanism for resistance to the third-generation cephalosporins. Awareness and the detection of these enzymes are necessary for optimal patient care. To determine the prevalence and the antibiotic sensitivity pattern of ESBL producing gram negative bacilli. A prospective study was conducted at a tertiary care teaching hospital. The ESBL producing organisms has been steadily increasing over the past years. The detection and treatment of these ESBL organisms are extremely limited. In the present study, to determine the Extended Spectrum Beta Lactamases producing organism which were isolated from various samples of Multispecialty hospitals in Chennai.
Key Words : Extended-spectrum- β lactamases, double –disk approximation test, Combination disk method.

1. Introduction

Infections caused by multidrug-resistant bacteria expressing extended-spectrum β-lactamase (ESBL) pose serious challenges to clinicians. ESBL producing bacteria are resistant to a broad range of β-lactams, including third generation cephalosporins, nosocomial infections caused by these organisms complicate therapy and limit treatment options [1]. Extended spectrum β-lactamase (ESBL) isolates were first detected in Western Europe in the mid-1980s. Since then, their incidence has been increasing steadily. ESBLs are able to hydrolyze 3 and 4 generation cephalosporins and monobactams. ESBL producing strains are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) [2]. Hospitals worldwide are faced with increasingly rapid emergence and spread of antibiotic resistant bacteria. Both antibiotic-resistant gram-negative bacilli and gram-positive bacteria are reported as important causes of hospital-acquired infections [3, 4]. Increasing antimicrobial resistance remains as one of the major problem in hospital as well as in community settings. This is mainly because of the acquisition of new mechanisms of antimicrobial resistance, poor infection control practices and improper use of antimicrobial drugs which in turn increase the emergence of newer antibiotic resistant strains. Antimicrobial drug resistance among the pathogens represents an ongoing worldwide therapeutic challenge. The growing bacterial resistant to antibiotics may lead to an increase in appropriate empirical antimicrobial treatment of infections with a delay in the correct therapy [5-7]. Appropriate use of antibiotics in health care setting is important. It improves morbidity and mortality rate. Judicious use of antibiotics by the clinicians requires ample knowledge about the mechanism of action of each antibiotic, bacterial antibiotic resistance profile, and to have thorough knowledge of which bacterial species is prevalent in that particular locality. Inappropriate administration of antibiotics in suboptimal doses and repeated use of single class of antibiotics for prolonged or reduced duration of time will also increase the prevalence rate of

drug resistance. Therefore, all clinical microbiology laboratories should perform internationally recognized and updated antimicrobial susceptibility testing methods. They should ensure the appropriate use of antibiotics which can play a major role in reducing the emergence and spread of drug resistant bacterial strains.

2. Materials and methods

2.1 Study design

This descriptive study was carried out in the department of Microbiology at Chettinad Hospital and Research Institute, Kelambakkam, Chennai. The strains were collected from clinical samples obtained from patients who attended the inpatient departments of medicine, surgery, obstetrics and gynecology and outpatient department of Chettinad Hospital and Research Institute. The study was conducted during the period from 2009 to 2013. Clinical samples collected from the patients include urine, pus, sputum, throat swab, pleural fluids, endotracheal aspirates, bronchoalveolar lavage, wound swab, central venous catheter tip and all body fluids. The samples were processed for the identification of organisms according to standard procedures. Laboratory work was carried out in the department of Microbiology in Chettinad Hospital and Research Institute, Chennai. All clinical samples were routinely cultured on MacConkey and blood agar plates at 37 °C aerobically for 18 hours. Gram negative isolates were further characterized by standard biochemical tests.

2.2 Conventional method of identification of Gram negative bacilli:

Identification of Gram negative bacilli was carried out based on colony morphology, Gram staining, Pigment production, growth at 37 °C, motility and other biochemical characters.

2.3 Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing of the isolated organisms was done by Kirby Bauer disk diffusion method as per the recommendation of the CLSI guidelines. (Clinical and Laboratory Standards Institute performance standards for the antimicrobial susceptibility testing- CLSI document.2011; M100-s20.Wayne PA.USA.)All the strains were tested for the antimicrobial susceptibility pattern. The isolates were tested for the sensitivity pattern to first and second line of drugs.

The isolated organisms were tested against Ampicillin (A-10µg), Amikacin(AK-30µg), cefepime (Cpm-30µg), ceftazidime (Caz-30µg), ciprofloxacin (Cf-5µg), cefotaxime(Ce-30µg), co-trimoxazole(Cot-23.75/1.25µg), gentamicin (G-10µg), Imipenem (Imp -10µg), meropenem (Mr-10µg), piperacilli-tazobactam (Pt-100µg/10µg), polymyxin-B (Pb—300 units), colistin, Aztreonam(Az-30µg), cefipime (Cpm-30µg), netilmicin (Net-30µg), Ofloxacin (Of-5µg), tobramycin (Tb-10 µg) were used and in case of urine samples nitrofurantoin(Nit-300µg), Norfloxacin (Nx-10µg) were also used.

2.4 Interpretation and Reporting of the Results

1. Using the published CLSI guidelines, the susceptibility or resistance of the organism to each drug tested were determined. For each drug, on the recording sheet whether the zone size is susceptible (S), intermediate (I), or resistant (R) is indicated based on the interpretation chart.
2. All the strains which showed a diameter of less than 27mm for cefotaxime and less than 25mm for ceftriaxone were selected for checking the ESBL production. Disc diffusion method as shown in Fig.1

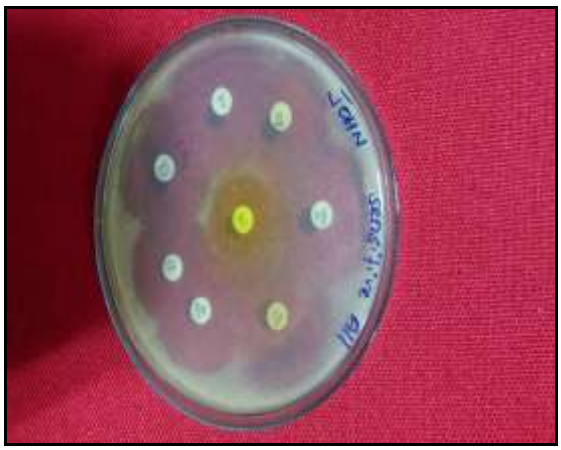

Fig.1 Disc Diffusion method

3. Phenotypic Identification of Esbl Production

3.1 Testing for the ESBL Production by MDDST Method

The ESBL production was tested by the Modified Double Disc Synergy Test (MDDST) by using a disc of Piperacillin/ Tazobactam (100/10 µg) along with cefotaxime. A lawn culture of the organisms was made on a Mueller-Hinton agar plate, as was recommended by CLSI (Clinical and Laboratory Standards Institute.) Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement M100-S19 [8]. A disc which contained Piperacillin/ Tazobactam (100/10 µg) was placed in the centre of the plate. The discs of Cefotaxime were placed 15mm apart to that of the Piperacillin/ Tazobactam (100/10 µg) [9] .Any distortion or increase in the zone towards the disc of Piperacillin/ Tazobactam (100/10 µg) was considered as positive for the ESBL production.

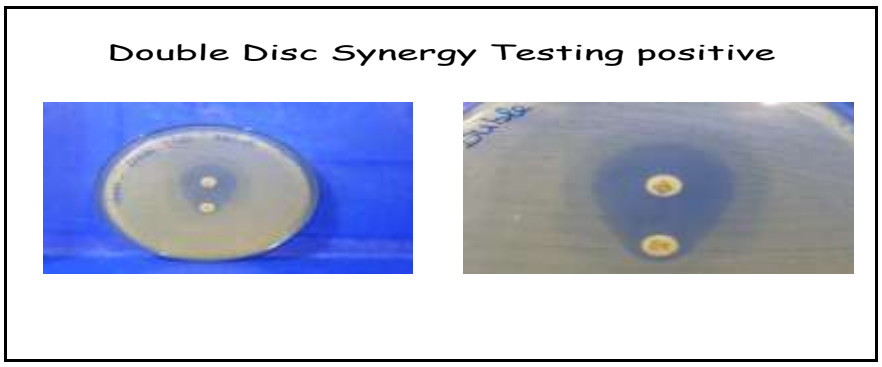

Fig.2 Double disc synergy

3.3 AmpC Detection

AmpC detection was carried out according to Jennifer et.al [10] All the isolates which showed a synergistic effect with cefotaxime only in MDDST were further tested for the AmpC enzyme production by AmpC disc test after an initial screening with a cephoxitin (30 µg) disc. A lawn culture of a 0.5 McFarland's suspension of ATCC *E.coli* 25922 was prepared on a Mueller-Hinton agar plate. A 30 µg cephoxitin disc was placed on the inoculated surface of the agar. A sterile plain disc (6mm) which was inoculated with several colonies of the test organism was placed beside the cephoxitin disc, almost touching it. After an overnight incubation at 37°C, the plates were examined for either an indentation or a flattening of the zone of inhibition, which indicated the enzyme inactivation of cephoxitin (positive result), or an absence of distortion, which indicated no significant inactivation of cephoxitin (negative result).

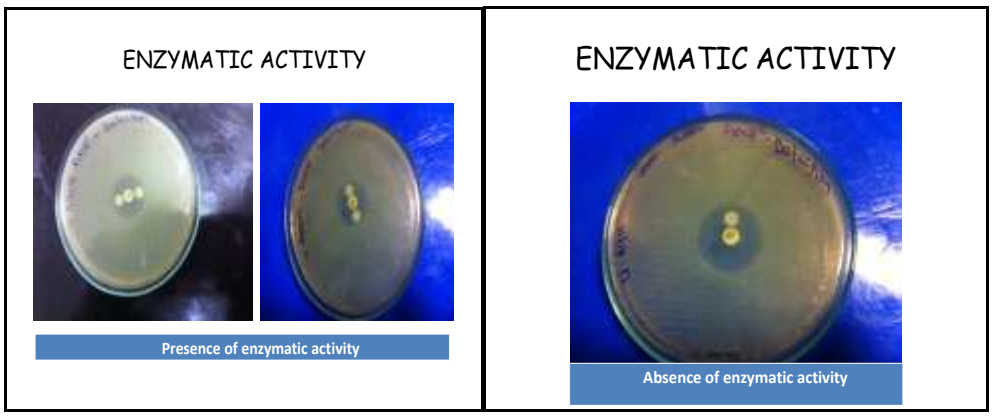

Fig.3. Enzymatic activity

4. Results and Discussion

A total of 6672 clinical samples obtained from Chettinad Hospital and Research Institute, a tertiary care hospital, during a period of 2009 to 2013 were included in this study. Sample type includes urine - 4272, Exudates - 1759 and Respiratory samples - 641. (Table-1)

Table 1: Type and Number of Clinical Samples Obtained From Patients

Type of Clinical sample obtained from patients	No. of patients	% of various clinical samples included in the study
Urine	4272	64.028%
Exudate	1759	26.036%
Respiratory	641	09.607%
Total	6672	100.0%

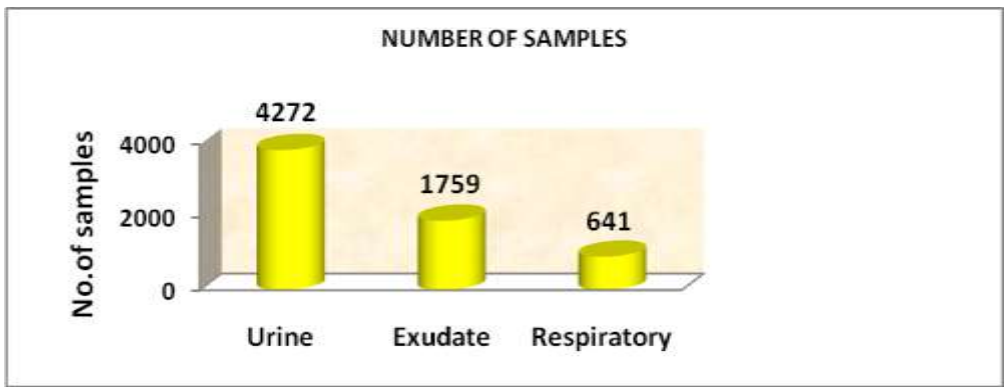

Chart 1: Type and Number of Clinical Samples Obtained From Patients

The distribution of the age group of the patients from whom the samples were obtained is shown in (Table-2).

Table 2: AGE Distribution of Patients from Whom Samples Collected

Age group	No. of patients	%
0 -10 years	530	7.9
11 - 20 years	383	5.7
21 - 30 years	1853	27.8
31 - 40 years	883	13.2

	41 - 50 years	1027	15.4
	51 - 60 years	886	13.3
	61 - 70 years	856	12.8
	71 - 80 years	216	3.2
	81 - 90 years	38	0.6
	Total	6672	100.0

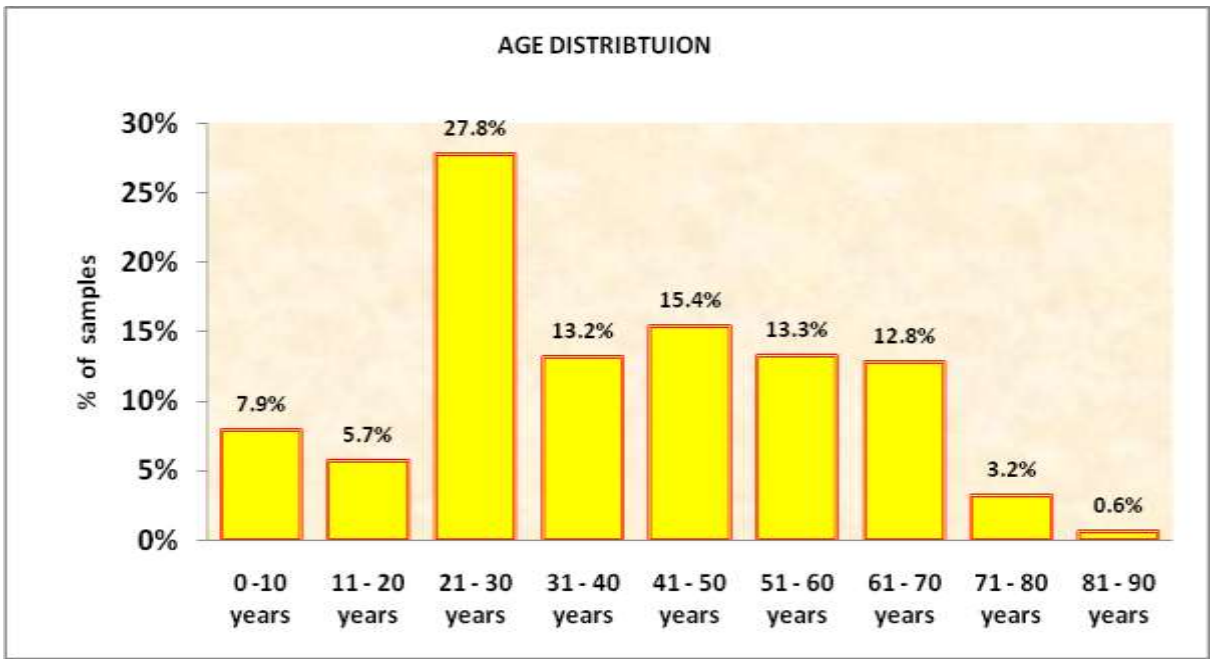

Chart :2 Age Distribution of Patients from Whom Samples Collected

Table 3: Significance for Age Distribution of Patients from Whom Various Samples Collected

Age distribution	**Type of clinical specimen collected**						**Significance for Age – wise distribution of clinical samples using Chi square test**
	Urine		**Exudate**		**Respiratory**		
	n	**%**	**n**	**%**	**N**	**%**	
0 -10 years	405	10.5	115	6.0	10	1.1	$\chi 2$=803.69 p=0.001***significant
11 - 20 years	270	7.0	99	5.2	14	1.5	
21 - 30 years	1391	36.1	397	20.8	65	7.1	
31 - 40 years	448	11.6	303	15.9	132	14.4	
41 - 50 years	421	10.9	411	21.6	195	21.3	
51 - 60 years	395	10.3	296	15.5	195	21.3	
61 - 70 years	407	10.6	216	11.3	233	25.5	
71 - 80 years	97	2.5	54	2.8	65	7.1	
81 - 90 years	19	.5	14	.7	5	.5	

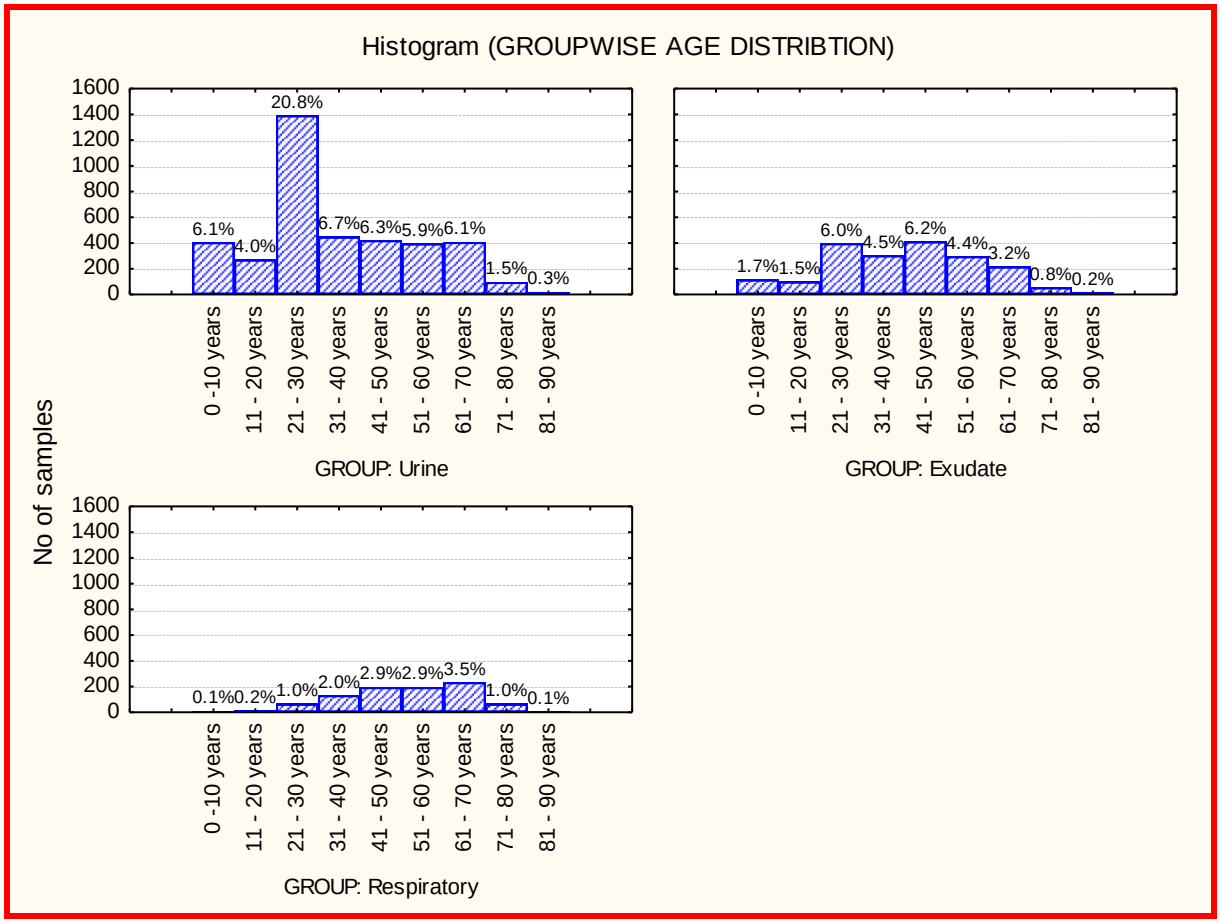

Chart 3: Histogram Showing The Age Distribution Of Patients For Various Clinical Samples

Age ranges from 0 to 90 years. 82.5% of the samples were obtained from patients with age between 21 -70 years. Average age is 39 years. There is a significant difference in age of patients from whom urine, exudate and respiratory were collected. It was confirmed using chi square test. $\chi 2=803.69$. p=0.001significant (Table: 4)

Table 4: Sexwise Distribution of Patients From Whom Clinical Speciemen Obtained

Sex	No. of patients	%
Male	2801	42.0
Female	3871	58.0
TOTAL	6672	100.0

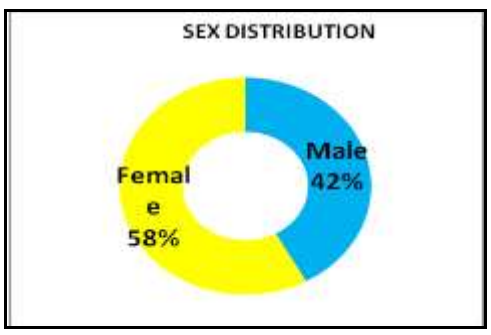

Chart : 4 Sexwise Distribution of Patients

Table 5: Sexwise Distribution of Patients and Various Clinical Specimens

Sex	Type of specimen						Significance of sex-wise distribution of patients using Chi square test
	Urine		Exudate		Respiratory		
	n	%	n	%	n	%	
Male	1146	29.7	1175	61.7	480	52.5	$\chi 2=582.06$ p=0.001*** significant
Female	2707	70.3	730	38.3	434	47.5	

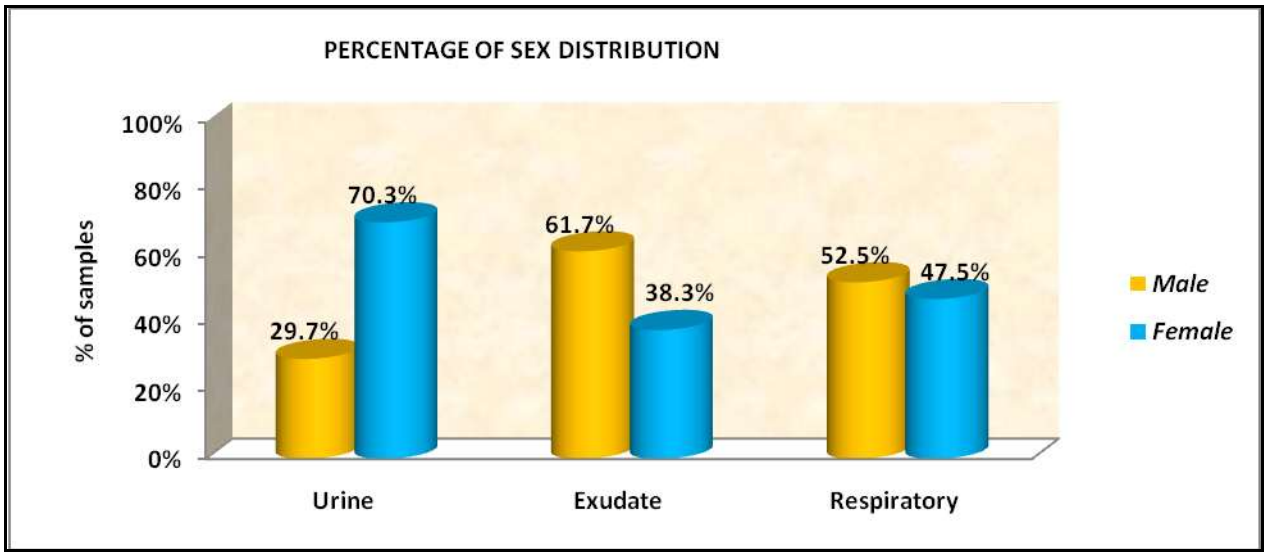

Chart : 5 Sexwise Distribution of Patients and Various Clinical Specimens

Table 6: Sexwise Mean Age of Patients from Whom Various Clinical Samples Obtained

Type of clinical sample	Sex	N	Mean age	Std. Deviation	Mean age difference	Student independent t-test
Urine	Male	1146	39.48	23.900	6.93 years	t=10.31 p=0.001*** significant
	Female	2707	32.55	16.585		
Exudate	Male	1175	42.60	18.207	4.61 years	t=5.34 p=0.001*** significant
	Female	730	37.99	18.418		
Respiratory	Male	480	52.28	15.761	0.65 years	t=0.63 p=0.52 not significant
	Female	434	51.63	14.663		

In urine sample, males average age is 39.48 years, females average age is 32.55 years .So the difference is 6.93 years, this difference is large and t=10.31 p=0.001which is statistically significant. In Exudates sample, males average age is 42.60 years, females average age is 37.99 years .So the difference is 4.61 years, this difference is large and t=5.34 p=0.001 statistically significant. In Respiratory sample, male's average age is 52.28 years, females average age is 51.63 years. So the difference is 0.65 years, this difference is large and t=0.63 p=0.52 which is statistically significant. Statistical significance was calculated using student independent t-test.

Table 7: Wardwise Distribution of Various Clinical Samples

	Urine		Exudate		Respiratory		Chi square test
	n	%	n	%	n	%	
*ICU	1037	26.9	508	26.7	207	22.6	$\chi 2$=1641.8 P=0.001 significant
Medicine	529	13.7	137	7.2	513	56.1	
Surgery	981	25.5	972	51.0	85	9.3	
Paediatric ward	179	4.6	84	4.4	26	2.8	
General OP	523	13.6	101	5.3	52	5.7	
*OBG	604	15.7	103	5.4	31	3.4	

*****Intensive care unit- ICU, OBG- obstetrics and Gynecology

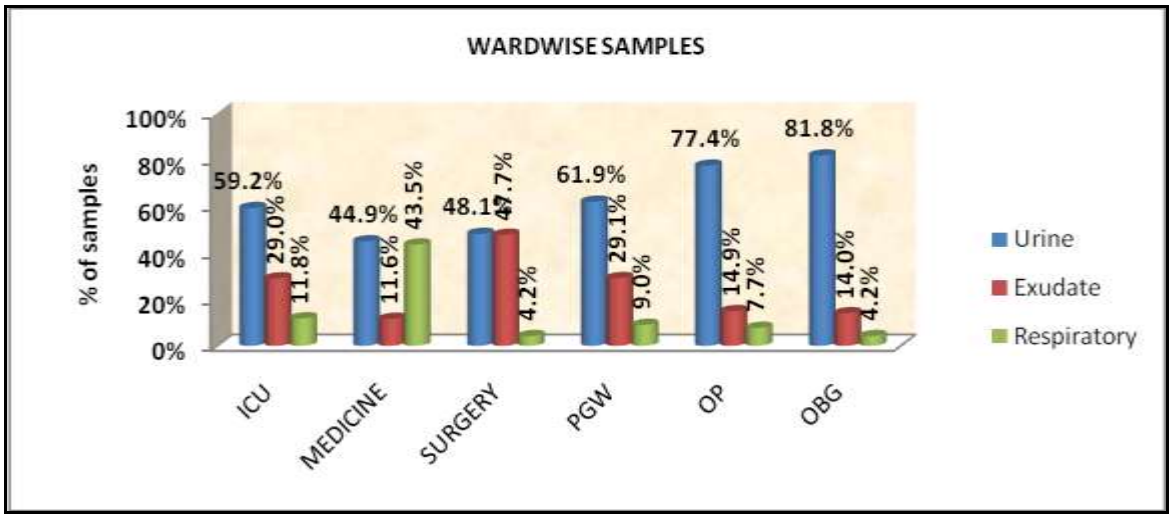

Chart: 6 Wardwise Distribution of Various Clinical Samples

A total of 1037(26.9%) urine samples were obtained from ICU's, 529(13.7%) from medicine wards, 981 (25.5%) from surgery wards, 179 (4 %) from pediatrics ward, 523 (13.6%) from outpatient department and 604 (15.7%) from OBG wards. A total of 508 (26.7%) exudates samples were obtained from ICU's, 137(7.2%) from medicine wards, 972(51.0%) from surgery wards, 84(4.4%) from pediatric wards, 101(5.3%) from outpatient departments and 103 (5.4%) from OBG wards. A total of 207 (22.6%) of respiratory samples were obtained from ICUs, 513(56.1%) from medicine wards, 85 (9.3%) were from surgery wards, 26(2.8%) were from pediatric wards, 52(5.7%) outpatient department and 31(3.4%) were from OBG. Urine samples and exudates samples were obtained more from ICU and surgery wards, whereas respiratory samples were more from ICU's and medicine units. Statistically there is a significant difference between urine, exudates and respiratory samples; it was confirmed using chi square test. $\chi 2$=1641.8. P=0.001 significant. (Table-7).

5. Conclusion

The ESBLs producing GNB occurs as saprophytes as well as commensals in human gut. They are innately resistant to many antibiotics. Among 2356 Gram negative bacilli isolated from various clinical samples *Escherichia coli* and *Klebsiella species* were the predominat bacterial isolates followed by *Acinetobacter species*, *Pseudomonas species*, *Citrobacter species*, *Proteus species*, and *Enterobacter species*. The emergence of Extended spectrum of beta lactamases among these isolates are of therapeutic challenge as these enzymes possess high hydrolytic activity towards higher generation of cephalosporins and even confer resistance to carbapenem group of drugs. Most of the ESBL producing organisms are multidrug resistant. Therefore, it is crucial to implement a revised strategy for empirical therapy, appropriate usage of Extended- spectrum cephalosporins and regular assessment of antibiotic resistance pattern to control the spread of ESBL producing organisms in both communities as well as hospital environments. Thus, ESBL production should be tested by

the conventional methods and should be reported along with routine antibiotic susceptibility testing by every clinical microbiology laboratory. From the present study, we can conclude that detection of ESBL's should be carried out as a routine by the phenotypic disc diffusion test as it is simple, cost effective and user friendly.

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