Prevalence and Control of Multi Drug Resistant (MDR) Nosocomial Pathogens Isolated from Hospital Wards (Surgical, Medical, Paediatric and Labor Unit)

1. ABSTRACT:

Introduction:
Nosocomial infection have increased and gained attention because of high isolation rates of multi drug resistant (MDR) organism in admitted and out patients in hospitals with complicated infectious ailments. The spread of multi drug resistant organisms among critically ill, hospitalized patients and subsequent epidemics, have become an increasing cause of concern. A recent manifestation of multi drug resistant organisms that has attracted public attention due to high mortality and morbidity rate is alarming.

Aims: To access the prevalence of multidrug resistant nosocomial pathogens in hospitalized patients.

Material & Method:
This was a hospital based cross sectional study from January 2013 to December 2017. Almost 700 hospitalized and out patients who acquired nosocomial infections (NI) were enrolled, with the permission of ethical and research review committee and with the informed consent to the patient and attendant. Clinical samples were analyzed for antibiotic sensitivity pattern by Kirby Baur method according to CLSI guidelines. [15] Extended spectrum of beta lactamases (ESBL) characterization was done by PCR method.

Results:
Data analysis showed that 62% were female patients and 38% were male patients included. Almost 37% prevalence of etiological agents was found. Predominant were *E.coli,* followed by *Klebsiella pneumoniae, Acinetobacter* spp, and *Staphylococcus aureus.* Antibiotic resistance rate was found very high i.e up to 55 - 90% against commonly prescribed antibiotics in hospitalized and out patients having nosocomial infection resulting complicated infections.

Conclusion:
Emergence of MDR strains in nosocomial infection (NI) is a matter of great concern and warrant investigation. There is need to adopt infection control strategies in public and private secondary tertiary care hospitals.

Key words
Multi Drug resistant (MDR), Nosocomial Infection (NI), Pathogens,

2. INTRODUCTION:

More than 2 million people, or approximately 5 to 10% of hospitalized patients, are affected by nosocomial infections (NI) with a death rate of estimated 90,000 deaths per year in United States. As well as to modify the disease burden regarding significant morbidity and mortality of nosocomial infections, high healthcare costs are incurred in managing nosocomial infections. [1]
Nosocomial infection develops within or after 48 hours of hospital admission or being discharged. [2] Patients admitted to the ward have been shown to be at particular risk of acquiring

nosocomial infection (NI) with high prevalence rate. [3] Hospital environment plays a significant role in the occurrence of nosocomial infection since it harbors a diverse population of microorganisms. [4]
Multiple drug resistant (MDR) organisms are bacteria and other microorganisms that have developed resistance to antibiotics. [5] The misuse and overuse of antibiotics is worldwide both in poor and developing countries it has also increased the rate of antimicrobial resistance around. [6]

Infections caused by multi-drug resistant microorganisms (MDR), often do not respond to conventional therapy and can result in prolonged illness and hospital stay as well as higher morbidity and mortality rates. Hospital acquired infections mainly occurred in surgical site infections (SSI), ventilator associated pneumonia (VAP), blood stream and urinary tract infection (UTI), and are commonly known as nosocomial infections (NI), mostly caused by extended spectrum of beta lactamases (ESBL) producing enteric pathogens and non-fermenting gram-negative organisms etc. [7]

Antimicrobial resistance among gram negative bacilli represents a major problem in nosocomial infection.[8] According to recent report, more than 30% of hospital acquired infections are due to gram negative bacteria in U.S, the majority of ventilator- associated pneumonia (47%) and UTI (45%) cases are associated with these bacteria. [9]

While antimicrobial agents are considered as a solution for infectious disease, resistance of microorganisms to various drugs has raised new problems, especially for hospital acquired infections therefore research should be conducted on risk factors related to the transmission of such infections. However, emergences of multi-drug resistance strains have left limited treatment options, so monitoring multi drug resistant organisms (MDROs) and the infections they cause in a healthcare setting warrant investigation for the wellbeing of vulnerable patient populations, and effectiveness of interventions. [1]

Several studies have identified general characteristics of patients that place them at high risk for acquisition of multidrug resistant (MDR) outbreak strains. [10] However, the diversity of risk factors suggests that separate investigations should be performed in each hospital setting. [1]

Therefore, identification of a microbe and determining susceptibility pattern are beneficial to the patient and assist in selection of therapy to avoid emergence of multidrug resistance organisms in hospital. [11] There are numerous reports describing the successful control of nosocomial outbreaks, but there is little data regarding control of the endemic setting. Hospital staff education was emphasized to improve their perception of and adherence to hand hygiene protocols, as well as to improve their understanding regarding the importance of controlling multidrug resistant (MDR) nosocomial infection. [14] Monthly education of new staff will perform by infection control staffs. Importance of personal protective equipment (PPE) sterilization of equipment for patients and caregivers wellbeing should be taken as of prime importance and exclusive to prevent cross-transmission between these patients in the hospital wards. The importance of clean environment for infected patients at least 3 times per day should be highlighted with the availability of alcohol-based hand rub sanitizer at all bed sides. [13] Campaigns and awareness sessions for hand hygiene should be conducted as hand colonization

contributes significantly transmission and can be controlled by follow biosafety and biosecurity methods and use of antiseptic- or alcohol-based soaps. [12]

3. MATERIAL AND METHODS:

3.1: Study design:

This study was a hospital based observational retrospective cross sectional study to determine the MDR nosocomial infection and its control.

3.2: Study setting:

Karachi is a metropolitan city of Pakistan, with more than 12 million population and hub of commercial activities. Large number of people from other parts of country lives and worked in Karachi due to employment opportunities. This study was conducted in "Chiniot general hospital" catering the health-care facilities, for indoor and outdoor patients including, Medicine, Pediatric, surgical, Gynecology and labor unit specialties.

3.3: Study population:

This is cross sectional study including in patients and out patients in surgical, medical, general unit with some serious infectious disease. All patients reporting some infectious condition were included; Patients with other morbid conditions were excluded. Study was also carried out in private secondary and tertiary care hospitals of Karachi city.

3.4: Sample size:

A total number of samples 700 patients were investigated.

3.5: Sampling method

For demographic analysis of contributing risk factors and data analysis surveillance form was distributed to patients or their attendant's. For any ethical issues informed consent was taken after face to face interview. The study was conducted at the Department of Microbiology, secondary tertiary care hospital Karachi. Clinical specimens obtain from patients with nosocomial infections included urine, blood, pus, sputum, ET-secretion, were collected and transported to laboratory. All the specimens were analyzed and diagnosed according to CLSI guidelines.

3.6: Ethical concern

Prior permission and informed consent from hospital's central ethical research committee was taken.

4. RESULTS:

Pathogenic bacteria have increasingly been shows resistant to antimicrobial therapy. Recently, resistance problem has been relatively much worsened in gram-negative bacilli causing infections and high mortality, almost exclusively in compromised hospital patients. It is known to be difficult to prevent emergence of multidrug resistant (MDR) infectious agents in hospitalized patients, because the organisms are ubiquitous in hospital environment, efforts to control resistant pathogens been not successful worldwide. We need concerted multidisciplinary

efforts to preserve the efficacy of currently available antimicrobial agents, by following the principles of antimicrobial stewardship.

700 patients admitted at private hospital catering the needs of tertiary care setting were recruited and 285 clinical isolates were recovered during the study period of four years (January 2013-December 2017). Details of these isolates are mentioned in [Table N0. 4].

Analysis of demographic data showed out of 700 samples 62%were females and 38% were males who presented with complications of infectious diseases [Table No. 1, Figure No. 1].

Table No. 1. Basic information of studied sample gender

Gender	N	%
Female	440	62
Male	260	38

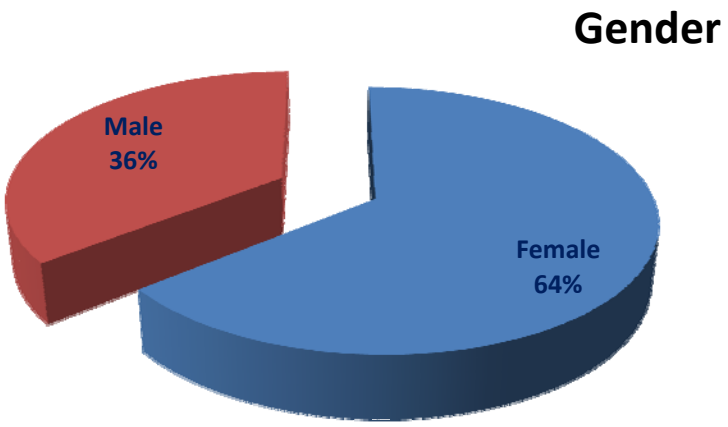


Fig No.1 Basic information of studied sample gender.

Data analyzed showed that age group 46- 60 was the most prominent among hospitalized patients with different ailments followed by 31-45 age groups.

Table No. 2 Basic Information of Studied Sample – Age Group

Age	N	%
1 – 15	86	12.3
16 – 30	97	13.85
31 – 45	115	16.4
46 – 60	194	27.18
61 – 75	110	15.71
76 – 100	98	14.0

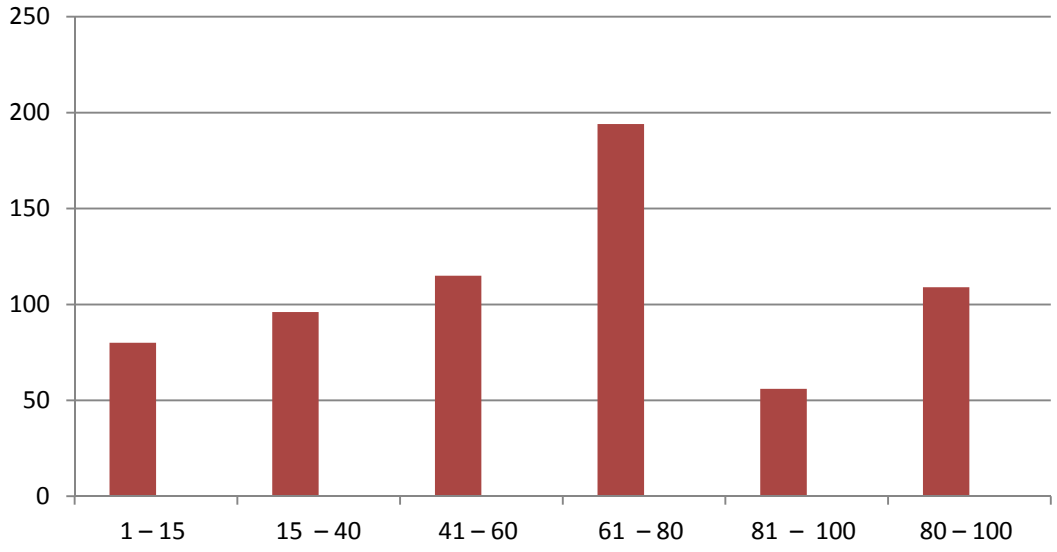


Fig No. 2 Basic Information of Studied Sample – Age Group

Out of total 700 samples, 285 were found positive for the presence of Spectrum of MDR pathogenic isolates. [Table No. 3, Figure No. 3]. Almost 41.9 % recruited patients were diagnosed with the presence of different pathogens.

Table No. 3: % age distribution of isolated Pathogens.

Growth	n	%
No Growth	377	58.1
Pathogen Diagnosed	285	41.9

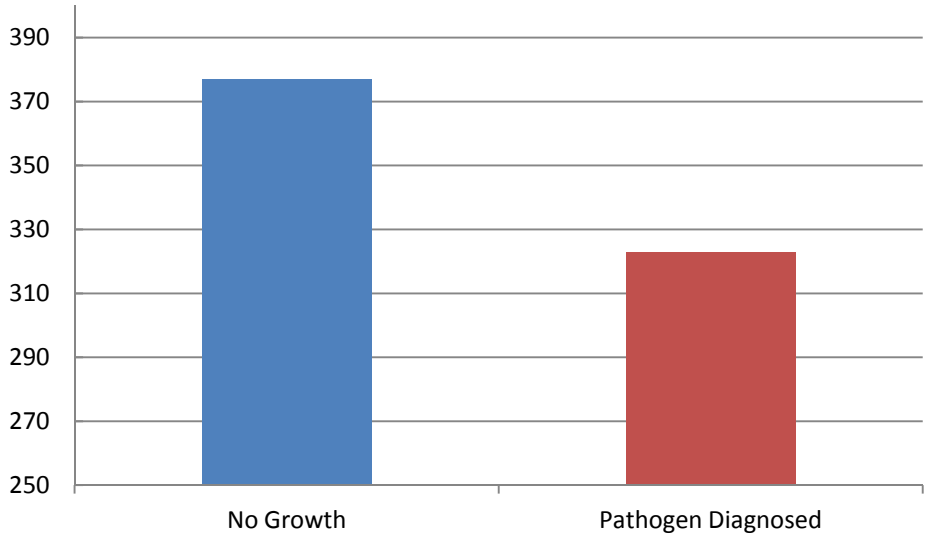


Fig No. 3: % age distribution of Pathogenic isolates.

It was found that 41.9% samples were found with the presence of multidrug resistant (MDR) pathogens. However 19.3% *Escherichia coli* strains were found predominant, followed by 17.54% *Staphylococcus aureus*, 17.19% *Klebsiella pneumonae*, *10.53% Salmoella typhi*, 9.47%*Pseudomonas aeruginosa*, 7.02% *Acinetobacter spp* others as shown in [Table No. 4, Fig No. 4]

Table No. 4 spectrum of MDR isolates

S No.	Pathogenic isolates	n	%
1	*Acinetobacter SPP:*	20	7.02
2	*Citrobacter freundii*	10	3.51
3	*Enterobacter spp:*	8	2.81
4	*Escherichia coli*	55	19.30
5	*Klebsiella pneumonia*	49	17.19
6	*Morganella morganii*	3	1.05
7	*Proteus mirabilis*	19	6.67
8	*Proteus Vulgaris*	3	1.05
9	*Pseudomonas aeruginosa*	27	9.47
10	*Pseudomonas spp:*	3	1.05
11	*Salmoella typhi*	30	10.53
12	*Staphylococcus aureus*	50	17.54
13	*Streptococcus pneumonia*	8	2.81

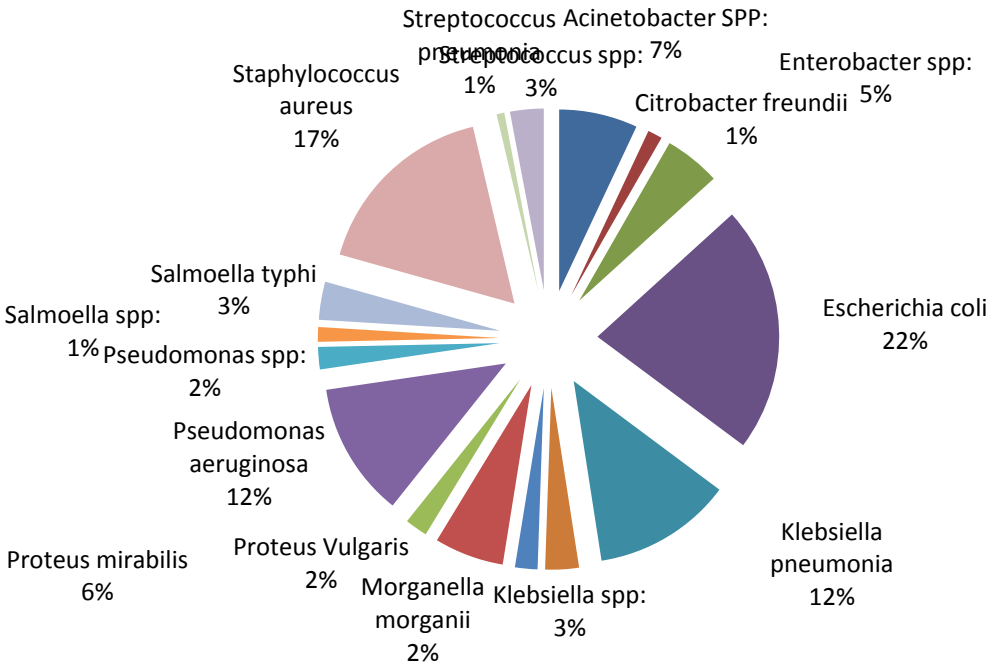


Fig: No. 4 spectrum of MDR isolate

Table No. 5 spectrum of Gram negative MDR susceptibility patterns of isolates

S No.	Isolated Pathogens	AMC	CRO	ATM	IPM	AK	CIP	FOS	SCF	Dox	TZP	C	CT	SXT	F
1	*Acinetobacter SPP:*	18.7	18.7	18.7	13	18.7	18.7	18.7	18.7	16	10.5	18.7	16.5	19.7	9
2	*Citrobacter freundii*	5	5	8	1	4	6	3	3	2	3	7	5	8	6
3	*Enterobacter spp:*	6	6	5	3	5	4	2	4	4	4	5	4	5	3
4	*Escherichia coli*	45	48	45	18	22	35	25	24	40	32	45	36	45	20
5	*Klebsiella pneumonia*	36	46	40	13	24	39	38	15	41	28	35	45	45	29
6	*Morganella morganii*	2	2	2	0	1	2	2	1	2	2	2	2	2	0
7	*Proteus mirabilis*	13	15	13	6	11	16	10	9	12	8	11	13	16	7
8	*Proteus Vulgaris*	3	3	3	1	2	3	1	1	3	0	3	3	3	0
9	*Pseudomonas aeruginosa*	22	20	23	10	17	19	19	16	23	20	25	21	26	14
10	*Pseudomonas spp:*	3	3	1	2	1	1	4	0	3	1	2	2	2	1
11	*Salmoella typhi*	25	24	27	12	19	24	19	21	26	17	28	27	28	16
	RESISTANT	178.7	190.7	185.7	79	124.7	167.7	141.7	112.7	172	125.5	181.7	174.5	199.7	105
	SENSITIVE	48.3	36.3	41.3	148	102.3	59.3	85.3	114.3	55	101.5	45.3	52.5	27.3	122

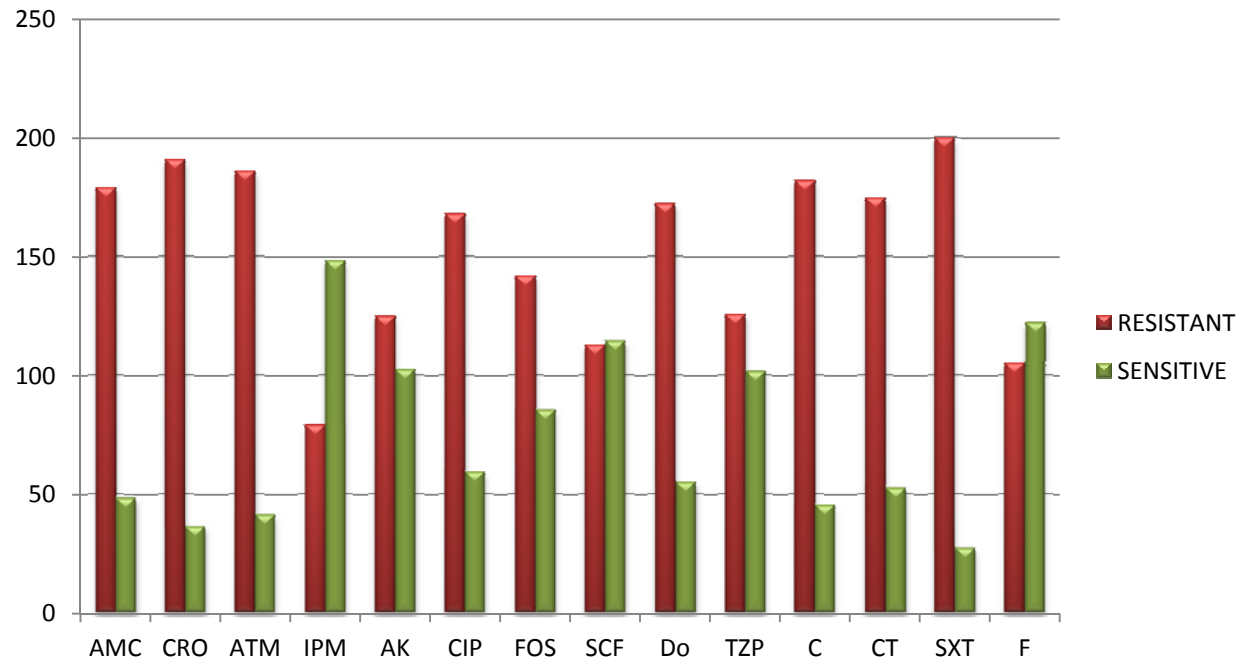


Figure No. 5: spectrum of Gram negative MDR susceptibility patterns of isolates

Table No 6: spectrum of Gram positive MDR susceptibility pattern of isolates

Isolated Pathogens	AMC	CRO	ATM	IPM	AK	CIP	FOS	SCF	Dox	TZP	C	CT	SXT	F	OX	LZD	VA	E
Staphylococcus aureus	46	40	47	15	48	35	25	39	32	28	41	47	46	41	34	28	25	43
Streptococcus pneumoniae	5	7	4	3	6	7	2	4	6	3	5	7	6	4	8	2	3	4
RESISTANT	51	47	51	18	54	42	27	43	38	31	46	54	52	45	42	30	28	47
SENSITIVE	7	11	7	40	4	16	31	15	20	27	12	4	6	13	16	28	30	11

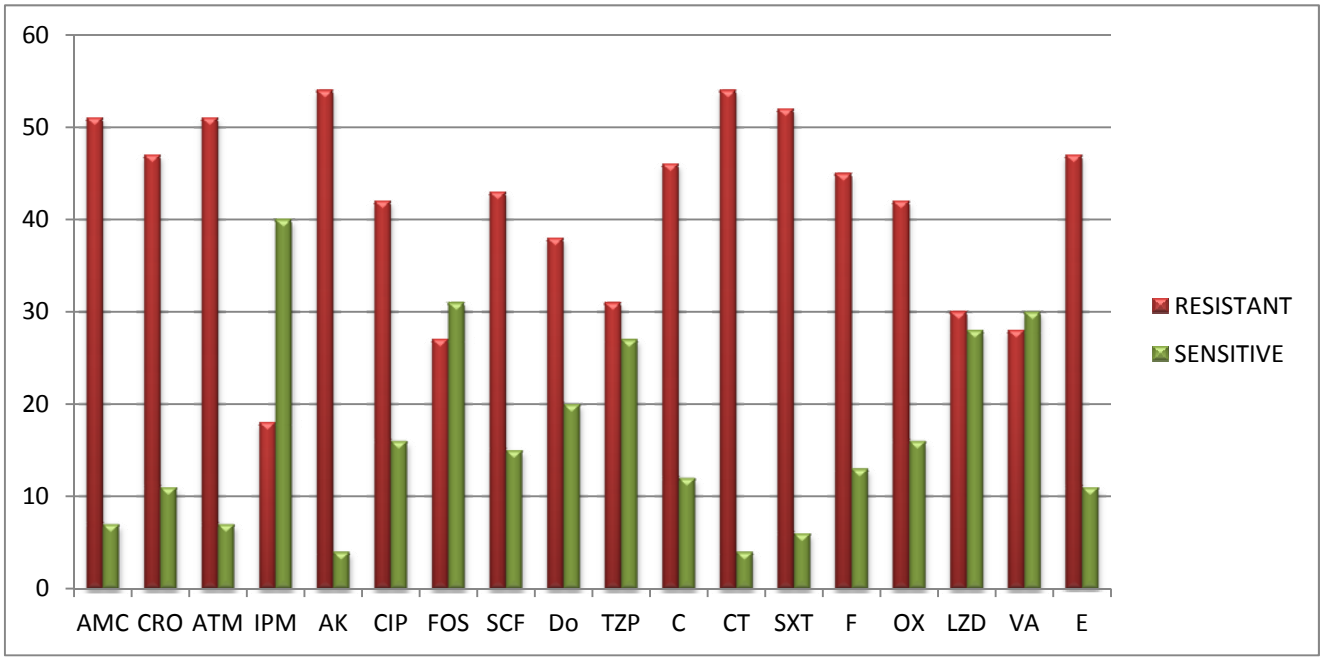


Figure No 6: spectrum of Gram positive MDR susceptibility pattern of isolates

1. Sensitivity Pattern of *E. coli:*

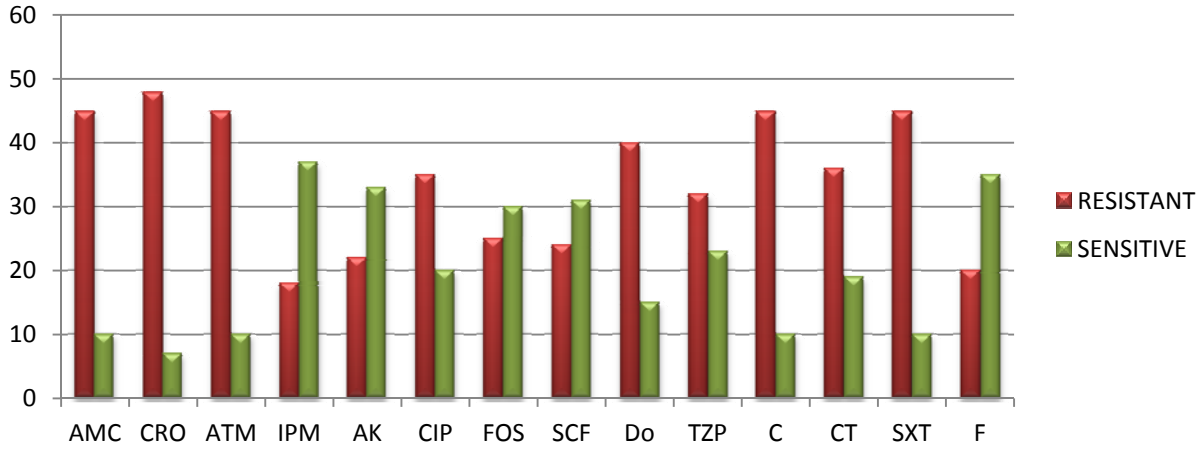
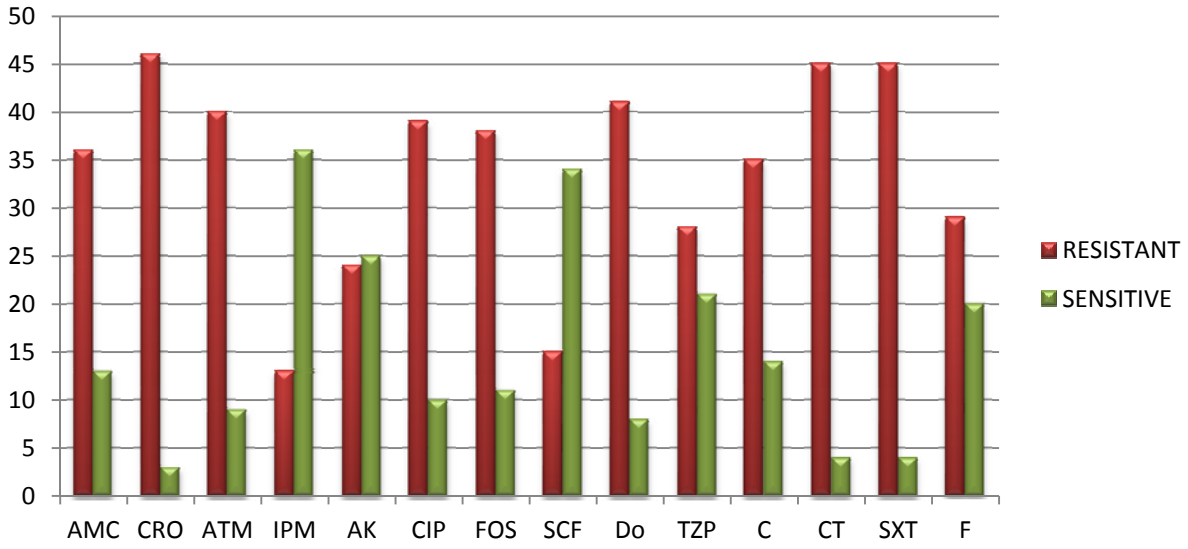


Figure No 7: Sensitivity Pattern of *MDR E. coli susceptible pattern of isolates.*

2. Sensitivity Pattern of *Klebsiella pneumonia:*

Figure No 8: Sensitivity Pattern of *MDR Klebsiella pneumonia susceptible pattern of isolates.*



3. Sensitivity Pattern of *S.aureus:*

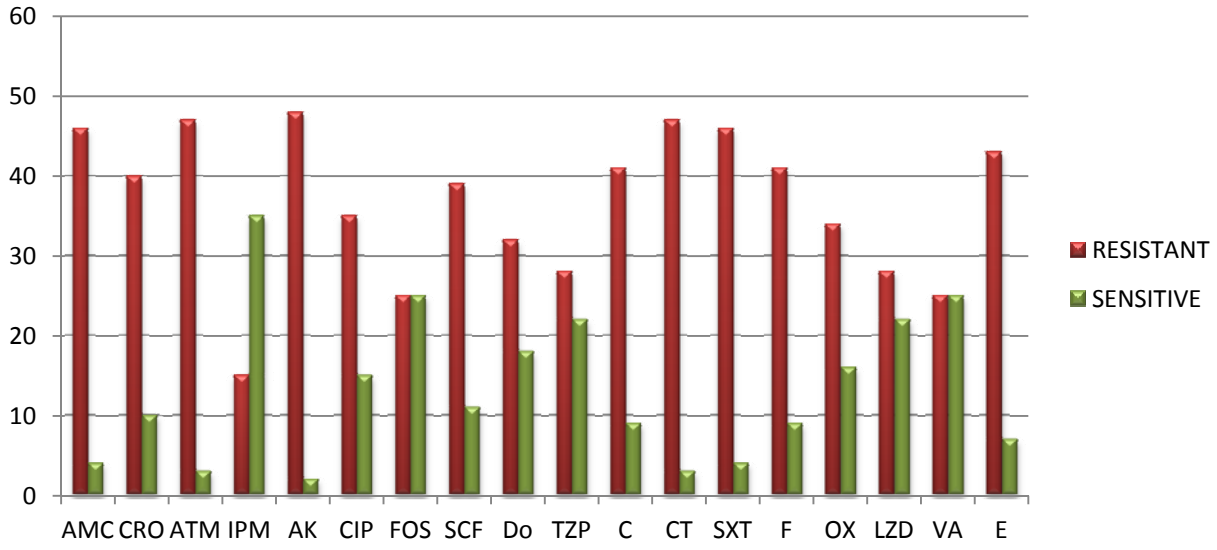


Figure No 9: Sensitivity Pattern of *MDR S.aureus susceptible pattern of isolates.*

4. Sensitivity Pattern of *Pseudomonas aeruginosa:*

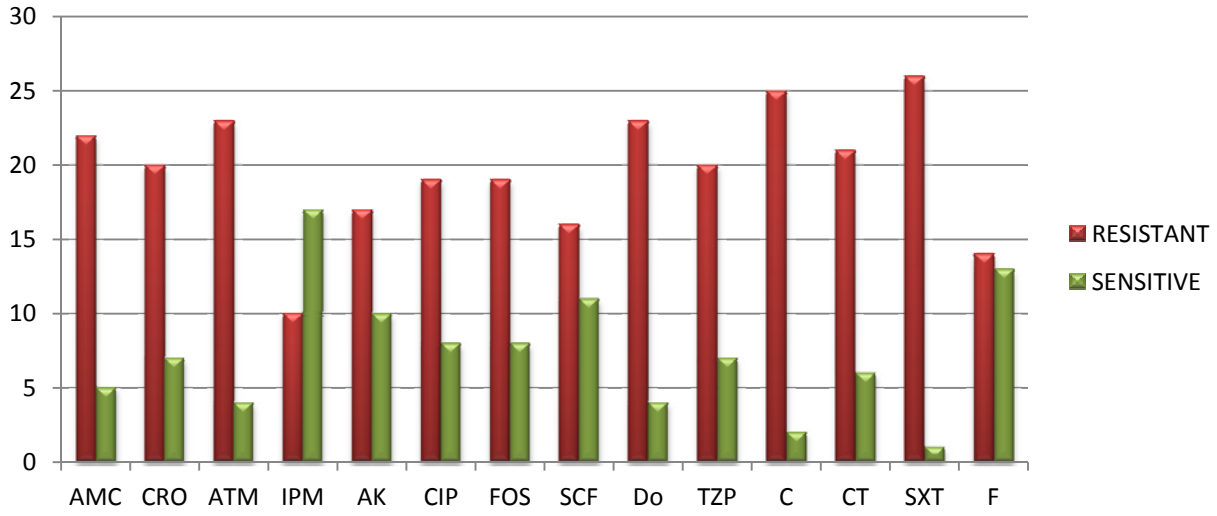


Figure No 10: Sensitivity Pattern of *MDR Pseudomonas aeuroginosa susceptible pattern of isolates.*

5. Sensitivity Pattern of *Acinetobacter SPP:*

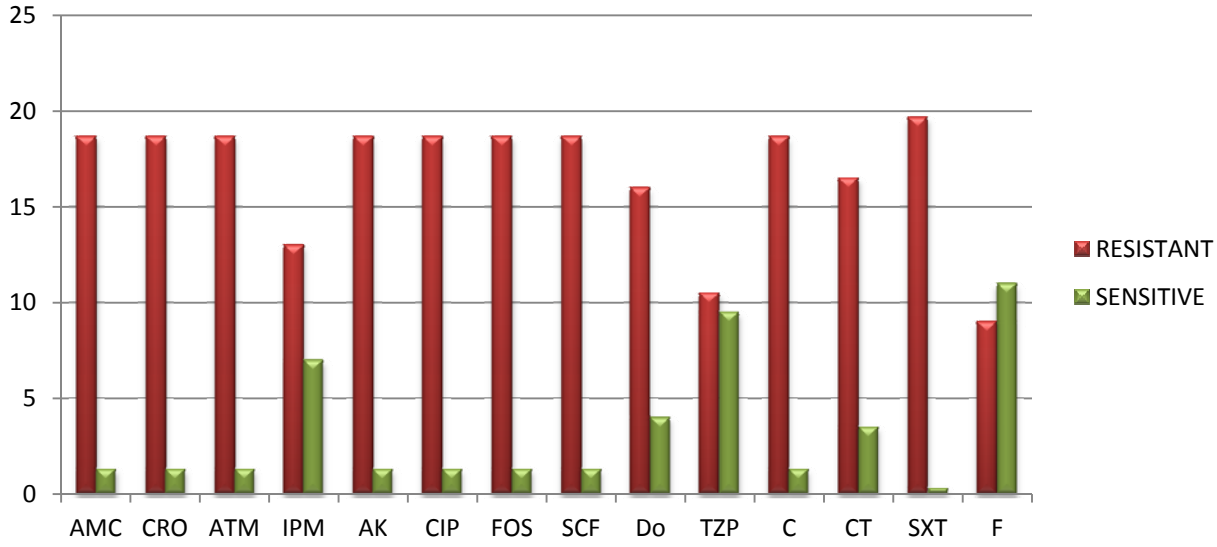


Figure No 11: Sensitivity Pattern of *MDR Acinetobacter SPP: susceptible pattern of isolates.*

5. DISCUSSION:

More than 2 million people, or approximately 5% to 10% of hospitalized patients, are affected by nosocomial infections with an estimated 90,000 deaths every year. [1, 2] In the United States alone the disease burden regarding significant morbidity and mortality is responsible for 8 million physician visits and more than 100 thousand admissions to the hospital per year. [2]

Antibiotics play vital role in the treatment of microbes in infectious diseases and eradication of infections. However, emergence and dissemination of multi-drug resistant strains as a result of overuse of antibiotics is a major concern among different groups of enterobacteraciae like (ESBL) ß-lactamase producing *E.coli*, *K.pneumoniae*, and many others. Multi-drug resistant strains of *E.coli* and *K.pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections. This alarming situation has increased frequently in last few years resulting in severe consequences i.e increased cost of medicines and mortality of patients.

According to demographic data analysis showed that male to female ratio (62%-38%) is very significant as more number of females patients were enrolled in this study may be due to frequent UTI infections and gynecological problems among female population .it was much lower in other reported studies from developed countries where almost 30% registered patients were female, where as in developing countries ratio is high probably due to low socioeconomic and low personal hygienic profile [Table No. 1, Fig No. 1]. The predominant age group suffering from nosocomial infection in this study is 46-60 [Table No. 2, fig No. 2].

The results does not correlate with the reported studies from developed countries where mostly sick persons acquiring nosocomial infections are more than 60 years of age. [16, 17]

Almost in 75% to 90% of the cases *E.coli* remains the leading cause in those patients who were hospitalized with a complicated urinary tract infection (UTI) and other ailments and is causing difficulties in treatment due to drug resistance towards commonly used drugs like ampicillin/ amoxicillin and Co-trimoxazole. In this study 19% *E.coli* [Table no 4, Fig no 4] were reported after data analysis, and showed high level of resistance rate (35 - 87%) against Augmentin, Ceftriaxone, Azethroneam, Ciprofloxacin, Doxicillin, Chloromfenicol, CT and Co-trimexazole. The most probable reason might be the overuse of antibiotics in other ailments where it is not recommended as in viral and parasitic infections.

One of the major gram-negative bacteria responsible for nosocomial infections is *Acinetobacter*. *Acinetobacter* may cause severe pneumonia and infections of the urinary tract, bloodstream, and other parts of the body. In this study 7.02% *Acinetobacter* [Table No. 4, Fig No. 4] cases were reported with (45 - 98%) resistance rate for first second and third generation antibiotics. A study carried out in Spain showed that more than 90% of *A.baumannii* infections were of nosocomial origin. [18] In this study 17% *K. pneumoniae* [Table No. 4, Fig No 4] considered to be responsible for (94 - 26%) of all hospital acquired urinary tract infections blood stream infections globally increasing resistance trends reported to multi Studies from both developed and developing countries. [19] The epidemiology of ESBL producing *K.pneumoniae* in the community had not

changed over the last 20 years with the emergence of virulent capsular serotypes. Our findings are consistent with other studies reporting interestingly when age as a risk factor. [20]
Staphylococcus aureus was the most prominent etiological agent among gram positive organisms 17.5 % [Table No. 4, Fig No. 4] reported in this study after data analysis. Almost all isolates were resistant (30 – 96%) to Augmentin, Ceftriaxone, Amakicin, Ciprofloxacin, Doxicillin, CT, Oxacillin and Erythromycin. Our study is in line with the report issued by U.S. National Healthcare Safety Network, where more than 30% of hospital-acquired infections were reported due to gram-negative bacteria and the majority of ventilator-associated pneumonia (47%) and urinary tract infection (45%) cases are associated with these bacteria. [21]

6. Conclusion:

Time for life saving antibiotics is running out due to resistance acquiring mechanisms. Despite the clear medical need for novel antibiotics without cross-resistance issues, antibacterial research and development pipelines are nearly dry, thus failing to provide the flow of novel antibiotics required to match the fast emergence and spread of MDR bacteria. In order to tackle this serious situation, there is also a need to refrain from such activities which are the main cause of nosocomial infection, and strategies should be planned to over count this situation.

7. ABREVIATION:

- MDR: multi-drug resistant
- NI : nosocomial infection
- ESBL: extended-spectrum beta-lactamase
- PCR: polymerase chain reaction
- *E.coli: Escherichia coli*
- *S.aureus: staphylococcus aureus*
- UTI: urinary tract infections
- U.S: united state
- CLSI: Clinical & Laboratory Standards Institute
- AMC: Augmintin.
- CRO: Ceftriaxone.
- ATM: Aztreonam.
- IPM: Imipenem.
- AK: Amikacin.
- CIP: Ciprofloxacin.
- FOS: Fosfomycin.
- SCF: Sulzone.
- DOX: Doxycycline
- TZP: Tazobactam
- C: CHLORAMPHENICOL
- CT: Colistin
- SXT: Sulphamethoxazole trimethoprim.
- F: Nitrofurantoin
- OX: Oxicilin.
- LZD: Linezolid
- VA: Vancomycin.
- E: Erythromycin.

8. REFERENCES:

1. Silveira MD. Fatores de risco, clonalidade, sazonalidade e prognóstico de colonização e/ou infecção por Acinetobacter baumannii em hospitais públicos na cidade de Bauru/SP.

2. Custovic A, Smajlovic J, Hadzic S, Ahmetagic S, Tihic N, Hadzagic H. Epidemiological surveillance of bacterial nosocomial infections in the surgical intensive care unit. Materia socio-medica. 2014 Feb;26(1):7

3. Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Therapeutic advances in drug safety. 2014 Dec;5(6):229-41.

4. Solomon FB, Wadilo FW, Arota AA, Abraham YL. Antibiotic resistant airborne bacteria and their multidrug resistance pattern at University teaching referral Hospital in South Ethiopia. Annals of clinical microbiology and antimicrobials. 2017 Dec;16(1):29.

5. Patwardhan RB, Dhakephalkar PK, Niphadkar KB, Chopade BA. A study on nosocomial pathogens in ICU with special reference to multiresistant Acinetobacter baumannii harbouring multiple plasmids. Indian Journal of Medical Research. 2008 Aug 1;128(2):178.

6. Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases. 2013 Feb 1;13(2):155-65.

7. Zivanovic V, Gojkovic-Bukarica L, Scepanovic R, Vitorovic T, Novakovic R, Milanov N, Bukumiric Z, Carevic B, Trajkovic J, Rajkovic J, Djokic V. Differences in antimicrobial consumption, prescribing and isolation rate of multidrug resistant Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii on surgical and medical wards. PloS one. 2017 May 3;12(5):e0175689.

8. Karageorgopoulos DE, Falagas ME. Current control and treatment of multidrug-resistant Acinetobacter baumannii infections. The Lancet infectious diseases. 2008 Dec 1;8(12):751-62.

9. Gao L, Lyu Y, Li Y. Trends in drug resistance of Acinetobacter baumannii over a 10-year period: nationwide data from the China surveillance of antimicrobial resistance program. Chinese medical journal. 2017 Mar 20;130(6):659.

10. Hawken SE, Snitkin ES. Genomic epidemiology of multidrug-resistant Gram-negative organisms. Annals of the New York Academy of Sciences. 2018 Mar 31.

11. Garner JS, Hospital Infection Control Practices Advisory Committee. Guideline for isolation precautions in hospitals. Infection Control & Hospital Epidemiology. 1996 Jan;17(1):54-80.

12. Dessie W, Mulugeta G, Fentaw S, Mihret A, Hassen M, Abebe E. Pattern of bacterial pathogens and their susceptibility isolated from surgical site infections at selected referral hospitals, Addis Ababa, Ethiopia. International journal of microbiology. 2016;2016.

13. Connor TH, Burroughs GE, McDiarmid MA, Mead KR, Power LA, Reed LD. NIOSH Alert: preventing occupational exposures to antineoplastic and other hazardous drugs in health care settings. Atlanta. DHHS (NIOSH) Publication. 2004:1-50.

14. Cheon S, Kim MJ, Yun SJ, Moon JY, Kim YS. Controlling endemic multidrug-resistant Acinetobacter baumannii in Intensive Care Units using antimicrobial stewardship and infection control. The Korean journal of internal medicine. 2016 Mar;31(2):367.

15. Johnson A, Renteria M, Hoban D, Sahm D, Dowzicky M, Hogan P. Antimicrobial Susceptibility Status of Multidrug Resistant (MDR) Enterobacteriaceae collected in Latin America: TEST Program Data (2012-2016). InINTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS 2017 Sep 1 (Vol. 50, pp. S99-S100). PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS: ELSEVIER SCIENCE BV.

16. More than 2 million people, or approximately 5 to 10% of hospitalized patients, are affected by nosocomial infections (NI) with a death rate of estimated 90,000 deaths per year. As well as to modify the disease burden regarding significant morbidity and mortality of nosocomial infections, high healthcare costs are incurred in managing nosocomial infections.

17. Ducel G, Fabry J, Nicolle L, World Health Organization. Prevention of hospital-acquired infections: a practical guide.

18. Ponce-de-Leon S. The needs of developing countries and the resources required. Journal of Hospital Infection. 1991 Jun 1;18:376-81.

19. Stone PW, Larson E, Kawar LN. A systematic audit of economic evidence linking nosocomial infections and infection control interventions: 1990-2000. American journal of infection control. 2002 May 1;30(3):145-52.

20. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infection Control & Hospital Epidemiology. 2008 Nov;29(11):996-1011.

21. Babypadmini S, Appalaraju B. Extended spectrum-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital. Indian Journal of Medical Microbiology. 2004 Jul 1;22(3):172.