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COMPARATIVE ANALYSIS OF ANTIBIOTIC SUSCEPTIBILITY AMONG BETA-LACTAMASE PRODUCING STRAINS FROM DIFFERENT CLINICAL SAMPLES IN PAKISTAN, LAHORE

[1]Fadia Waheed, [2]Dr. Saba Riaz, [3]Muhammad Farhan Zafar Chaudhry

[1]Microbiology and Molecular Genetics Punjab university, Lahore, Pakistan, Fadia.waheed1@gmail.com

[2] Microbiology and Molecular Genetics Punjab university, Lahore, Pakistan, saba.mmg@pu.edu.pk

[3] Department of Microbiology, Government College University Faisalabad, Pakistan,Zfarhan322@gmail.com

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Abstract:

Increasing trend of antimicrobial drug resistance in extended spectrum beta-lactamase (ESBLs) producing bacteria is one of the major problems to the health sectors these days. Most of the bacteria produce beta-lactamase enzymes which inactivate the beta-lactam ring of the antibiotics and become resistant to that drug. The aim of the study was the comparative analysis of antibiotic susceptibility among beta-lactamase producing strains from different clinical samples in Pakistan, Lahore. The present study was conducted at Citi Lab and Research Centre. All specimens received in the lab were processed according to Clinical Laboratory Standard Institute (CLSI, 2006). Identification of strains was performed using biochemical battery and analytical profile index. ESBLs were phenotypically examined by antibiotic susceptibility (AST) test, double disk synergism (DDST) and combination disk synergy test (CDST). Out of 300 strains isolated from different clinical samples (urine, wound and blood) 157 isolates belonged to Enterobacteriaceae family. Phenotypic analysis showed 59/157(37.5%) were ESBL producing strains. These strains include E. coli, Klebsiella pneumonia, Morganella morganii, Citrobacter spp, Proteus spp. Urine revealed 41% ESBLs producers following wound 16%, blood 2%. Data presents that ESBLs producers were predominant in the females as compared to males following age variation groups, vary from area to area, gender variation in health care facilities. Amikacin, Tazobactams and Sulzone are still the drugs of choice for such infections. Proper antibiogram profile evaluation is very significant for managing such infection.

***Key words**: Enterobacteriaceae, Extended spectrum beta-lactamases, Susceptibility, AST, DDST, CDST.*

Corresponding author:

FadiaWaheed,

Microbiology and Molecular Genetics Punjab university, Lahore, Pakistan,Fadia.waheed1@gmail.com

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INTRODUCTION:

Antimicrobial agent is a key factor to control the growth of microbes; those arecausing serious problems worldwide; these microbes include viruses, bacteria, fungi etc. But most common of them are bacteria, which are spreading day by day and becoming drug resistant. This phenomenon is known as antimicrobial resistance, which is the ability of the microbes to withstand against the antimicrobial drugs. Antimicrobial resistance is rapidly spreading and causing major public health problems and made the treatment difficult. These microbes become resistant by producing those effective agents which alters the structure of antimicrobial agents or by modifying the target site. Antibiotic treatment options for the ESBLs are limited and bacteria resistant to the most of the generations of the drugs as, first line second line and even the fourth generation Cephalosporin [1]. Antimicrobial resistance was not so much problem in the past but due to the intelligent nature of the microbes made them able to fight against the antimicrobial agents[2]. Fighting ability of bacteria against drugs made them multidrug resistant [3]. Extended spectrum β-lactamases (ESBLs) production, or production of carbapenemase in gram negative bacteria are simple markers for multidrug resistance [4]. β-lactamases are the enzymes that are produced by the gram-negative bacilli mostly *Escherichia coli* and *Klebsiella pneumoniae*. These bacteria have the ability to hydrolyze the beta-lactam ring of the beta lactam antibiotics e.g. penicillin. ESBLs are placed in ambler's class A and bush's 2be group [5]. ESBLs were first reported in 1983s from Germany in the *Klebsiella* and later in *E. coli*, also in *Enterobacteriaceae* family. The gene encoding the new β-lactamase is *blaSHV-1*, made due mutation at the one point in the parental ones and become resistant to the cephalosporin's [6]. ESBLs are identified by their ability to hydrolyze penicillin's by producing penicillinases, cephalosporin by producing cephalosporinase, oxyimino-thiazolylcephalosporin include third and fourth generation cephalosporins, aztreonam, monobactams but not cephamycin and carbapenems [7]. They have innate super powers to fight with antibiotics [8]. Fosfomycin is active against urinary tract infections caused by ESBLs producing E.coli [9]. A beta-lactamase producing bacterium not only protects ourselves from the antibiotics such as penicillin but also protects the antibiotic susceptible bacteria [10]. ESBLs are originated by the point mutations at the one-seven pointin β-lactamase (*bla*) genes from TEM and SHV types. ESBLs are plasmid encoded periplasmic enzymes produced by *Enterobacteriaceae* family, fermenter or non-fermenters (*Acinetobacter spp* and *Pseudomonas spp*) [11]. ESBLs contain serine residues at their active site, which attack the amide bond in beta-lactam ring of drugs and hydrolyze them and make the bacteria resistant to these drugs [12].International travel, adoption, trade and immigration all facilitate the globalization of antimicrobial resistance [2]. World health leaders have described these antibiotic-resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world and being a threat to undo decades of advances in our ability to treat disease. It is challenging our whole understanding of how we control communicable diseases[13]. Prevalence of ESBLs increasing day by day, this happened due to the mobile genetic elements, such as integrins, insertion sequences, and transposons play important role in dissemination of ESBLs genes *blaTEM*-type ESBLs genes acquired by mutation in the parent *blaTEM-1* and *blaTEM-2*, produce *TEM*-type ESBLs in E. coli. These genes occurred in the transposons. The *blaSHV*-type ESBLs are chromosomal origin, parent *blaSHV-1* genes, which identified in K. pneumoniae. They became plasmid mediated by the insertion sequences from chromosome to plasmid [14]. At the end of 1970s most *E.coli* and *K.pneumoniae* reported as plasmid mediated, ampicillin hydrolyzing β-lactamases e.g. *TEM-1*, *TEM-2* and *SHV*[15].

MATERIALS AND METHODS:

A total of 300 samples were collected from the Citi lab in front of Jinnah hospital. Sample includes blood, urine, and wound for the research purpose to estimate the ESBLs ratio. Routine culture medium such as blood agar, chocolate agar, MacConkey for all routine samples and CLED for the urine specimen were used following CLSI criteria(CLSI, 2006).Glycerol stocks were prepared. Broth was inoculated and allowed to grow for 2:30-3:00 hours, after that took 300ul of glycerol and 700ul of broth culture in 1.5ml of Eppendorf and Stored tubes in refrigerator. In order to study different macroscopic features of the selected strains a small inoculum of each strain was picked and plated or and four streaked the culture and then incubated at 37ºC for 24hrs. The purified colonies obtained were studied for morphologic characteristics including colony size, consistency, shape, elevation, margins, color and pigments diffusing into the medium. The colonies were observed visually. Gram staining was performed for microscopic examination following biochemical

battery (Catalase Production Test, IMVIC, Oxidase Test, Citrate Utilization Test, Indole Production Test, Methyl Red-Vogues Proskauer Test, Triple Sugar Iron Test, API-20E TEST), PHENOTYPIC TESTS (Double-Disc Synergy Test, Combination Disc Test).

RESULTS:

About 300 samples of body fluids (urine, wound, and blood) were collected from the City laboratory, Lahore. They were further processed on routine culture media (MacConkey's agar, Blood agar, Chocolate agar and CLED) media. Work focuses on ESBL *Enterobacteriaceae* so 157/300 (52.3%) *Enterobacteriaceae* were isolated, of which 59/157(37.5%) *Enterobacteriaceae* were suspected ESBLs. Purified colonies from 24 hours old cultures were incubated at 37°C on MacConkey's agar. These were examined under microscope to characterize them on the basis of morphological features (colony size, color, shape, consistency, elevation and margins). *E. coli* and *Klebsiella spp* were the main lactose fermenters. While *E. coli* showed different morphology from *Klebsiella spp* in this respect that it has smaller and dry colonies and *Klebsiella spp* has larger and very moist colonies. Biochemical characterization was done for identification of strains at specie level. Therefore, MIU (motility, indole and urease) Test, Citrate Utilization Test and TSI (triple sugar iron) Test were performed and results recorded on the basis of media color change, H_2S production and motility pattern. Consequently, *E. coli* being motile, indole positive and lactose-fermenting was found different from *Klebsiella spp*, which was non-motile, lactose fermenting and utilize citrate on Simon's citrate medium. On the other hand, *Proteus spp* was identified by its H_2S production turning the media black. Finally results obtained after the application of the described tests, *Escherichia coli* (38:64.4%), *Klebsiella spp* (4:6.7%), *Morganella morganii* (7:11.86%), *Citrobacter spp* (4:6.7%) and *Proteus mirabilis* (5:29.5%). Selected *Enterobacteriaceae* were further screened for the ESBLs production by phenotypic Detection Techniques namely; DDST and CDST. Double disk synergy means the mutual effect of two antibiotics, if one is in-active then other drug makes it active due to the combine effect. DDST is a preliminary test for the ESBLs detection that detects by synergism (in terms of key hole production) among the cephalosporins when applied to the strains. Five cephalosporin (ATM, CTX, CAZ and CRO against AMC) were selected and placed 18mm apart from each other. Results revealed 37.57% (59/157) ESBLs as per detection limit of DDST. While synergism between the applied set of drugs can be seen in terms of positive (detected as suspected ESBLs) and negative (detected as suspected non-ESBLs) results (Figure No 1 a & b). Combination disk synergism showed the combine microbicidal effect of more than one drugs against the test microorganism. In CDST, we tested the inhibitory capacity of inhibitors against the ESBLs enzyme production by using two of the cephalosporins (CTX and CAZ) along with their inhibitory supplements i.e., CTC and CZC respectively (spaced at 30mm center to center distance) (Figure No:1 a & b).

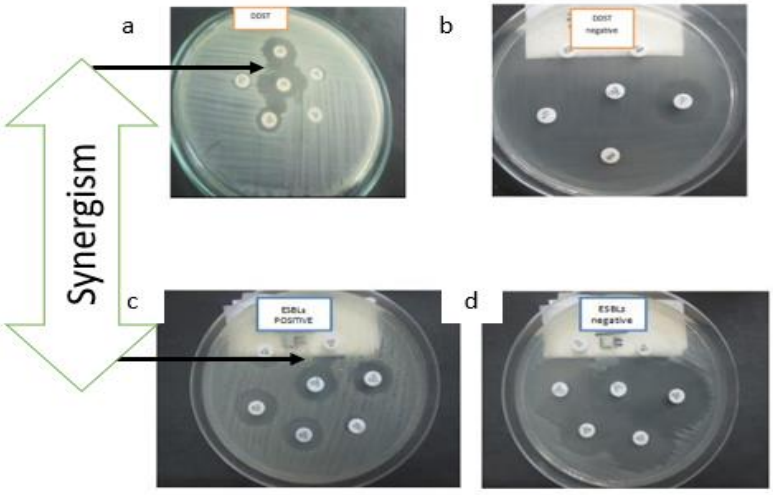

Figure No 01: a) Double disk synergism ESBLs positive results, **b)** Double disk synergism ESBLs negative results, **c)** CDST ESBLs positivity, **d)** CDST ESBLs negativity

Prevalence of ESBLs varies from sample to sample which we observed from ESBLs/Non ESBLs in samples (urine 41%/59%, wound 16%/ 84% and Blood 2%/98%) which indicate that uropathogenic bacteria are more commonly ESBLs producers. Resistance pattern and occurrence varies among males and females in different specimens. As in case of Urine samples ESBLs occurrence is more common in females as compared to males while for wound samples it is more common among males but equal in both males and females in case blood samples. *E. coli* strains were most commonly isolated followed by *Morganella morganii*, *Citrobacter*, *Proteus mirabilis*, and *Klebsiella spp*. Highest percentage of *E. coli* was isolated from urine than wound with a least percentage among blood samples. ESBLs production with respect to age varies among males and females. It was noted that the occurrence of ESBLs quite higher in females as compared to males in the urine sample. There are 34.14% (14/41) chances of ESBL occurrence in females of age 21-30 years and 4.87% (2/41) in males of same age group. Similarly, 29.2% (12/41) females of 41-50 years of age carry ESBLs when only 12.19% (5/41) males of same age group do so (Figure No 2 (a &b). Females are more common indicated in this research data for the infection threat and this above result shows the highest infection rate in the females at the age group of 21 year -30year and then 41year-50 year. Infection occurrence depends upon the age and gender also which is more frequently occurs in the younger age. The infection among male's percentage increases as the age increases. Infection occurrence depends upon the age, and bacterial attack increases (Figure No 1 c & d). If we compare male's ratio with the females then this data showed the different results with respect to the age causing the UTI infection. Males were more targeted by the infectious agents as they become old and females at early years of their age. *E. coli* was more common to producing ESBLs in urine sample. *Morganella morganii* was isolated more often after the *E. coli* and then *Citrobacter spp*, *Klebsiella species* and at the last Proteus mirabilis which was last seen to produce ESBLs (Figure No:2 a & b).

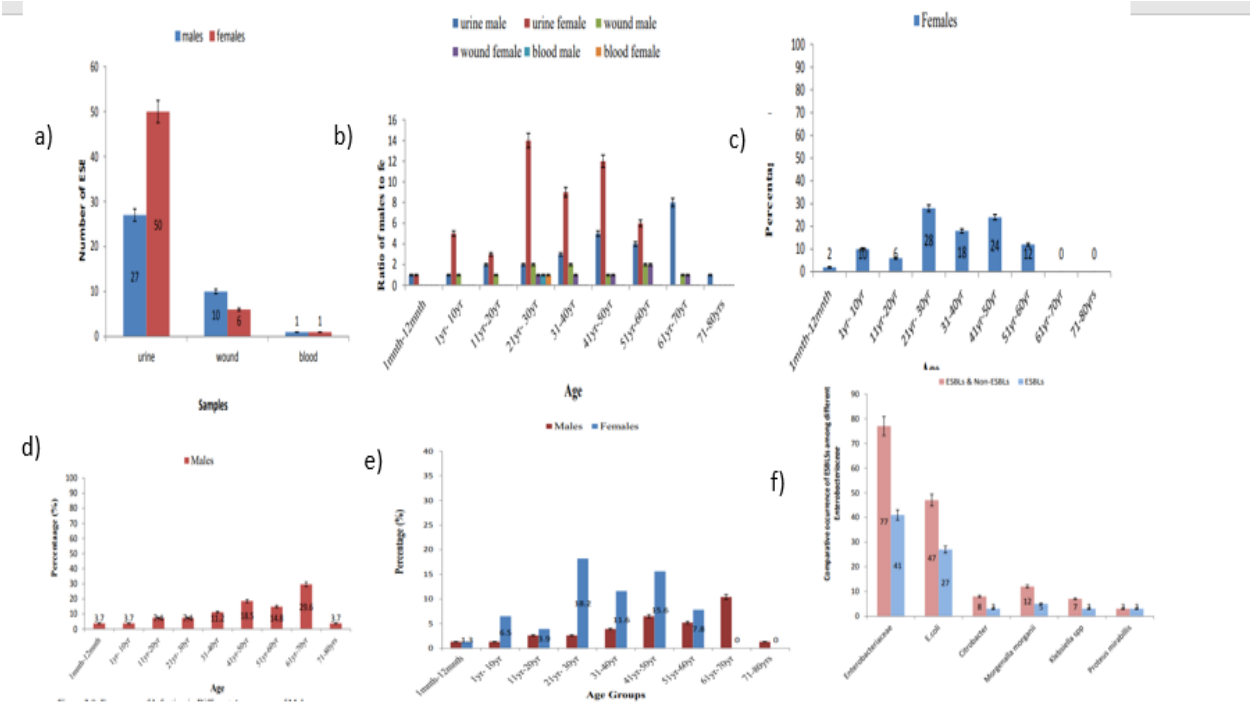

Figure No 2: **a)** Comparison of ESBLs among different samples with respect to Gender, **b)** Comparison among the clinical samples with respect to the age related to gender producing ESBLs, **c)** Frequency of infection in different Age groups of females, **d)** Frequency of infection in different age groups of males, **e)** Comparison of infection rate among Males and Females, Depending upon Age, **f)** Comparison of ESBLs among different strains isolated from urine.

Antibiogram pattern of all ESBLs producing strains were checked against 19 drugs of different groups and concentrations. A high number of drugs showed resistant to most of the drugs and revealed that these strains left no choice of treatment. In the study, ESBLs producing strains were resistant even to third generation of cephalosporin. High resistance >95% to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Gentamicin, Tetracycline and Trimethoprim was noticed while, other drugs faced less resistance and to some strains were highly sensitive such as amikacin, imipenem <2. *E. coli* in the urine isolate showed more resistance as compared to the wound and blood. In the *Citrobacter spp* isolates from urine and blood resistance pattern was almost same as that in the *E. coli* because both were resistant to the same drugs but variation comes at the percentage level was observed from the data. ESBLs *Citrobacter spp* was isolated from urine and blood so comparison was made in both urine and blood but not with the wound. *Citrobacter spp* were highly resistant >92% to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Gentamicin, Ciprofloxacin, Tetracycline and Trimethoprim, while other drugs encountered less resistance and some such as Amikacin, Meropenem, Imipenem < 1% had strong effect on strains. *E. coli* in the urine isolate showed more resistance as compared to the blood. As the *Pseudomonas spp* is non-lactose fermenting organism and results showed that only one ESBLs isolated from all 300 clinical samples from the urine only and it showed the resistance against the ampicillin, aztreonam, gentamicin, ciprofloxacin which was confirmatory drug for Pseudomonas spp and also ceftazidime >95%, while other drugs are susceptible and are the choice of treatment (Figure No 2c, d, e & f).

In the *Morganella morganii* isolates from urine and wound, resistance pattern was almost same as that in the *E. coli* because both are resistant to the same drugs and belongs to the same family as gram negative bacilli but variation at the percentage level was observed from the data. ESBLs *Morganella morganii* was isolated from urine and wound, so comparison was made in both urine and wound but not with the Blood. In the data showed that *Morganella morganii* from wound are highly resistant to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Ceftazidime, Tetracycline and Trimethoprim >90%, while to other drugs strain showed less resistance and some are highly sensitive to drugs such as amikacin, meropenem, imipenem <2%. *Morganella morganii* in the wound isolate showed more resistance as compared to the urine. In the *Klebsiella spp* isolates from urine and wound resistance pattern was almost same as that in the *E. coli* because both are almost resistant to the same drugs and belongs to the same family as gram negative bacilli but variation at the percentage level was observed from the data. ESBLs *Klebsiella spp* was isolated from urine and wound, so compared was made in both urine and wound but not with the blood. *Klebsiella spp* were highly resistant to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Ceftazidime, while Gentamicin, Tetracycline, Ciprofloxacin and Trimethoprim <3% faced higher resistance from strain in wound other than that from urine. Other drugs encountered less resistance and some are highly sensitive such as Amikacin, Meropenem, Imipenem. *Klebsiella spp* in the wound isolate showed more resistance as compared to the urine *Klebsiella isolates*. In the *Proteus spp* isolates from urine and wound resistance pattern observed which was similar to the other gram negative *Enterobacteriaceae* but variation at the percentage level compares resistance faced by the drugs. ESBLs *Proteus spp* was isolated from urine and wound, so comparison was made in both urine and wound. Proteus spp were highly resistant to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Ceftazidime, while Gentamicin, Tetracycline, Ciprofloxacin and Trimethoprim were at higher resistance >95% in wound other than urine and other drugs showed less resistance and some were less resistance such as Amikacin, Meropenem, Imipenem. These results also showed that *Proteus spp* in the wound isolate showed more resistance as compared to the urine *Proteus spp* (Figure No 3 a, b & c).

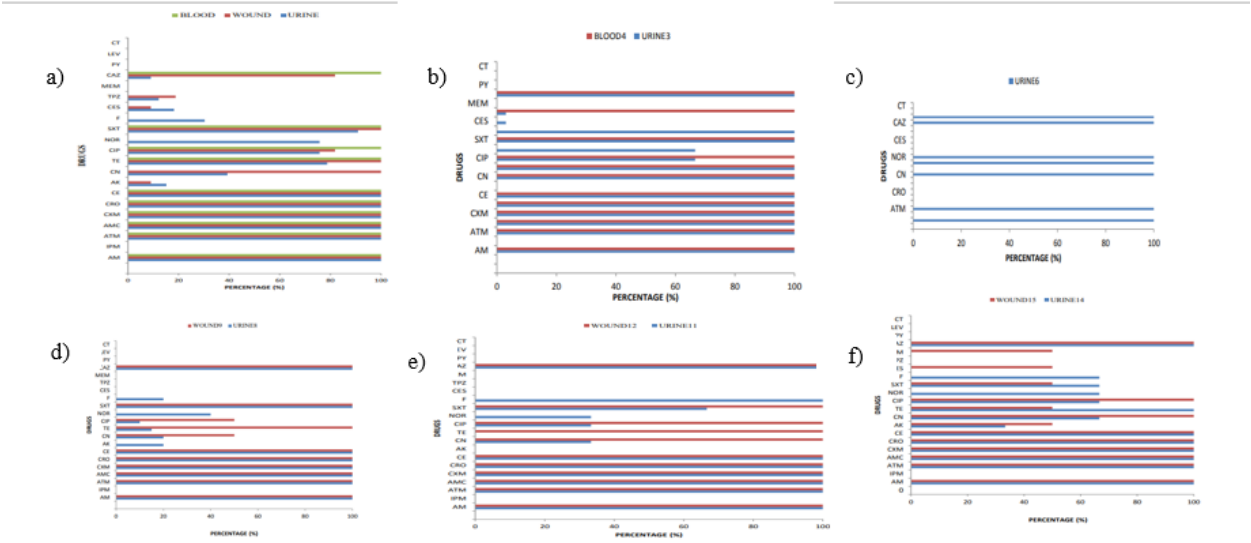

Figure No 3: a) Drug Resistant Pattern in *E.coli*, **b)** Drug Resistant pattern in *Citrobacter spp*, ***c)*** Drug Resistant pattern in *Pseudomonas aeruginosa,* **d)** Drug Resistant Pattern in *Morganella morganii*, **e)** Drug Resistant pattern in *Klebsiella spp*, ***f)*** Drug Resistant pattern in *Proteus spp*

DISCUSSION:

Multi drug resistance (MDR) is one of the worrisome issues of the century because of its lack of treatment. With the passage of time, it is more difficult to treat the MDR due to adaptation of bacteria according to new drug trends. This problem made concern people more worried about the health future due to the limitation of treatments. Not only have these bacteria become resistant but also spreading all over the society worldwide. This bacterial resistance is a great hurdle in the treatment of infections such as nosocomial and health care associated infections [16].This resistance pattern of bacteria raised the mortality and morbidity rates among patients. Resistance also affects the cost of treatment due to the extension of the treatment options that are limited[17]. Extended spectrum beta-lactamases are the enzymes produced by the bacteria especially by the gram negative *Enterobacteriaceae* family such as *Escherichia coli, Klebsiella spp, Citrobacter spp, Proteus spp, Morganella morganii*etc. These enzymes made the bacteria resistant to the beta-lactam antimicrobial agents which are the most common treatment for the bacterial infections. The rate of bacterial resistance increasing worldwide day by day and is alarming for the clinicians, researchers and laboratory persons. The origin of ESBLs phenomenon began in Western Europe, where the first clinical use of expanded-spectrum β-lactam antibiotics was employed. However, in a limited period of time, just a few years, ESBLs had also been monitored in the United States, Asia and many countries of Europe [18]. Rate of bacterial resistance vary from country to country, area to area and even between the clinical samples, health facilities, gender health status and age groups plays very significant role. Beta-lactamases enzymes fight against the beta-lactam ring of the penicillin group antibiotics such as third generation cephalosporin. These enzymes made bacteria resistant to antibiotics by the mutation because they are numerous and mutate themselves in response to the high dose of the antimicrobial agents. When a disease is treated with the high pressure of antimicrobial agent to cure as soon as possible then bacteria fight against these agents intelligently to survive and made them stable. Bacteria produce certain enzymes to hydrolyze the antimicrobial agents such as beta-lactamases produce by the Enterobacteriaceae family which hydrolyze the beta lactam ring by mutate the gene encoding these enzymes such as TEM and SHV found from the *Escherichia coli* and *Klebsiella species* respectively [19].Time is necessary to kill the β-lactams activity because it has a positive correlation between its efficacy and the time require to the drug exceeds its MIC value during dosing intervals [20].To overcome the bacterial infective activity drug needs time depends upon the dose of the drug as in high concentration reaches its target faster than the lower concentration but chances of resistance increases due to the bacterial resistance activity against that drug

[21].compared the results of patients with *P.aeruginosa* infections which were treated with the piperacillin-tazobactam by 2dosageregimens (3.375g IV for 30 minutes every 4-6 hours VS 3.375g IV for 4 hoursevery 8hours). Patients who received therapy for high time had lower mortality rates and less hospital stays as compared to patients who received therapy for less time longer hospital stays. Here, total 300 clinical samples were collected from the city lab and research center, 525-A Maulana Shaukat Ali Road near Jinnah Hospital Lahore for my research of M.SC on the topic of Comparative analysis of antibiotic susceptibility among beta-lactamase producing strains from different clinical samples in Pakistan, Lahore, 157 strains were isolated as *Enterobacteriaceae* member from the clinical sample's urine, wound and blood. Some strains other than *Enterobacteriaceae* was isolated such as Staphylococcus aureus which were MRSA, *Staphylococcus saprophyticus, Enterococcus* etc. Majority of them were NON-ESBLs. Generally, here focus on ESBL *Enterobacteriaceae* members. Urine was more common ESBLs producing clinical specimen following wound or blood in which number of ESBLs producers varied from specimen to specimen and also organism to organism. Here total ESBLs 41/59(69.4%) isolated from urine, 16/59(27.1%) from wound, 2/59(3.3%) from blood in which *E. coli, Klebsiella spp, Citrobacter spp, Morganella morganii* and *Proteus mirabilis* isolated. However, *E. coli* 27/59(45.7%), *Citrobacter spp* 3/59(5%), *Morganella morganii* 5/59(8.4%), *Klebsiella spp* 3/59(5%), and *Proteus mirabilis*3/59(5%), from wound *E. coli*11/59(18.6%), *Klebsiella spp*1/59(1.6%), *Proteus mirabilis* 2/59(3.3%), *Morganella morganii* 2/59(3.3%),from blood *E. coli* 1/59 (1.6%), *Citrobacter spp* 1/59 (1.6%) was isolated. A study conducted at Hyderabad Hospital that 660 clinical samples from which 125 were isolated as ESBLs producers including *E. coli, Klebsiella spp* and other organisms. Here, again it was noted that urine was most common for the ESBLs production then comes wound and then blood. Kumar reported that *E. coli* isolated from different clinical samples such as 67/125(53.6%) from urine, 21/125(16.8%) from blood and 37/125(29.6%) from the wound and other exudates (Kumar et al., 2014). Comparison of different reports with our work reveled that UTI was most common infectious. 157 *Enterobacteriaceae* comprise *E. coli, Citrobacter spp, Proteus mirabilis, Morganella morganii, Klebsiella spp, Enterobacter spp, Pseudomonas spp,* and *Salmonella typhi*. 59 were isolated as the ESBLs comprise *E. coli, Citrobacter spp, Morganella morganii, Klebsiella spp, Proteus mirabilis,* but *E. coli* was most commonly isolated. It was noticed that ESBLs isolated in highest frequency from the urine samples then wound and blood. E. coli was predominant uro-pathogen. Total 41 ESBLs isolates were obtained from the urine, 16 ESBLs from the wound, and at the last too much smaller number of ESBLs isolated from the blood clinical samples, showed ESBLs commonly present in UTI. Mumbai Maharashtra reported that urinary tract infections (UTI) were more common infection in general population. Out of 59 ESBLs *E. coli* were 27(65.8%), *Citrobacter spp* 3(7.3%), *Klebsiella spp* 3(7.3%), *Morganella morganii* 5(12.1%) and *Proteus mirabilis* 3(7.3%) showed that *E. coli* is more commonly isolated from the urine samples, at the second *Morganella morganii* and then *Proteus mirabilis, Klebsiella spp, Citrobacter spp* were isolated in equal number from the urine samples. It was noted in this study that colony forming unit which is 10^5cfu/ml causing the UTI infection. Same report was given in the central Mumbai that 10^5cfu/ml considered as the bacteremia [22]. In Central Mumbai 112 clinical samples were processed in which most commonly isolated bacteria was *Escherichia coli* 90(80%), *Klebsiella spp* 18(16.07%) and *Proteus mirabilis* 1(0.8%). Other reports also had the similar results about the *E. coli* frequency isolated from the UTI [23]. Noteworthy occurrence of ESBLs was quite common in females as compared to males. Age noted was 21-30 years cause 34.14% (14/41) in females and 4.87% (2/41) in males, 41-50 years cause 29.2%(12/41) in females and 12.19% (5/41) in males. Study data showed that females were more targeted by the uropathogenic bacteria, because severe infections. UTI was more common infection which is related to the age, gender, or other physical or chemical factors of our body. Females are more common indicated in this research data for the infection threat and showed the highest infection rate inthe females at the age group of 21-30 years and at the second rank 41year-50year. Infection occurrence depends upon the age and gender which was more frequently occurs in the younger age, as the age increases infection rate becomes severe and also increases. In general, here data revealed highest infection in females at younger age. Females of young reproductive age and males of old age were more prone to infection [24]. So, infection rate increases as the age increases because of the low immunity level and risen the attack of the pathogens due to the absence of the fighting

ability as the age increases. Here again data suggest that infection rate among males increases as the age increases. Infection occurrence depends upon the age, and bacterial attack increases.Females were predominant with respect to causing the infection which indicated that UTI were more common among the females. It was noted that females were more common to cause UTI infections at the younger age, showed younger age of females is highly risky to the bacterial attack which is increases among the young women. If we compare male's ratio with the females then this data showed the different results with respect to the age causing the infection. Males are more targeted by the infectious agents as they become old and females at the younger, there is a great difference to causing infection between males to females, bacterial attack increases on the females as they become young may be due to the immunity weakness at that age which is not related to the males because this immunity risk increases in the males as they become old. Due to female anatomy as bacteria enter from the bowel to the urethra and females become more at the risk of bacterial attack. In another reported in the India that females are predominant from the males in causing the UTI infections and are at high risk of more bacterial attack. It was reported that females are 59% causes UTI infections with respect to the males which are41% indicate that females more commonly cause UTI infections, half of the females haveat least one infection in their lives [25]. *E. coli* MDR had more serotypes and its own sub species, each sub specie has its own pattern of causing disease and also multidrug resistant pattern that's why E. coli is more common to producing ESBLs. Data analysis showed that all the ESBLs organisms show highest resistance to the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Gentamicin, Ciprofloxacin, Cephradine, Trimethoprim for the lactose-fermenting *Enterobacteriaceae* and *Pseudomonas spp* is especially resistant to the PY (Carbenicillin). At the specie level analysis of drug susceptibility data showed that in the wound sample *E. coli* is highly resistant (100%) to Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Gentamicin, Tetracycline, Trimethoprim, and 81% resistant to the Ceftazidime and Ciprofloxacin. It is sensitive to the Imipenem, Amikacin, Meropenem, Sulzone, Norfloxacin, Nitrofurantoin (30%), it was same for the E. coli isolated from the urine except Gentamicin is 39.3%, Tetracycline is 78.7%, Norfloxacin is 75.7%, and Nitrofurantoin is 30.3%, while the other sensitivities are also same in both urine and wound. Resistant pattern for *E. coli* in the blood was unusual in which all the drugs showed 100% resistance includes Augmentin, Ceftriaxone, Cephradine, Tetracycline, Ciprofloxacin, Aztreonam, Cefuroxime, Ampicillin, Ceftazidime, and Trimethoprim while the sensitivities is same except for Norfloxacin and Nitrofurantoin because these drugs are special for urine samples. Mandal et al., (2012) reported the drug resistance among the uropathogenic he mentioned that *E. coli* is highly resistant to the Ampicillin (80.6%), Ceftriaxone (60.5%) and 59.3% in all other isolates of *E. coli*. Earlier it was mentioned that E. coli from the blood showed 100% resistance to the drugs mentioned and same results were noted for the Citrobacter spp isolated from the blood. Resistant pattern for Citrobacter spp was also unusual like *E. coli* in the blood in which all the drugs showed 100% resistance includes Augmentin, Ceftriaxone, Cephradine, Tetracycline, Ciprofloxacin, Aztreonam, Cefuroxime, Gentamicin, Tazobactam, Ampicillin, Ceftazidime, and Trimethoprim while the sensitivities is same except for Norfloxacin and Nitrofurantoin because these drugs are special for urine samples. While in the urine showed 100% resistance towards the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, Gentamicin, Tetracycline, Trimethoprim, Ceftazidime and Ciprofloxacin (66.6%) and Norfloxacin (66.6%) and sensitive results noted towards the Imipenem, Amikacin, Meropenemetc. *Pseudomonas spp* was isolated from the urine sample as ESBLs not from blood or wound. Pseudomonas spp showed 100% resistance towards the Ampicillin, Aztreonam, Gentamicin, Ciprofloxacin, Norfloxacin and Ceftazidime and especially for the Carbenicillin. It was reported from the Maharashtra that *P. aeruginosa* was resistant to the ampicillin and ceftriaxone while 66.6% and showed high resistance towards the aztreonam, gentamicin and ciprofloxacin and highly sensitive towards the tazobactam [26]. *Morganella morganii* also isolated from the urine and wound and observed for the sensitivity pattern and noted that these isolates showed 100% resistance towards the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, and Tetracycline in urine, Trimethoprim, and Ceftazidime while all other drugs noted sensitive towards the Nitrofurantoin, Norfloxacin, Amikacin, Meropenem, Sulzone and Tazobactam. *Klebsiella spp* isolated from the urine and wound and high resistance were observed in the wound instead of urine.it is noted that *Klebsiella spp* from both urine and wound resistant to the same drugs except few variation such as 100% resistance towards the Ampicillin, Aztreonam, Augmentin,

Cefuroxime, Ceftriaxone, Cephradine, Gentamicin from urine (33.3%) and from blood 100% and same for the Ciprofloxacin, Tetracycline in case of urine is sensitive to the *Klebsiella spp* or in wound case 100% resistant, Trimethoprim 66.6% for urine and 100% in wound case, and 100% resistant to the Ceftazidime. It is sensitive to the imipenem, Amikacin, Meropenem, Sulzone, Norfloxacin for urine is 33.3% and in wound case 100% sensitive, Nitrofurantoin noted100% resistant to in urine sample. In India *Klebsiella pneumonia* was 85-95% resistant to the cefotaxime and 37-53% resistant to the ceftazidime from this resistant percentage values they concluded that ESBLs enzyme was cefotaxime's which hydrolyze the cefotaxime and make bacteria resistant to that drug [27]. *Proteus spp* also isolated from the urine and wound and observed for the sensitivity pattern and noted that these isolates showed 100% resistance towards the Ampicillin, Aztreonam, Augmentin, Cefuroxime, Ceftriaxone, Cephradine, and Tetracycline in urine and 50% in wound, Trimethoprim 66.6% in urine and 50% from wound, and Ceftazidime, while all other drugs noted sensitive towards the Nitrofurantoin and Norfloxacin showed 66.6% in urine, Amikacin, Meropenem, Sulzone and Tazobactam. Maharashtra, noted that Proteus spp showed resistance towards the Gentamicin, and Ciprofloxacin [25]. Proper antibiogram profiling in different hospitals, health sectors and different geographical areas with periodic passage of time is very crucial. It helps clinicians in designing the new panel of drugs depending on type of infection every year. The comparison of antibiogram developed by clinical ESBL isolates showed that there was high rate of resistance. This is alarming that regardless of isolation source beta-lactamase producers were equally harmful to living organisms. Such work helps clinical laboratory standard institute for developing the new panel of drugs for this geographical area in recent years.

CONCLUSION:

ESBLs Varies from Area to Area, Depending Upon Gender, Age, Health Care Facilities. Amikacin, Tazobactam And Sulzone Are Still the Drugs of Choice for Such Infections. Proper Antibiogram Profile Evaluation Is Very Significant for Managing Such Infection.

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