Prevalence of Blactx-M Producing Gram-Negative Bacterial Pathogens from a Teaching Hospital in Dera Ghazi Khan

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Abstract- Bacterial infections and antimicrobial resistance cause significant mortality and morbidity. Extended-spectrum lactamases (ESBLs) are enzymes that help bacteria resist monobactams and cephalosporin. ESBLs hydrolyze cephalosporin and aztreonam and are inhibited by clavulanic acid. Gram-negative bacteria, *K. pneumonia, E. coli, A. baumannii*, and *Pseudomonas spp.* Linked to blood infections. ESBL acquisition is rising worldwide. ESBL-producing strains cause public health problems. ESBLs include blaTEM, blaSHV, and the blaCTX-M group. Study to assess antimicrobial resistance & frequency of ESBL genes from clinical samples at a hospital in Dera Ghazi Khan. Samples were collected and cultured. After identifying bacteria with Gram Staining and biochemical testing, we determined antimicrobial resistance and ESBL gene frequency. *Salmonella typhi* caused most cases of typhoid fever (48.2%), followed by *Klebsiella pneumoniae* (15.2%), Escherichia coli (10.7%), and *Acinetobacter baumannii* (9.8%). Less common pathogens were *Serratia marcescens* and *Burkholderia* cepacia (4.5% each). ESBLs gene prevalence was analyzed via primer-specific PCR and Gel Electrophoresis. 100% of marcescent isolates tested positive for blaTEM and K. Pneumonia with a high prevalence of blaTEM and blaSHV (88.2% and 64.7% respectively). E. coli carried prevalent blaCTX-M (83.3%), blaSHV (16.7%), and blaTEM (66.7%). This study focuses on strategies to limit the spread of antimicrobial resistance genes and find alternative treatments.

Index Terms- Extended spectrum–lactamases, Multidrug-resistant, blaCTX-M enzymes, Antibiotic-resistant

I. INTRODUCTION

A major worldwide health concern of the twenty-first century, antimicrobial resistance (AMR) affects all aspects of health, including humans, animals, and the environment (Kamran et al., 2022).Resistance is primarily fueled by selective pressure brought on by the overuse of antibiotics. Access to over-the-counter antibiotics and increased antibiotic use worldwide may help to explain some of the rising AMR burden (Teklu et al., 2019). Antibiotic effectiveness is being reduced or eliminated due to the evolution of antibiotic-resistant microorganisms. Antimicrobial resistance rates have risen during the past ten years, and a rise in the incidence of multidrug-resistant (MDR) isolates in clinical settings has also been seen (Sangare et al., 2015). Additionally, the term "extreme drug resistance" has been used to describe isolates for which no effective treatments are available. Intense research is also being done on rapidly developing and describing new resistance mechanisms and identifying nearby genes and genetic platforms that effectively facilitate the capture, transfer, and expression of resistance determinants (Haque et al., 2021). These investigations also examine the genetic platforms that correspond to the resistance determinants' origins (Nagvekar, Sawant, & Amey, 2020). Antimicrobial resistance is a problem that affects people and animals all over the world and poses a serious risk to their health. (Puvača & de Llanos Frutos, 2021). Even though this problem has been caused by the overuse and abuse of antibiotics, several other variables have contributed to its development (Anand et al., 2021). Bacteria have a unique property to build up usual antibiotic resistance due to long-term use as a result of gene changes, transmutation, and transportable hereditary material (plasmid, transposons, and integrons) due to improper use of antimicrobials (either due to over-prescription, shortened treatment duration, or insufficient dose rate of antibiotics). When it comes to tracking AMR, time is important. The longer action is postponed, the more difficult it is to maintain control in the long run (Harbarth et al., 2015). Start of the 1990s, a little number of countries spread action plans and national strategies to mitigate this warning, and some countries successfully decrease antibiotic utilization in animals and humans (Puvača & de Llanos Frutos, 2021). Furthermore local estimates of AMR. But most countries confronted this problem only more recently (Capita & Alonso-Calleja, 2013). Antimicrobial resistance in bacteria is a growing problem worldwide, just like the prevalence of bacterial infections

(Nguyen, Ho, Nguyen, & Nguyen, 2021). The synthesis of beta-lactamase is one of the most prevalent and widely used of these methods. The diversity of beta-lactamases that can kill Gram-negative bacteria is growing at an alarming rate (ESBLs). The extended-spectrum-lactamases (ESBLs) are a family of plasmid-encoded enzymes that can break down third-generation antibiotics. ESBLs. One of the major sources of drug-resistance genes, especially those involved in ESBL synthesis, which imparts resistance to all beta-lactam antimicrobials, is gram-negative bacteria. The almost 3 million cases of antibiotic-resistant illnesses that occur annually in the United States result in roughly 35,000 annual deaths (Anwar et al., 2022). AMR is responsible for about 33,000 annual deaths in Europe. Cost and impact estimates for antimicrobial resistance (AMR) have been especially difficult to make in low and medium-income countries. AMR could shorten people's lives by even more years in places where children's deaths are typically caused by bacterial illnesses (Betelhem, Shubisa, & Bari, 2022; Goksoy et al., 2021). As a global issue, AMR is most pressing for the world's poorest countries (Raheel, Orabi, & Tag, 202; Betelhem, Shubisa, & Bari, 2022). Pathogens that are resistant to antibiotics are believed to have originated in South Asia. The Asia region is predicted to have high growth in antibiotic resistance, making it a national and global hazard High rates of antibiotic resistance in Pakistan, a developing country in South Asia, are a major international and regional health concern (Ahmad, Samad, & Hamza, 2023; Ahmed, Riad, Diab, Mansour, & El-Mossalami, 2022). Extensively drug-resistant (XDR) and multi-drug-resistant (MDR) bacteria have been discovered in Pakistan in recent years (Ntondini, Lenetha, & Dzogbewu, 2021). Because human health and animal health, in addition to the viability of ecosystems, are inextricably linked, the "One-Health" concept is essential for finding a solution to this problem. Antibiotic resistance has been connected to the extensive use of antibiotics as well as their improper usage in human medicine, agriculture (including animal farming), and industry (Harbarth et al., 2015; Rossi, Efendi, Rahmayuni, & Yoenissa, 2022a, 2022b; Riaz et al., 2023). This article will attempt to provide light on the origins of antimicrobial resistance, its history of development, and the factors that have contributed to its continued existence over time. Antimicrobial resistance is the key factor contributing to the problem (Mannan et al., 2021; ; Javed et al., 2021). As a result of increased awareness of the evolutionary underpinnings of antibiotic resistance, it may be able to design novel techniques to address this critical public health issue. Antibiotic resistance is a problem because it makes bacteria less susceptible to being treated with antibiotics. The majority of countries contain organisms in their populations that carry ESBL enzymes, most notably the blaCTX-M enzymes. Enterobacteriaceae is a family of bacteria that also includes bacteria with high levels of antibiotic resistance and germs that regularly cause infections in hospitals and the community. *Klebsiella pneumoniae* and *Escherichia coli* are both members of this family of bacteria (Nazir et al., 2021; Sarwar et al., 2021). This resistance profile poses the greatest substantial threat to the public's health in terms of both infection prevention and treatment (Do, Byun, & Lee, 2021). Bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* are among the most common causes of urinary tract infections (UTIs). One of the most common bacterial species, *Escherichia coli*, is responsible for a wide variety of bacterial diseases that can affect people as well as other animals the family Enterobacteriaceae is responsible for what is considered to be one of the most significant bacterial infections in the world today for therapeutic purposes. As was mentioned by (De Francesco et al., 2019). One of the most common types of gram-negative bacteria, *Klebsiella pneumoniae* (*K. pneumoniae*), is capable of causing opportunistic infections such as pneumonia, sepsis, and inflammation of the urinary tract. Many gram-negative bacteria, except for *Escherichia coli*, which is a member of the family Enterobacteriaceae and is typically not harmful to people, are capable of infecting people in various parts of the body, such as the kidneys, the central nervous system, and the gastrointestinal tract. *Escherichia coli* is a member of the Enterobacteriaceae family and is typically not harmful to people (Dale, Dey, Delhey, Kempenaers, & Valcu, 2015). *Klebsiella pneumoniae* is a bacterium that is typically considered to be non-threatening. Even though the bacteria are found in your intestines and feces, they may pose a health risk if they spread to other areas of your body. Lung, bladder, brain, liver, eyes, blood, and wound infections caused by *K. pneumoniae* may be life-threatening (Bengmark, 2013). *Klebsiella pneumoniae* is both a commensal and an opportunistic pathogen in the gastrointestinal system. This pathogen may cause a variety of illnesses, including pneumonia, urinary tract infection, and bacteremia, and it can spread systemically to secondary locations (Linhares, Raposo, Rodrigues, & Almeida, 2013). Hospital-acquired infections are caused by *K. pneumoniae*, which accounts for around 10% of all infections, with cases of pneumonia accounting for 11.8 percent of all infections. It is becoming more dangerous owing to an increase in the number of antibiotic-resistant strains, of which a subset is resistant to all antibiotics now available (Jacob et al., 2013). Antibiotic resistance in biofilm increased exponentially. The blaCTX-M gene was conjugated by seven isolates from ESBL-producers, with transfer frequencies ranging from 2.22 104 to 7.14 102 trans conjugants/recipient cell during the planktonic stage to 3.04 103 to 9.15 101 during the biofilm stage. In biofilms, blaCTX-M transfer frequency was significantly higher than in planktonic conditions, and co-transfer of ciprofloxacin resistance was discovered in five isolates (Emes, Naylor, Waage, & Knight, 2022).This research shows that the wide spread of -lactam and fluoroquinolone-resistant genes in environmental environments is directly related to the presence of biofilm-forming ESBL-producing enteric bacteria with a high frequency of resistance gene transfer. This type of multidrug resistance poses a significant risk to both animal and human health as it continues to proliferate (Dale, Dey, Delhey, Kempenaers, & Valcu, 2015).Urinary tract infections (UTIs) acquired in the community are highly common in less developed countries. These infections are often difficult to treat because the pathogenic bacteria have evolved antibiotic resistance. UTI is a distinct risk factor for renal cell carcinoma and bladder cancer. Women are more likely to get urinary tract infections (UTIs) than men. UTIs, which are also responsible for a considerable amount of morbidity and significant amounts of money spent on medical care, can affect a sizeable portion of the world's population. 90% of urinary tract infections are brought on by uropathogenic *E. coli* (UPEC). It is generally accepted that the overuse of antibiotics in both human and veterinary medicine is the key factor that contributes to the development of multiple drug resistance (MDR) in UPEC. Somily et al. (2014) stated that ESBL bacteria are increasingly resistant and a major public health risk.

This study examined ESBL-producing *E. coli* and *K. pneumoniae*. Antibiotic susceptibility in a hospital. *E. Coli* & *K. Pneumoniae* Samples were sent to the King Khalid University Hospital lab (2006-2010). Test strips confirmed the ESBL status of strains with certain antibiotics. 268 ESBL-producing pneumonia were detected from 17,105 samples.

II. Materials, Methods, and Interpretations

Study Design

Descriptive cross-sectional. 112 patients with positive culture were included in the study. Department of Microbiology, Government College University Faisalabad.

Clinical specimens

The clinical specimen (Blood, Urine, Pus, CSF) were collected from patients admitted at various public and private sector tertiary care hospitals of Dera Ghazi Khan during the period of six months.

Bacterial Isolation

The samples were initially cultured in Tryptic Soy Broth (TSB) and were sub-cultured on routinely used culture media including McConkey agar, Blood agar, and Chocolate agar (Oxoid, UK). The presumptive identification of bacterial isolates was completed based on colony morphology, and culture characteristics.

Interpretations

Table 1: Interpretation of the TSI Test for the Members of Enterobacteriaceae

Name of the organism	Slant	Butt	Gas	H_2S
Escherichia	Yellow	Yellow	Yes	No
Enterobacter	Yellow	Yellow	Yes	No
Klebsiella	Yellow	Yellow	Yes	No
Shigella, Serratia	Red	Yellow	No	No
Salmonella, Proteus	Red	Yellow	Yes	Yes
Pseudomonas	Red	Red	No	Yes

Catalase test

This test is used for evaluating the capability of a microorganism to break down hydrogen peroxide into oxygen and water. Take a drop of hydrogen peroxide on a glass slide. Mix a colony of microbial growth in it with the help of an inoculation loop. Bubble formation is indicative of a positive result.

Oxidase Test

`Cytochrome oxidase produced by bacteria oxidizes the phenylenediamine in the reagent to produce a deep purple color. *E. coli* is oxidase negative.

Vitek identification of isolates

Vitek uses a fluorescent and turbidimetric methodology for the identification and susceptibility of microorganisms respectively. Four types of Vitek are generally used, ID-GN (Gram-negative Bacilli identification), and ID-GP (Gram-positive Cocci identification).

Antimicrobial Susceptibility Tests

Isolates were tested for antibiotic susceptibility using broth dilution. Per CLSI recommendations, strains were classified as susceptible or resistant to tested antibiotics. The inhabited area is the organism's sensitivity to the antibiotic; continued growth is resistance. Make culture inoculum at 0.5 McFarland in sterile saline. Swab inoculum onto Mueller Hinton agar plates. Put antibiotic discs onto sterile Mueller Hinton agar plates. Incubate plates upside down at 37°C for 24 hours. Measure antibiotic disc zones. Check measurements against CLSI 2015 standards for antibiotic effectiveness. Bacterial isolates not inhibiting antibiotic disc retested for resistance verification (Kassim, Omuse, Premji, & Revathi, 2016).

Figure 1: Antimicrobial Sensitivity Testing of isolates

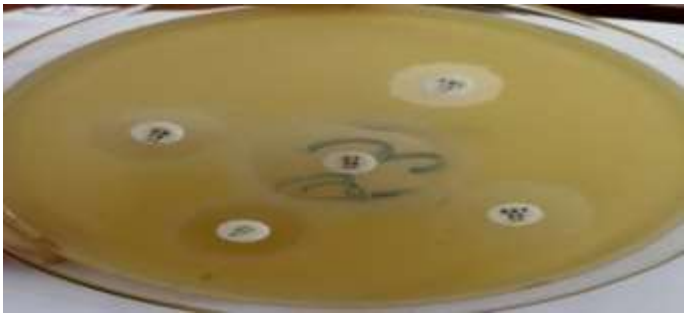


Figure 2: Antibiotic sensitivity testing by disk diffusion method

ESBL Confirmation

Isolates were tested using disc diffusion with various antibiotics to check for ESBL enzyme production. Enzymes identified per CLSI recommendations. TEM and SHV are susceptible to cefotaxime, while CTX-M is susceptible to ceftazidime.

Extraction of the Genomic DNA

Bacteria were grown in nutrient broth and incubated for 24 hours at 37 Celsius. 2 ml broth was centrifuged for 5 minutes at 8000 rpm. Pellet was re-suspended in IXBPS at PH 7.4, washed, and subjected to DNA extraction with a FATG DNA extraction kit.

DNA Quantification

DNA's optical density was measured using a Calibri spectrophotometer at 260 and 280nm. DNA was diluted 1:50 in 90µl diluent by adding 2µl DNA. DNA was quantified using standard procedure. The formula for DNA quantification was used. DNA conc. OD at 260nm x dilution factor x 50 = DNA in µg/l. 260/280 ratio > 1.8 = pure DNA. DNA stored at -20C for processing.

Primer Sequence for ESBLs Genes Detection Responsible for Antibiotic Resistance.

Phenotypically confirmed isolates were further confirmed by using species-specific primer and the presence of ESBLs genes through the Polymerase chain reaction technique. A thermal cycler (Bio-Rad Laboratories, Inc. California, United States) was used.

Table 2: Concentration of antibiotics used for AST as per CLSI guidelines

Sr. No.	Antibiotic	Concentration (µg)
1	Gentamicin	10
2	Tobramycin	10
3	Amikacin	30
4	Imipenem	10
5	Meropenem	10
6	Ciprofloxacin	5
7	Levofloxacin	5
8	Piperacillin-tazobactam	100/10µg
9	Ticarcillin-clavulanic acid	75/10
10	Cefotaxime	30
11	Ceftriaxone	30
12	Ceftazidime	30
13	Cefepime	30
14	Trimethoprim-sulfamethoxazole	1.25/23.75µg
15	Ampicillin-sulbactam	10
16	Doxycycline	30

Bacterial Identification

Gram Staining

Gram staining was used to check the morphology of isolates. The following protocol was used for Gram staining, Bacterial culture, Glass slide, Spirit lamp, Wire loop, Diamond pen to label the slides, Staining rack, Distilled water and Reagent set for Gram staining.

Motility Test

The organism's motility was checked by wet mount preparation as per the standard guidelines and observation of the visible movement of bacteria under a microscope was checked.

Triple Sugar Iron (TSI) Agar Test

Test to differentiate Enterobacteriaceae based on sugar fermentation and Hydrogen Sulphide production. TSI contains sugars, including 1% Lactose, 1% Sucrose, and 0.1% Glucose, in a 10:10:1 ratio. Phenol red indicates acidification. Contains Ferrous Sulphate for iron. Sugar fermentation produces acid, lowering the PH and causing the indicator to turn yellow. No sugar fermentation, and no color change in phenol red. Enterobacteriaceae ferments sugar, turns butt yellow. Forming ferrous sulfide with Thiosulfate as an electron acceptor (El-Zanfaly & Kassim, 1983).

III. WRITE DOWN YOUR STUDIES AND FINDINGS

Amplification Procedure

ESBL gene amplification was detected using this procedure. In a PCR cuvette, 1µl DNA template was added, followed by 10 pico-mole of respective primers. 10µl distilled water as negative control. 15µl 2X Master mix (NEB, UK) added. BioRad-T100 was programmed for one cycle of DNA denaturation at 94 °C for 5 minutes. Cycle repeated 35x @ 94°C for 30 sec. DNA annealed at 50°C for 40 sec. 72°C, 45 sec. Final 5-minute cycle at 72 °C.

Gel Electrophoresis and UV Transillumination of Amplicons

The solidified gel was placed in a gel tank already filled with TAE buffer. 1µl of 6X loading dye was placed in each well. About 5µl of each DNA sample was loaded in separate wells and electrophoresis was done at 140 volts for 45 minutes. UV transillumination was used to visualize DNA bands.

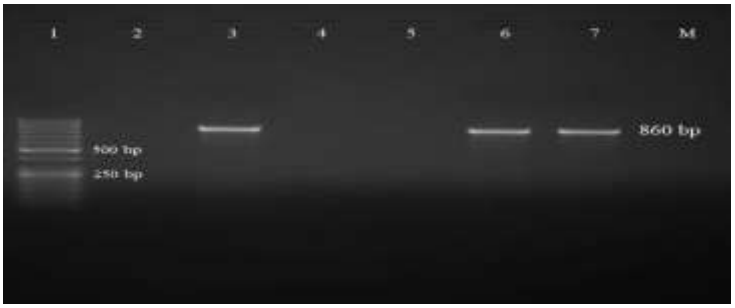


Figure 3: Gel Electrophoresis of Amplified TEM gene

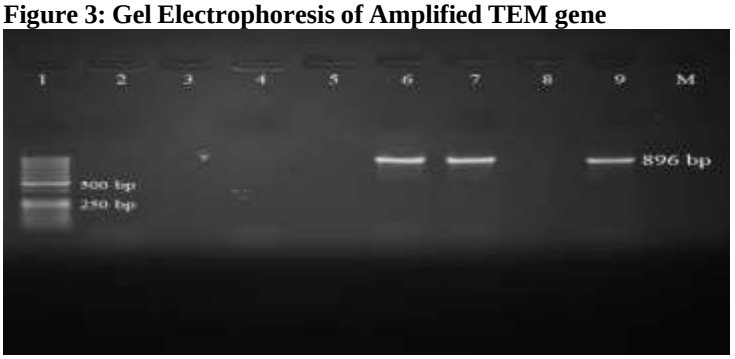


Figure 4: Gel Electrophoresis of Amplified SHV gene

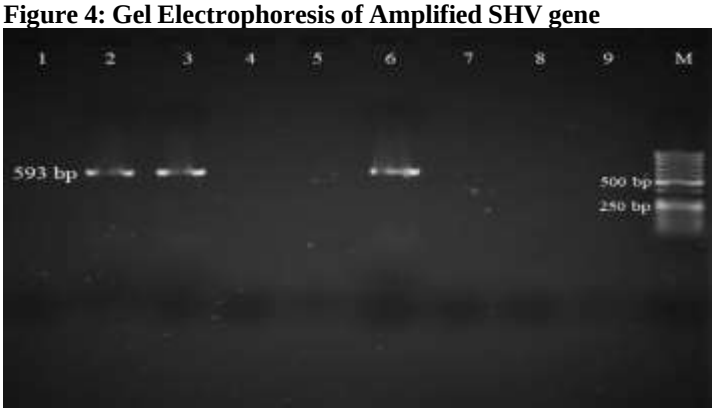


Figure 5: Gel Electrophoresis of Amplified blaCTX-M gene

phenotypic identification of the genus Acinetobacter was done based on various biochemical tests.

Percentage Distribution of isolates in patients

During the current study, the clinical samples were collected from both genders: males and females. As for the distribution of the sample is concerned, male patients have more prevalence of bacterial infections (62.5%) than females (37.5%). (Figure 5). The clinical samples were processed for isolation, identification, and molecular characterization of bacterial pathogens at the Microbiology laboratory. *Salmonella typhi*, the causative agent of typhoid fever was the most frequently isolated pathogen (48.2%), followed by *Klebsiella pneumoniae* (15.2%), *Escherichia coli* (10.7%), and *Acinetobacter baumannii* (9.8%). The least prevalent pathogens were *Serratia marcescens* and *Burkholderia cepacia* (4.5% each). (Figure 6)

Gender-wise distribution of isolates

As for the distribution of the pathogens is concerned, male patients have more prevalence of bacterial infections (70) than females (42). According to the results, *Salmonella typhi* was the most prevalent both in males and females, (47.6%) in females and (48.6%) in males. The least prevalent bacteria was *Pseudomonas aeruginosa* (0%) in females and *Serratia marcescens* (2.9%) in males. *Klebsiella pneumoniae* was (19.0%) in females and (12.9%) in males, *Escherichia coli* was (14.3%) in females and (8.6%) in males, *Acinetobacter baumannii* was (7.1%) in females and (11.4%) in males and *Burkholderia cepacia*n was (4.8%) in females and (4.3%) in males. (Figure 7).

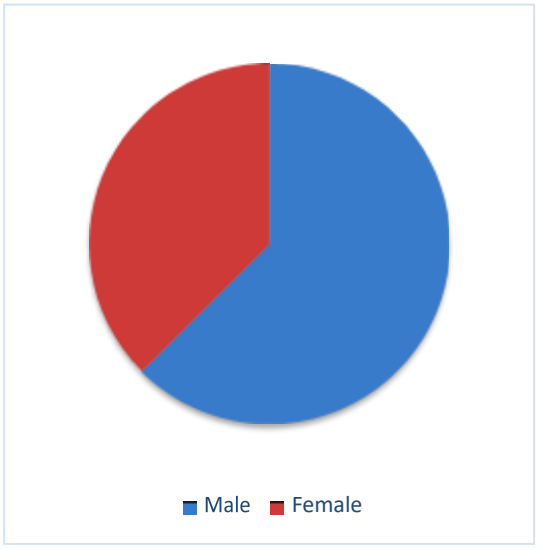
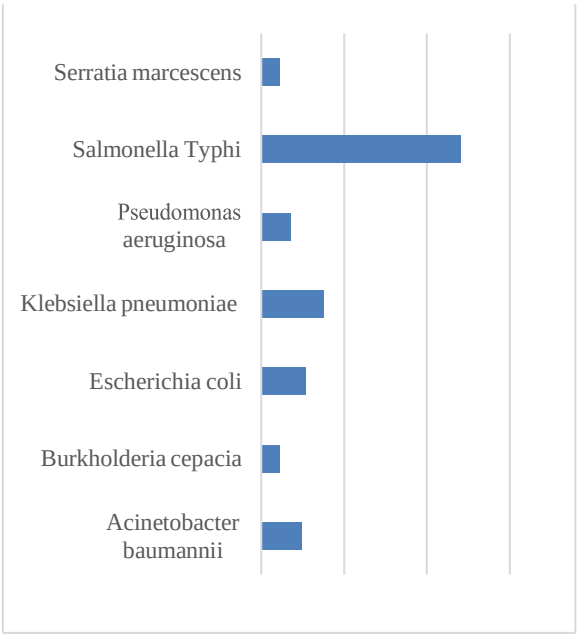


Figure 6: Gender-wise isolation of bacterial isolates

Figure 7: Percentage Distribution of Gram-Negative Bacteria



Percentage susceptibility of *Salmonella typhi*

Percentage susceptibility of *Salmonella typhi* shows that *S.* Typhi was more susceptible to three drugs Meropenem, Imipenem, and Azithromycin (100%) each. Intermediate for three drugs Cefepime, Cefotaxime, and Ceftriaxone (50%) each, and *S.* Typhi was least susceptible to Ciprofloxacin (22.2%). (Figure 8)

Table 3: Gender-wise distribution of various bacterial species

Bacterial Species	**Gender**	
	Male	**Female**
Acinetobacter baumannii	8	3
Burkholderia cepacia	3	2
Escherichia coli	6	6
Klebsiella pneumoniae	9	8
Pseudomonas aeruginosa	8	-
Salmonella typhi	34	20
Serratia marcescens	2	3
Grand Total	**70**	**42**

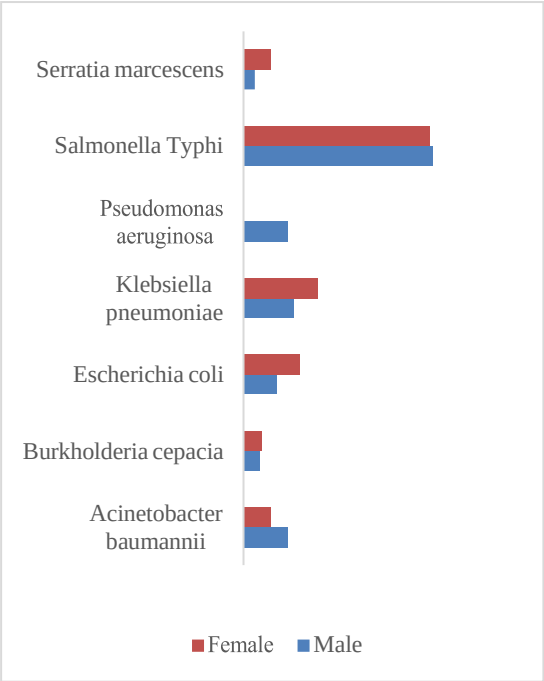


Figure 6: Percentage distribution of bacterial species in male and female patients

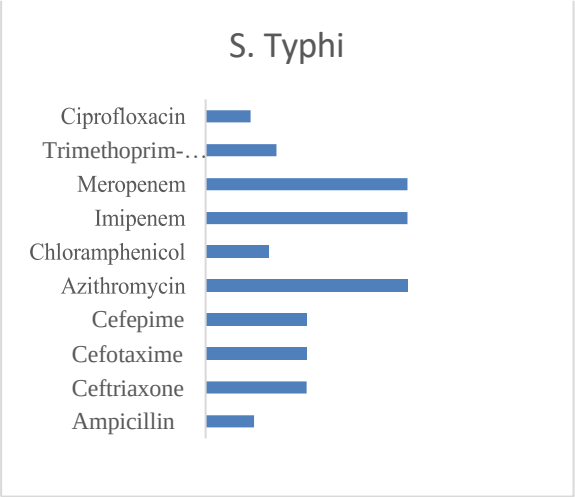


Figure 7: Percentage susceptibility of *Salmonella typhi* isolates to various antimicrobial agents

Percentage susceptibility of *Burkholderia cepacia*

Burkholderia cepacia was sensitive to chloramphenicol (100%) followed by Trimethoprim-sulfamethoxazole (60%), Meropenem (40%) and *Burkholderia cepacia* were least susceptible to the rest of the antibiotics (0%) each. (Figure 9)

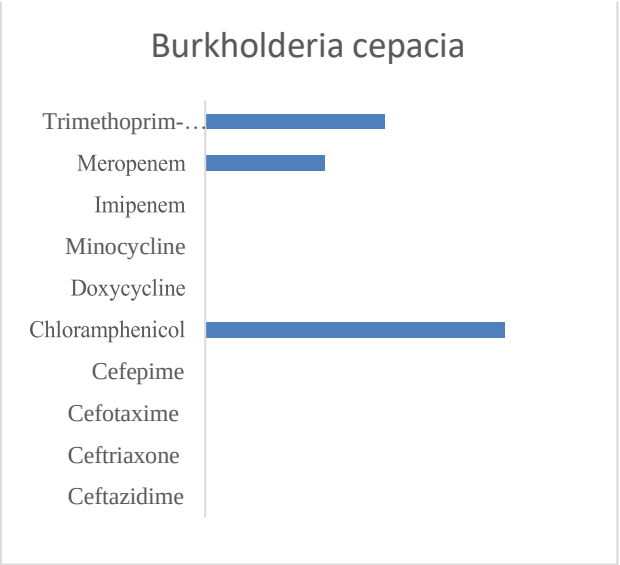


Figure 8: Percentage susceptibility of *Burkholderia cepacia* isolates to various antimicrobial agents

Percentage susceptibility of *Klebsiella pneumoniae*

According to antimicrobial susceptibility testing, *Klebsiella pneumoniae* was most susceptible to Doxycycline (35.3%), followed by Ciprofloxacin (17.6%), Trimethoprim-sulfamethoxazole, Meropenem, Imipenem, Gentamicin, Amikacin (11.8%) each. Cefepime was (5.9%) susceptible and the rest of the antibiotics were (0%) susceptible. (Figure 10)

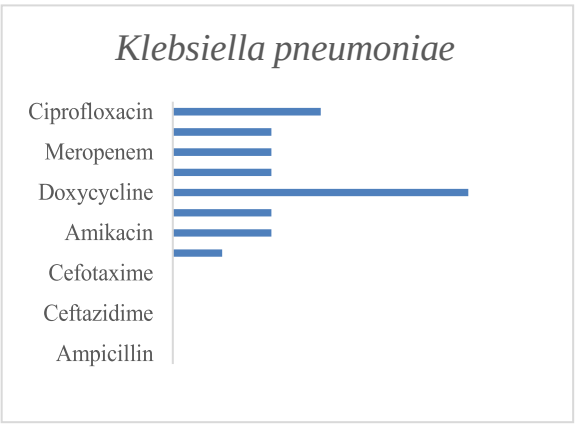


Figure 9: Percentage susceptibility of *Klebsiella pneumoniae* isolates to various antimicrobial agents

Percentage susceptibility of *Acinetobacter baumannii*

Acinetobacter baumannii was most susceptible to minocycline (63.3%), followed by Doxycycline (54.5%), Gentamycin, Amikacin (45.5% each),

Trimethoprim-sulfamethoxazole (36.4%), Ciprofloxacin (27.3%), Cefepime (18.2%). *Acinetobacter baumannii* was least susceptible to the rest of the antibiotics (9.1%) (Figure 11).

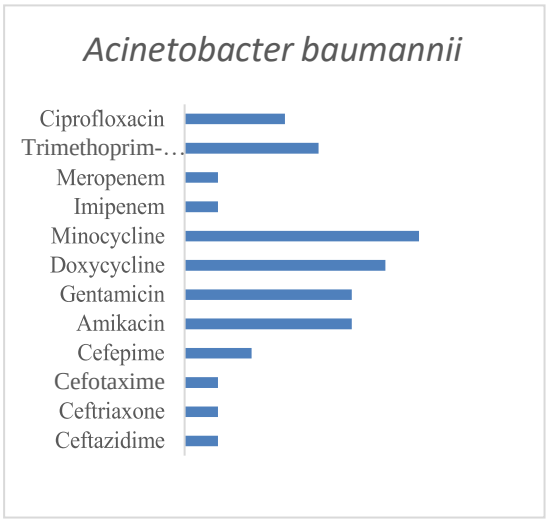


Figure 10: Percentage susceptibility of *Acinetobacter baumannii* isolates to various antimicrobial agents

Percentage susceptibility of *Serratia marcescens*

Serratia marcescens was most susceptible to Tigecycline (100%), followed by Trimethoprim-sulfamethoxazole (80%), Meropenem, Imipenem, Minocycline, Doxycycline, and Amikacin were (40% each) susceptible, Gentamicin was (20%). *Serratia marcescens* was least susceptible to the rest of the antibiotics (0%) (Figure 12).

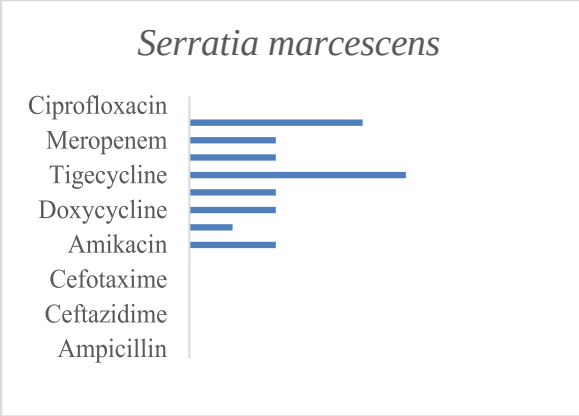


Figure 11: Percentage susceptibility of *Serratia marcescens* isolates to various antimicrobial agents

Percentage susceptibility of *Escherichia coli*

The susceptibility graph of *Escherichia coli* shows that *E. coli* was most susceptible to Tigecycline, Imipenem, and Meropenem (100%) each, Ciprofloxacin and Minocycline was (83.3%), Amikacin (33.3%) and rest of the antibiotics are least susceptible (16.7%) each (Figure 13).

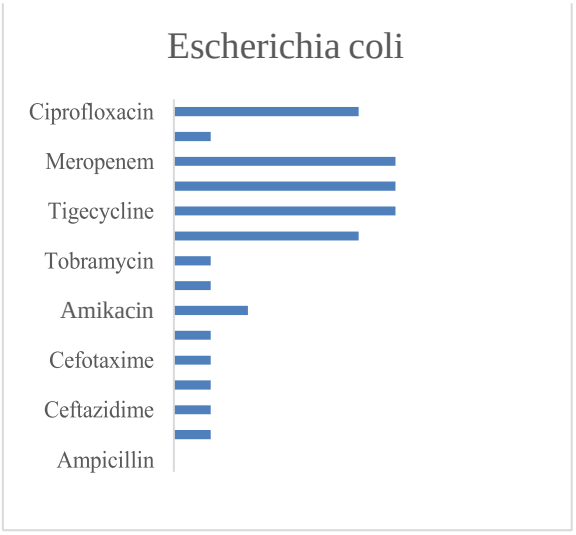


Figure 12: Percentage susceptibility of *Escherichia coli* isolates to various antimicrobial agents

Percentage susceptibility of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa shows (100%) susceptibility against Meropenem, Imipenem, and Amikacin. The least (50%) susceptibility against Ciprofloxacin and Trimethoprim-sulfamethoxazole, Gentamicin (75%), and the rest of the antibiotics was (62.5%) (Figure 14).

Figure 13: Percentage susceptibility of *Pseudomonas aeruginosa* isolates to various antimicrobial agents

Table 4: The overall antimicrobial susceptibility profiling of bacterial isolates

Antimicrobial agents	*S.* Typhi		*B. cepacia*		*K. pneumoniae*		*A. baumannii*		*S. marcescens*		*E. coli*		*P. aeruginosa*	
	S. (%)	R. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)	S. (%)
Ampicillin	13 (24.1%)	41 (75.9%)	NT	NT	0 (0%)	17 (100%)	NT	NT	0 (0%)	5 (100%)	0 0.0%	12 100.0%	NT	NT
Amoxicillin-clavulanic acid	NT	NT	NT	NT	0 (0%)	17 (100%)	NT	NT	0 (0%)	5 (100%)	2 16.7%	10 (83.3%)	NT	NT
Ceftazidime	NT	NT	0 (0%)	5 (100%)	0 (0%)	17 (100%)	1 (9.1%)	10 (90.9%)	0 (0%)	5 (100%)	2 16.7%	10 (83.3%)	5 (62.5%)	3 (37.5%)
Ceftriaxone	27 (50%)	27 (50%)	0 (0%)	5 (100%)	0 (0%)	17 (100%)	1 (9.1%)	10 (90.9%)	0 (0%)	5 (100%)	2 16.7%	10 (83.3%)	5 (62.5%)	3 (37.5%)
Cefotaxime	27 (50%)	27 (50%)	0 (0%)	5 (100%)	0 (0%)	17 (100%)	1 (9.1%)	10 (90.9%)	0 (0%)	5 (100%)	2 16.7%	10 (83.3%)	5 (62.5%)	3 (37.5%)
Cefepime	27 (50%)	27 (50%)	0 (0%)	5 (100%)	1 (5.9%)	16 (94.1%)	2 (18.2%)	9 (81.8%)	0 (0%)	5 (100%)	2 16.7%	10 (83.3%)	5 (62.5%)	3 (37.5%)
Amikacin	NT	NT	NT	NT	2 (11.8%)	15 (88.2%)	5 (45.5%)	6 (54.5%)	2 (40%)	3 (60%)	4 33.3%	8 (66.7%)	8 (100%)	0 (0%)
Gentamicin	NT	NT	NT	NT	2 (11.8%)	15 (88.2%)	5 (45.5%)	6 (54.5%)	1 (20%)	4 (80%)	2 (16.7%)	10 (83.3%)	6 (75%)	2 (25%)

Tobramycin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2 (16.7%)	10 (83.3%)	NT	NT
Azithromycin	54 (100%)	0 (0%)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Chloramphenicol	17 (31.5%)	37 (68.5%)	5 100.0%	0 0.0%	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Doxycycline	NT	NT	0 (0%)	5 (100%)	6 (35.3%)	11 (64.7%)	6 (54.5%)	5 (45.5%)	2 (40.0%)	3 (60.0%)	NT	NT	NT	NT
Minocycline	NT	NT	0 (0%)	5 (100%)	NT	NT	7 (63.6%)	4 (36.4%)	2 (40%)	3 (60%)	10 (83.3%)	2 (16.7%)	NT	NT
Tigecycline	NT	NT	NT	NT	NT	NT	NT	NT	5 100.0%	0 (0%)	12 (100%)	0 (0%)	NT	NT
Imipenem	41 (100%)	0 (0%)	0 (0%)	5 (100%)	2 11.8%	15 (88.2%)	1 (9.1%)	10 (90.9%)	2 (40%)	3 60.0%	12 (100%)	0 (0%)	8 (100%)	0 (0%)
Meropenem	41 (100%)	0 (0%)	2 (40%)	3 (60%)	2 11.8%	15 (88.2%)	1 (9.1%)	10 (90.9%)	2 (40%)	3 (60%)	12 (100%)	0 (0%)	8 (100%)	0 (0%)
Trimethoprim-sulfamethoxazole	19 (35.2%)	35 (64.8%)	3 (60%)	2 (40%)	2 (11.8%)	15 (88.2%)	4 (36.4%)	7 (63.6%)	4 (80%)	1 (20%)	2 (16.7%)	10 (83.3%)	4 (50%)	4 (50%)
Ciprofloxacin	12 (22.2%)	42 (77.8%)	NT	NT	3 (17.6%)	14 (82.4%)	3 (27.3%)	8 (72.7%)	0 (0%)	5 (100%)	10 (83.3%)	2 (16.7%)	4 (50%)	4 (50%)

Percentage Distribution of ESBL Production

All the isolates of *B. cepacia*n, *K. pneumoniae*, and *S. marcescens* were positive for ESBLs (100%), 83% of the *E. coli* were positive followed by *Salmonella* Typhi (50%).

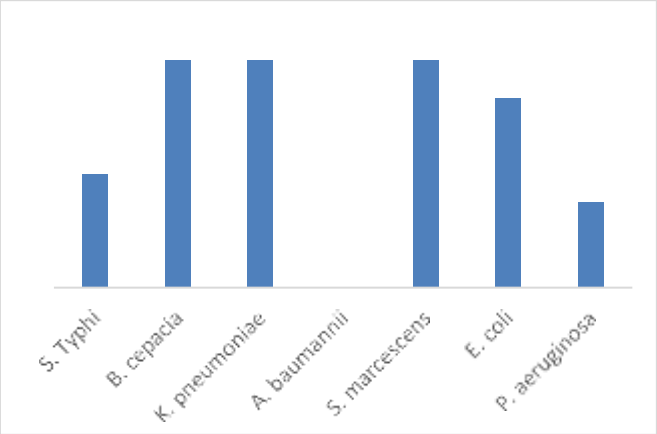


Figure 14: Percentage production of ESBL among the bacterial isolates

Distribution of ESBL genes

The prevalence of ESBLs genes among isolates was determined through primer-specific PCR and Gel Electrophoresis. In *S. marcescens*, 100% of isolates tested positive for the occurrence of TEM, followed by *K. pneumonia* where TEM and SHV were prevalent (88.2% and 64.7% respectively). In the case of *E. coli* CTX-M (83.3%) , SHV (16.7%), and TEM were prevalent (66.7%) (Figure 16).

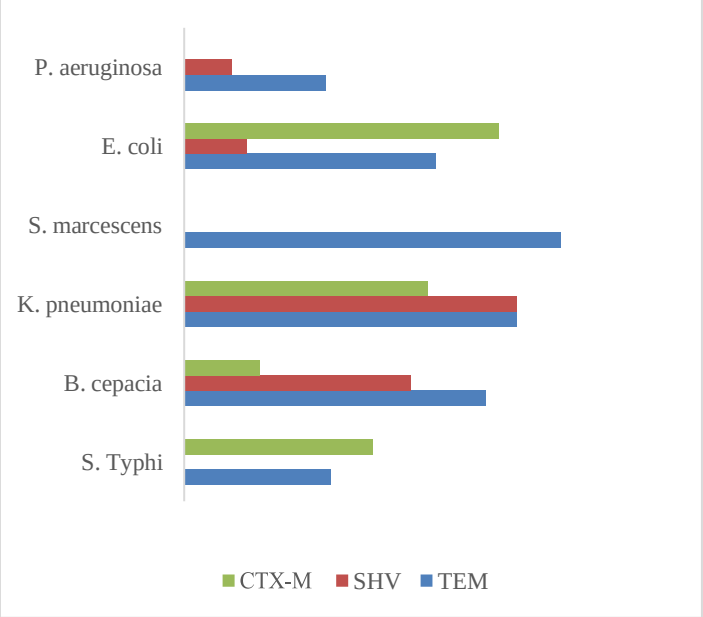


Figure 15: Percentage distribution of ESBL genes among the bacterial isolates

IV. CONCLUSION

Bacterial resistance is a serious health threat, as ESBL enzymes resist certain antibiotics. ESBLs hydrolyze cephalosporin and aztreonam but can be inhibited by clavulanic acid and affect gram-negative microorganisms like *K. pneumoniae* and *E. coli*, *A. Baumannii* & *Pseudomonas* spp. Microbes may cause bloodstream infections, making ESBL infections a major public health concern. Study examines ESBL prevalence and antibiotic resistance in clinical specimens from Dera Ghazi Khan healthcare facility focusing on BlaTEM, BlaSHV, and BlaCTX-M. Samples were gathered, cultured, Gram stained, and biochemically tested to identify bacteria. Antibiotic susceptibility and presence of ESBL genes were also analyzed. *S. typhi* caused 48% of typhoid cases followed by *K. pneumoniae* at 15% and 5% per pathogen. ESLs gene frequency detected through PCR and Gel Electrophoresis. BlaTEM and blaSHV were 88% prevalent. Pneumonia had a prevalence of 2% and 647%. N/A (not applicable) Proper diagnosis and treatment needed due to resistance genes blaCTX-M at 83. 3% blaSHV gene at 16.7% & 66% blaTEM gene. 7% E. coli rate. Reduce antimicrobial resistance, and find new treatments with global strategies.

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DECLARATION

Authors declare no conflict of interest

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