

DEVELOPMENT OF MULTIPLEX PCR ASSAY TO DETECT THE MULTIDRUG RESISTANCE IN BACTERIAL PATHOGENS

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with GRADE "A++" by NAAC
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This is to certify that this project report is the Bonafide work of Jayameenaa P (40230014) and Pooja Nath(40230033) who have done the project work entitled "DEVELOPMENT OF MULTIPLEX PCR ASSAY TO DETECT THE MULTIDRUG RESISTANCE IN BACTERIAL PATHOGENS" under our supervision from October 2023 to April 2024.

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We, Jayameenaa P (40230014) and Pooja Nath (40230033) hereby declare that the project report entitled **“DEVELOPMENT OF MULTIPLEX PCR ASSAY TO DETECT THE MULTIDRUG RESISTANCE IN BACTERIAL PATHOGENS”** done by us under the guidance of **Dr. Saqib Hassan** (internal guide) and **Dr. S. Usha Nandhini** (external guide) at the department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai is submitted in the partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Multidrug resistance is a global threat because most of the pathogenic bacteria have become resistant to several antibiotics, making them difficult to treat. Several bacteria, like *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and many other pathogens, can cause infectious diseases that could be difficult to treat due to the multidrug-resistant phenotype shown by these bacterial pathogens. The most common antibiotic resistance-coding genes present across different species were found to be variants of beta-lactamase genes like blaOXA, blaKPC, blaNDM, blaCTX-M, and blaTEM etc that facilitated resistance to several generations of different classes of antibiotics like carbapenems and extended-spectrum beta-lactamase. Other non-bla-associated genes also provide resistance to certain antibiotics like quinolones and fluoroquinolones. These resistant genes evolved in these bacteria over the years due to exposure of bacteria to low doses of antibiotics that could have happened through antibiotic residues from pharmaceutical and agricultural practices. For this project we have shortlisted the common multidrug resistance genes like blaOXA-48, blaCTX-M1, blaNDM-1, blaKPC-2 and qnrB and designed the primers for these particular genes. And then these primers were used for running PCR and development of the multiplex assay for detection of the resistance genes in bacterial samples.

Keywords: Multidrug resistance (MDR), Multiplex PCR, ESBLs, blaOXA-48, blaKPC-2, blaNDM-1

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LIST OF ABBREVIATIONS

AMP	Ampicillin
ARG	Antibiotic Resistance Gene
CHDL	Carbapenem, Hhydrolysing class D β -Lactamases
CAZ/AVI	Ceftazidime/Avibactam
CIP	Ciprofloxacin
CNSE	Carbapenem non-susceptible <i>Enterobacteriaceae</i>
CPE	Carbapenemase producing <i>Enterobacteriaceae</i>
CRAB	Carbapenem-resistant <i>Acinetobacter baumannii</i>
CRKP	Carbapenem-resistant <i>Klebsiella pneumonia</i>
CTX	Ceftriaxone
ECC	<i>Enterobacter cloacae</i> complex
ESBL	Extended-spectrum beta-lactamases
IPM	Imipenem
MALDI	Matrix Assisted Laser Desorption/Ionization
MBL	Metallo-beta-lactamase
MCRPEC	mcr-1-positive <i>Escherichia coli</i>
MDR	Multi drug resistant
MDR-PA	Multidrug <i>Pseudomonas aeruginosa</i>
MEM	Meropenem
PCR	Polymerase chain reaction
PFGE	Pulsed Field Gel Electrophoresis
SNP	Single nucleotide polymorphism
ST	Sequence type
TMP/SMX	Trimethoprim/sulfamethoxazole
TS	Trimethoprim/sulfamethoxazole
TZP	Tazobactam/ piperacillin
VAG	Virulence-associated genes
VAP	Ventilator-associated pneumonia
WGS	Whole genome sequencing
XDR	Extensively drug resistant

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CHAPTER 1

INTRODUCTION

Over time, the prevalence of multidrug resistance in microorganisms has increased, posing a grave and worrying risk to the public's health (Prestinaci *et al.*, 2015). Multidrug resistant (MDR) organisms include bacteria, viruses, and fungi that have acquired resistance to multiple antimicrobial agents, thereby rendering them refractory to conventional therapeutic interventions involving antibiotics and antiviral medications (Salam *et al.*, 2023).

Multidrug resistance is primarily due to confluence of genetic, evolutionary, and environmental variables in the pathogens (Jacopin *et al.*, 2020). In the face of genetic phenomenon, frequently originating from genetic alterations within the population conferring tolerance to various antibiotics. These mutations may happen randomly and build up in the genome with time (Davies, 1996). The frequency of antibiotic resistance genes (ARGs) in MDR pathogenic bacteria can vary significantly based on a number of variables, including geographic location, healthcare practices, and the particular bacterial species in issue (Prestinaci *et al.*, 2015). Beta-lactamases, extended-spectrum beta-lactamases (ESBLs), carbapenemases, aminoglycoside resistance genes, and fluoroquinolone resistance genes are a few of the common antibiotic resistance genes found in bacterial species (Shaikh *et al.*, 2015).

High rates of resistance against the common antibiotics used to treat common bacterial infections have been observed globally, indicating the need to change treatment options (Nwobodo *et al.*, 2022). According to United Nations (UN) projections, up to 10 million fatalities might be brought on by superbugs and related types of antimicrobial resistance by the year 2050, equaling the yearly death toll from cancer worldwide (de Kraker *et al.*, 2016). According to reports by the Global Burden of Disease 2019 antimicrobial resistance partners, led by the Institute for Health Metrics and Evaluation and the University of Oxford, 7.7 million global deaths were determined to be related to bacterial infection (Murray *et al.*, 2022). That amounts to 13.6%, or 1 in 8, of all deaths worldwide. The majority of these deaths were caused by *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Ikuta *et al.*, 2022). Among the illnesses that contributed to these deaths were mostly lower

respiratory infections, bloodstream infections, peritoneal and intra-abdominal infections, as well as other illnesses (Zhang *et al.*, 2024). A multimodal strategy is required to address the rising danger posed by MDR pathogens, starting with the creation of diagnostics and treatments specifically designed for such pathogens. Providing rapid diagnosis, infection management, and the prevention of future resistance lowers healthcare costs, boosting global health security, and thereby encouraging responsible antibiotic usage, which will provide a better solution. In this review, we have reported on the frequency of MDR genes, which result in resistance to third and fourth-generation antibiotics, including carbapenemases and ESBLs, among high-risk bacterial pathogens such as *E. coli* and *K. pneumonia*, among others in India.

1.1 CLASSIFICATION OF BETA-LACTAMASES

The *Enterobacteriaceae* family consists of various bacterial species that are significant clinically, as antibacterial resistance is quite prevalent in these species. Among antimicrobial-resistant bacterial species with the potential to produce enzymes such as ESBLs and carbapenemases, the group of enzymes under beta-lactamases was found to have the greatest mortality rates and a longer treatment period (Wilson and Török, 2018). ESBLs are a group of enzymes that are major resistance determinants of beta-lactam antibiotics like penicillins, cephalosporins, and monobactams, especially in the gram-negative members of the *Enterobacteriaceae* family. Bacterial pathogens that secrete ESBLs still remain susceptible to carbapenems. It is important to classify such enzymes to understand their resistance mechanism and pattern. These enzymes are produced with the help of enzymes present in the genome or in the plasmids of the bacterial strains. The most significant β -lactamase genes are variants of the genes CTX-M, SHV, TEM, VEB, GES, PER, TLA, and OXA (Abrar *et al.*, 2019).

Beta-lactamases are classified into four molecular classes, namely A, B, C, and D. The classification was done based on molecular size and the homology between the active-site amino acid motifs, known as the Ambler classification. Class A includes Narrow Spectrum (PSE), CARB, ESBLs (TEM, SHV, CTX-M), carbapenemase (GES), KPC, SME, IMI/NMC-A, SHV-38, and SFC-1. The class includes metallo-beta-lactamase (MBL): Subclass B1-IMP, VIM, SPM, IND, NDM, DIM, GIM, SIM, Subclass B2-CphA, Sfh-I, ImiS, Subclass B3-AIM, CAU-1, GOB-

FEZ-1, Class C includes AmpC and Extended-Spectrum AmpC (ESAC), and Class D includes ESBLs and Carbapenem-hydrolysing class D beta-lactamases (CHDLs). Another classification used for beta-lactamases is called the Bush-Jacoby-Medeiros functional classification (Bush, 2018). It is based on the biochemical and functional properties of beta-lactamase enzymes produced by bacteria. It classifies the beta-lactamase enzymes into three groups according to their ability to degrade beta-lactamase substrates. Groups 1, 2, and 3 are cephalosporinase, serine beta-lactamase, and metallo-beta-lactamase, respectively.

Table 1.1: Prevalence of MDR genes in Enterobacteriaceae family.

β-lactamases	Class	Activity	Organism	References
BPS/PenA	A	ESBL	<i>Burkholderia pseudomallei</i>	(Kaderabko va et al., 2022; Philippon et al., 2016)
BKC - 1	A	Carbapenemase	<i>K. pneumoniae</i>	(Kaderabko va et al., 2022)
BLAC	A	Narrow spectrum	<i>Mycobacterium tuberculosis</i>	(Bhattacharya et al., 2021; Kaderabko va et al., 2022)
CTX - M	A	ESBL	<i>Enterobacteriaceae</i>	(Bradford, 2001; Kaderabko va et al., 2022)
KPC	A	ESBL/Carbapenemase	<i>K. pneumoniae</i>	(Kaderabko va et al.,

				2022; Philippon <i>et al.</i> , 2016)
TEMP	B1	Carbapenemase	<i>P. aeruginosa</i>	(Bradford, 2001; Kaderabko <i>va et al.</i> , 2022; Sacha <i>et al.</i> , 2008)
NDM	B1	Carbapenemase	<i>Enterobacteriace</i>	(Bush & Bradford, 2020; Kaderabko <i>va et al.</i> , 2022)
VIM	B1	Carbapenemase	<i>P. aeruginosa</i> , <i>Enterobacteriaceae</i>	(Halat & Moubareck, 2022; Kaderabko <i>va et al.</i> , 2022; Sacha <i>et al.</i> , 2008)
AIM	B3	Carbapenemase	<i>P. aeruginosa</i>	(Kaderabko <i>va et al.</i> , 2022; Sacha <i>et al.</i> , 2008; Salimraj <i>et al.</i> , 2016)
AmpC/EC	C	Narrow spectrum/ESBL	<i>Enterobacteriaceae</i>	(Kaderabko <i>va et al.</i> ,

				2022; Livermore, 1995)
DHA-1	C	ESBL	<i>Morganella morganii</i>	(Kaderabko va et al., 2022; W. Song et al., 2011)
CMY	C	ESBL	<i>K. pneumoniae</i>	(Kaderabko va et al., 2022; W. Song et al., 2011)
OXA -10	D	ESBL	<i>P. aeruginosa</i>	(Bradford, 2001; Kaderabko va et al., 2022; Sacha et al., 2008)
OXA 48	D	Carbapenemase	<i>K. pneumoniae</i>	Bush & Bradford, 2020; Kaderabko va et al., 2022)

1.2 EMERGENCE OF DRUG RESISTANCE IN *ENTEROBACTERIACEAE* FAMILY

The *Enterobacteriaceae* family is a diverse group of gram-negative bacteria consisting of multiple genera and species. *E. coli*, *Klebsiella*, *Enterobacter*, *Salmonella*, *Pseudomonas*, and many more organisms are members of this family. They pose resistance to many classes of antibiotics, like carbapenems (Bandy *et al.*, 2022; Gao *et al.*, 2020; Hubbard *et al.*, 2020; Ramsamy *et al.*, 2020; Stewart *et al.*, 2018; Zhao *et al.*, 2023) penicillins (Cummins *et al.*, 2020; Paskeh *et al.*, 2020), cephalosporin derivatives (Abrar *et al.*, 2019; Bandy *et al.*, 2022; Cummins *et al.*, 2020; Gao *et al.*, 2020; Hamza *et al.*, 2020; Hubbard *et al.*, 2020; Naziri *et al.*, 2020; Pławińska- czarnak *et al.*, 2022; Stewart *et al.*, 2018; Xihui *et al.*, 2023; X. Yang *et al.*, 2020; Yuan *et al.*, 2021) Carbapenemase- producing *Enterobacteriaceae* (CPE), specifically OXA-48 and its gene variants, are predominantly reported by many researchers against cephalosporin-derivates (Stewart *et al.*, 2018).

Additionally antibiotic resistance factors originating in aquaculture settings, particularly integrated agriculture-aquaculture systems, which may endanger public health. A significant number of *Enterobacteriaceae* isolates showed resistance to cephalosporins and carbapenems due to the presence of the carbapenemase gene blaKPC, highlighting the need for comprehensive control measures against antibiotic resistance (Hamza *et al.*, 2020).

A study conducted in a Korean hospital found that there were no notable variances in sequence types, patterns of antimicrobial resistance, or virulence factors between extra-intestinal pathogenic *E. coli* isolates from blood and urine specimens of urinary tract infection patients, this suggests that both blood and urine samples exhibit equivalent levels of virulence factors and antimicrobial resistance (Kim *et al.*, 2021). Gram-negative bacterial isolates from wound infections were MDR, XDR, and carbapenem-resistant. They showed a resistance of above 40% towards carbapenems and other 3rd- and 4th-generation cephalosporins (Bandy *et al.*, 2022).

In a randomized study, microbial alterations on skin after administration of systemic antibiotics was investigated to study four antibiotic regimens doxycycline (20 mg or 100 mg), cephalexin, or trimethoprim/sulfamethoxazole (TMP/SMX). Skin and oral sites, as well as stool samples were used before and after 1 year of antibiotics treatment. Doxycycline and TMP/SMX treated subjects showed greater

changes to skin microbes then other regimens, along with fluctuations in oral and stool microbiota. The study revealed that skin microbiome is largely affected by antibiotics and can develop persistent antibiotic resistance resulting from overpricing (Jo *et al.*, 2021).

Research was carried out in Saudi Arabia to investigate the bacterial diversity of wound infections and the associated antimicrobial resistance, revealing that the most common pathogens detected were *Staphylococcus species*, *E. coli*, and *K. pneumoniae*. (Bandy *et al.*, 2022). This shows the prevalence of MDR genes is more common (Table 1.1), and there is a need to focus critically on the pattern of MDR genes, especially in bacterial pathogens, to improve antibiotic treatment outcomes.

CHAPTER 2

LITERATURE SURVEY

2.1 PRESENCE OF MDR GENES IN *E. coli*

In a study by (Ikuta *et al.*, 2022), *E. coli* was the one among the five bacterium that claimed more than 6 lakh death in India in 2019. A similar clinical study by (Abbasi *et al.*, 2020) analysed diarrheagenic *E. coli* isolated from paediatric patients with gastroenteritis, finding a high prevalence of MDR genes, AmpC genes, and ESBL genes, with blaTEM and blaCTX-M15 being the most common, and in the case of AmpC, blaCIT and blaDHA were predominantly present. In another study using paediatric patients, PCR assays revealed that the major subtypes of blaCTX-M are M-15, M-14, M-55, and M-90. Among these, the subtype ST131 was more dominant in producing the ESBL gene in *E. coli* (Patil *et al.*, 2019).

Similarly (Abrar *et al.*, 2019)., showed that the most prevalent ESBL-producing genes were bla CTX M, bla OXA, bla TEM, and bla SHV in *E. coli* in a study. Also (Manandhar *et al.*, 2020) in a PCR-based analysis using clinical samples, showed that blaTEM, bla CTX M1, and bla CTX M8 were predominantly present in the *E. coli* isolates, and the most prevalent blaOXA family genes were found in *Acinetobacter* spp. and blaOXA and blaOXA 5 in *enteric bacilli*.

And (Patil *et al.*, 2019) in another study used MDR-gram-negative bacteria and PCR sequencing to show the coexistence of the beta-lactamase-producing genes and the plasmid-borne colistin resistance mcr-1 gene. The blaCTX M gene was most prevalent, along with blaTEM, blaSHV, blaKPC, and blaOXA48 (Patil *et al.*, 2019). In a study using ESBL-producing *E. coli* from urinary tract infections, the prevalent MDR genes were blaCTX M, blaSHV (63%), and blaTEM (11.1%). ESBL producers showed greater resistance to cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole, and tetracycline compared to non-ESBL producers (Naziri *et al.*, 2020).

In a study of the Thai population, 50% of the clinical bacterial isolates showed MDR-*E. coli* with commonly present carbapenemase MDR-A genes blaIMP, blaVIM, blaVIM bla OXA-40-like, and blaOXA-58-like (Yungyuen *et al.*, 2021). A study conducted using high-resolution bioinformatic methods showed the global spread of CTX-M-55-positive *E. coli*, especially in Asia. bla CTX-M-55; orf477 (Tn2); IS Ecp1; and IS 26 (IS 15DI) - hp - hp - blaCTX-M-55, orf477, hp-blaTEM-IS 26,

Tn2 and hp-IS 26 were found to be dominant in humans and animals, indicating potential rapid dissemination of blaCTX-M-55 harboring *E. coli* (Yang *et al.*, 2023).

In a study utilizing genome sequencing and analysis of CTX-M-producing *E. coli* isolates from river water, it was demonstrated that *E. coli* can effectively retain blaCTX-M genes in environmental water sources (Gomi *et al.*, 2022). The presence of a class 1 integron-borne blaVIM-4 gene with a 170-bp duplication in the 3' end of the gene, which was found in a rectal swab of a patient infected with *E. coli* and suffering from osteomyelitis in Colombia with no travel records, was believed to be caused by the blaVIM-4 gene in an IncA/C-type plasmid, potentially leading to the spread of VIM-4 carbapenemase in Colombia (the first case of blaVIM-4 in South America) (Rada *et al.*, 2022).

A study was conducted to detect the spread of ESBL *E. coli* in Ethiopia. 54.8% of isolates were ESBL-EC positive, additionally, 96% of these strains exhibited resistance to more than one class of antibiotics. The blaCTX-M-15-M-15 gene was detected in most of them. Genes producing resistance towards aminoglycosides and ciprofloxacin (CIP) (*aac*(6')-Ib-cr), phenicols (*cat*B3, sulphonamides (*sul*1, trimethoprim (*dfr*A17), and macrolides (*mph*(A)) were also detected. ESBL genes were prevalent, and dissemination of blaCTX-M-15 was seen in high-risk *E. coli* clones (Sewunet *et al.*, 2022). An investigation of the incidence of MDR-associated blood stream infections (BSI) and the mortality risk in adult cancer patients was made at an oncology hospital in Iran. The study discovered that gram-negative bacteria, particularly *E. coli*, were the most prevalent strains and that Central Line-Associated BSI (CLABSI) was more frequent in patients with solid tumors (Amanati *et al.*, 2021).

An analysis of MDR-*E. coli* revealed that producers of ESBL synthesized a kind of carbapenemase that had different degrees of resistance to certain antibiotics, with the help of genes such as bla IMP, bla VIM, bla KPC, and bla OXA (Paskeh *et al.*, 2020). A research was conducted to assess how the New Delhi Metallo- β -Lactamase-1 gene (bla NDM-1) expression varied in clinical isolates (*K. pneumonia* TH-P12158), transformant strains *E. coli* DH5 α -bla NDM-1 and *E. coli* BL21 (DE3)-bla NDM-1, following exposure to imipenem (IPM). The investigation found that even minimal IPM doses could induce a lasting memory of resistance in bla NDM-1 positive strains, leading to alterations in bla NDM-1 expression (Zhao *et al.*, 2023). The phenotypic difference in the presence of the carbapenem gene in chromosomes

and plasmids is very poorly known. An examination was conducted to reveal the significance of the location of the drug-resistant gene (blaNDM). The wild-type strain *E. coli* M17, which harbors the blaNDM gene on both its plasmid and genome, served as the basis for generating mutants Δ pblaNDM and Δ cblaNDM, where the chromosome- or plasmid-bound blaNDM was specifically knocked out. Compared to WT or cbla NDM, pbla NDM showed reduced hydrolysing ability against IPM and gene expression. It has been discovered that carbapenemase genes placed on plasmids offer greater levels of carbapenem resistance than those located on chromosomes (Sakamoto *et al.*, 2022).

A study conducted in Nigeria found that clinical isolates exhibited resistance to various antibiotics, including ampicillin (AMP), trimethoprim/sulfamethoxazole, ceftriaxone(CTX), gentamicin, and meropenem (MEM), with 102 cases being MDR; half of these cases showed the ESBL phenotype, while the blaCTX-M-15 gene was most commonly present, along with other genes such as blaCMY-2, blaOXA-1, blaNDM-1, blaNDM-5 and blaCTX-M-15; additionally, the prevalence of ST131 was observed. (Xu *et al.*, 2022). In a study in Quetta, bacterial isolates were collected from urine samples. 16S rDNA gene approach identified isolates as *E. coli* and other bacterial species (*K. pneumonia*, *P. aeruginosa*, *Proteus mirabilis*), which were MDR to many antibiotics, including carbapenems. PCR detected the presence of blaNDM-1, and the strains were susceptible to only tigecycline and polymyxins, suggesting XDR (Pirkani *et al.*, 2020).

For a study, *E. coli* isolates were collected from rectal swabs of patients from hospitals. The isolates contained blaNDM-4 gene, which is a rare blaNDM variant and was situated on a 46.5-kb IncFIA a self-transmissible plasmid. The strains were identified as belonging to ST405 and phylogenetic group D, which are widely linked with antimicrobial resistance on a global scale. Additionally, these strains carried 14 recognized resistance genes, including the ESBL gene blaCTX-M-15. The isolates exhibited merely 1 or 2 SNP disparities, suggesting a common origin and cryptic transmission within hospital environments. A 14.6-kb segment carrying blaNDM-4 was flanked by two copies of IS26, that indicated the possibility of forming a composite transposon capable of facilitating the mobilization of blaNDM-4 (Zhang *et al.*, 2018).

In a study, a 145-kb IncC MDR plasmid (pK71-77-1-NDM) was sequenced, which contains genes resistant to last-stage antibiotics, including carbapenems. The

study showed that the plasmid can transfer to genetically diverse clinical *E. coli* strains, though the fitness cost of the host is often low. It was seen that plasmids are stable under non-selective conditions in different genetic backgrounds. A low conjugation frequency and high fitness cost were observed in the *E. coli* ST73 background, suggesting the cause of low antibiotic resistance in this strain compared to other *E. coli*. It was revealed by bioinformatic analysis that the ST73 background carried fewer plasmids than other *E. coli*. The study helped to understand the variability in plasmid-host associations (Gama *et al.*, 2020).

A study characterized both ESBL and carbapenemase genes in *Enterobacter* in Iran with samples collected from inpatients. A high prevalence of MDR was seen, and many were ESBL-positive and non-susceptible to carbapenems. blaCTX-M, blaTEM, blaSHV, blaIMP, and blaNDM were the genes detected using PCR. This study was the first to report the presence of blaNDM in *Enterobacter gergoviae* in Iran (Khashei *et al.*, 2020).

A study was conducted in Kolkata, India, to characterize MDR commensal *E. coli* (CE) with blaNDM-5 positivity isolated from diarrheal stools. Genes encoding carbapenemases and other additional antimicrobial resistance were identified through PCR. The bla-NDM-positive CE strain was resistant to colistin and other antibiotics. CIP (>32 mg/L) and IPM (8 mg/L) both had higher MICs. Screening for ARGs indicated the presence of Int-1, blaTEM, blaCTX-M3, blaOXA-1, blaOXA-7, blaOXA-9, tetA, strA, aadA1, aadB, sul2, floR, mph(A), and aac(6')-Ib-cr in the CE strains. The IncFII plasmid closely resembles the blaNDM-5 gene, representing the first documented instance of carbapenem resistance associated with blaNDM-5 gene, with the capacity to transfer this gene to other enteric pathogens (Chowdhury *et al.*, 2022).

A research study in Lebanon conducted with *E. coli* isolates from poultry farms as it was the major bacterial strain showed the presence of bla TEM and CTX, including ESBL and ampC genes (Dandachi *et al.*, 2018). In another wild animal study, ESBL-producing *E. coli* commonly presents bla CTX-M1 in both people and animals (Guyomard-Rabenirina *et al.*, 2020). In Ecuador, another investigation revealed the presence of ESBL/Ampc and mcr genes in *E. coli* resistant to third generation cephalosporins(3GC-R) sourced from grill causing human enteritis with blaCTX-M variants being frequently detected alongside identification of blaCMY-2. (Ortega-Paredes *et al.*, 2020).

In a Chinese study, *E. coli* strains acquired from a dairy farm demonstrated widespread resistance, with the majority displaying resistance to sulfamethoxazole/trimethoprim, followed by cefotaxime, AMP, CIP, and tetracycline. Resistance to amikacin, florfenicol, Fosfomycin, and gentamicin was observed to a lesser extent. However, all strains remained susceptible to meropenem, tigecycline, and colistin sulphate. ARGs detected were *sul2*, *blaTEM*, *gyrA*, *tet(B)*, *aph(3)-I*, *floR*, and *fosA3*, which were predominant among several classes of antibiotics. Among the virulence-associated genes (VAGs), *ompA* was the most common, followed by *ibeB*, *traT*, *ompT*, *fyuA*, *iroN*, and *irp2*. The study suggested that the environment around dairy farm could potentially serve as a reservoir for mastitis-causing pathogens among animals, resulting in the horizontal transfer of ARGs and virulence genes carrying bacterial strains to humans via tainted milk and meat, surface water, and agricultural crops (Shoaib *et al.*, 2023).

In a study in South China, *mcr-1*-positive *E. coli* (MCRPEC) of swine origin was detected to have resistance to third and fourth generation cephalosporins, and some were resistant to carbapenem. MCRPEC exhibited high resistance to IPM and MEM, both of which are prohibited for use in livestock. Furthermore, it was found that plasmid-carrying resistance genes were present alongside the *mcr-1* gene of the MCRPEC isolates, including the ESBL genes such as *blaCTX-M14*, *blaCTX-M24*, *blaCTX-M123*, *blaOXA-1*, plasmid-mediated AmpC (pAmpC) gene *blaCMY-2*, carbapenem resistance gene *blaNDM-5*, and non- β -lactamase antimicrobial resistance genes *qnrA*, *qnrB*, *qnrS*, *aac (6')-Ib-cr*, *tetA*, *tetB*, *sul1*, *sul2*, *floR*, *aadA* (Yuan *et al.*, 2021).

A recent study examined the characteristics of plasmids in *E. coli* that are resistant to extended spectrum cephalosporins (ESC) in healthy pigs in the time period from 2013 to 2016. The study found that the resistance was caused by a specific plasmid called IncI1 ST-3 plasmid (pCTXM1-MU2) that encoded *blaCTXM-1*. The study also discovered the presence of atypical enteropathogenic *E. coli* (aEPEC) that carried multiple LEE pathogenic islands. Resistance to aminoglycosides, sulphonamides, and trimethoprim due to the pCTXM1-MU2 plasmid was observed, also the plasmid was highly stable and transferable. Additionally, the study revealed that ESC resistance can persist even after the removal of selection pressure, leading to the emergence of resistant *E. coli* in both commensal and EPEC isolates. (Abraham *et al.*, 2018).

PCR analysis was used in a study in Bangladesh to investigate *E. coli* isolates from cloacal swabs of live broiler chickens, revealed uniform resistance of the isolates to multiple antibiotics due to presence of various genes. The highest resistance was seen against colistin, followed by gentamicin and levofloxacin, and tetracycline(tet(A)) was most prevalent gene and then followed by ampicillin(blaTEM), streptomycin(aadA1), erythromycin(ere(A)) (Azad *et al.*, 2019). Several studies so far have reported MDR genes in pathogenic *E. coli*, especially in clinical settings, as well as prevalence in domestic animals that can invariably enter humans, thus proving the severity of the situation that needs critical focus.

2.2 PRESENCE OF BLA GENE IN *K. pneumonia*:

The blaKPC gene is frequently embedded in the Tn4401 transposon. The blaKPC gene was first detected in *P. aeruginosa*, in the UC-CHRISTUS Clinical Hospital followed by its detection in *E. coli* and other pathogens. Research was conducted to understand its genetic transfer and dissemination mechanisms through multi-locus sequence typing, plasmid analysis, PFGE, and whole-genome sequencing (WGS). High-risk sequence types like *K. pneumoniae* ST11, *P. aeruginosa* ST654, and *E. cloacae* ST114 were found, and a few *E. coli* isolates were found to be clonal. The truncated blaTEM-1 gene was present upstream of blaKPC, followed by a sequence similar to ISEc6 (84%). The study uncovered the presence of the downstream blaKPC-truncated ISKpn6 gene, in conjunction with the inverted repeat right sequence of Tn4401. It indicated that the multispecies KPC outbreak stemmed from horizontal gene transfer rather than clonal dissemination (Wozniak *et al.*, 2021).

In a study in China, clinical isolates from UTI infected patients, showed out of 17,966 strains isolated 1534 isolates of *K. pneumonia* were identified preceded by *E. coli*. There was reduction in ESBL-producing *K. pneumonia* by 10% in 2019 while carbapenem resistant strains increased by 20% in 2017 along with growth in resistance to amikacin. A stable resistance pattern was seen towards ceftazidime (CAZ) and aztreonam (AVI) (Ding *et al.*, 2021). A study was conducted using clinical samples to analyse antimicrobial resistance in *Klebsiella* sp, virulence factor gene serum resistance, hypermucoviscosity and the ability to form biofilms. Urine strains showed high ESBL production and high resistance to fosfomycin and other antibiotics. Also, virulent uge gene was prevalent in urine strains along with higher

biofilm-forming ability than other strains isolated from blood or respiratory samples (Ballén *et al.*, 2021). In a study in Pakistan using 178 ESBL producing *K. pneumoniae*, among inhibitor-resistant beta-lactamase enzymes, carbapenemases, or ampC type beta-lactamases at least one was present in most of the isolates as showed by (Heinz *et al.*, 2019).

In a study using using Pulsed Field Gel Electrophoresis (PFGE) and Multilocus sequence typing (MLST) 43 out of 45 blaOXA-48-positive *K. pneumoniae* were analysed. Broad spectrum beta-lactam antibiotics (CTX, CAZ, cefepime, and piperacillin/tazobactam) did not work on these isolates. Of these, 42 were resistant to carbapenems such as doripenem, ertapenem, MEM, and IPM. The study also discovered that the isolates, the majority of which were ST1, coproduced at least one ESBL or Ampc beta lactamase (C. H. Wang *et al.*, 2021).

In another study using carbapenemase and ESBL producing *K. Pneumonia*, blaCTX M is highly detected in all isolates, followed by bla – KPC, bla SHV, bla-OXA- 48 (Patil *et al*, 2019). A study in Belgium discovered a new class D carbapenemase called OXA-427 in many *Enterobacteriaceae* species. Researchers conducted a comparative examination of two plasmids harboring the blaOXA-427 gene that were isolated from a strain of *K. pneumoniae* and a strain of *Enterobacter cloacae* complex in order to explore the dissemination of this gene. The backbone of the two IncA/C2 plasmids that contain blaOXA-427 is identical, however in the *K. pneumoniae* strain, this plasmid cointegrates with an IncFIIb plasmid to create a 321-kb Megaplasmid with several multi resistance areas (Harboring *et al.*, 2018).

A study investigated genomic epidemiology of XDR *K. pneumonia* in Africa in clinical samples form ICU patients. All isolates were resistant towards beta-lactams (OXA-1, NDM-1, CTX-M-15, SHV-1, TEM-1B) as well as other antibiotics. Except for one isolate all other isolates belonged to ST152 with novel sequence type, ST3136. The isolates had same plasmid multilocus sequence type (IncF [K12: A-: B36]) and the capsular serotype (KL149), suggesting epidemiological linkage. The presence of blaNDM-1 was linked to carbapenem resistance in the isolates, suggesting that clonal dispersion and horizontal transfer are the root causes of carbapenem resistance (Ramsamy *et al.*, 2020). Using PCR and DNA sequencing, the virulence and resistance of a *K. pneumoniae* isolate from a sepsis patient in a hospital in Recife-PE, Brazil, were studied. They found presence of blaNDM-1,

aac(6')-Ib-cr and acrB resistance genes, as well as cps and mrkD virulence genes for the first time (Scavuzzi *et al.*, 2019).

A study with Ventilator-associated pneumonia (VAP) a nosocomial infection was studied from endotracheal aspirate samples using MALDI-TOF-MS and antibiotic susceptibility testing. The isolates contained carbapenem-resistant gram-negative pathogens including *Acinetobacter baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. The main antibiotic resistance genes responsible for conferring resistance to carbapenems in VAP were identified as blaNDM-1 and blaVIM (Kumari *et al.*, 2021). Drug resistance, joint efficiency and gene environment of blaNDM-1 harboring *K. pneumoniae* was investigated in a study using VITEK-2 compact kit. And fifty percent of them showed presence of blaNDM-1 gene. Plasmids p11106 and p12 in *K. pneumoniae* were found to be similar to pNDM-BTR. Also, blaNDM-1 gene was located at orf00037 and ble at orf00038. Two tnpA genes were present upstream and downstream of orf00052. It was concluded, blaNDM-1 containing bacteria exhibit MDR which is transmitted through plasmid conjugation. And presence of ble and tnpA gene suggest that blaNDM-1 originates from *A. baumannii*. retained in *K. pneumoniae* over a long period by transposition of mobile elements (Xiang *et al.*, 2020).

A study examined the association between the blaNDM gene and plasmids in *K. pneumoniae* by analyzing the variants of blaNDM, replicon types, and conjugative transfer regions among 171 blaNDM harboring plasmids out of 4,451 *K. pneumoniae* plasmids. Most dominant among them were blaNDM-1 and blaNDM-5. More than half 50% of the blaNDM-harboring plasmids of *K. pneumoniae* were categorised as IncF plasmids, distributed across China, USA and Vietnam (Zeng *et al.*, 2022).

NDM-5 is continuously emerging and often present in carbapenemase producing *Enterobacteriaceae* (CPE). In a study, isolates collected contained *K. pneumoniae*, *Klebsiella aerogenes* and *E. coli*. PFGE used to determine *K. pneumoniae* and *K. aerogenes* as homologous clones. The blaNDM-5 gene was found on a 46f-kb plasmid and exhibited stability in both clinical isolates and transconjugants without any noticeable plasmid loss. Plasmid sequences pNDM-K725, pNDM-CR33, and pNDM-Z214 showed significant similarity, although they differed from pNDMZ244. It was concluded that dissemination of the IncX3-type plasmid was found to be the cause of horizontal gene transfer of blaNDM-5 gene to

other *Enterobacteriaceae* in pediatric patients (Tian *et al.*, 2020). In another study of survey of the isolates of *Enterobacteriaceae* and *K. pneumoniae*, the survey results revealed that *oqxAB* gene which codes for fOqxAB efflux pump was found to mediate the MDR in bacteria. Quinoxalines, quinolones, tigecycline, nitrofurantoin, and several other detergents and disinfectants are becoming less effective or less effective against these MDR bacteria (Li *et al.*, 2019).

In China, carbapenem-resistance in *K. pneumoniae* was found related to *blaKPC-2* gene and its copy number affected KPC enzyme expression. In a clinical study CR-KP isolates of *K. pneumoniae* were collected and on screening *blaKPC-2* gene was found. Some had *blaNDM-1* gene, and few had both the genes. ST11 clone of *K. pneumoniae* was dominant. The isolates showed 100% susceptibility towards polymyxins and an intermediate susceptibility to tigecycline (75.7%) but were resistant to all other antimicrobials during DST. Some isolates showed elevated minimal inhibitory concentration to IPM. It was concluded that carbapenem pressure can increase copy number of KPC enzymes in carbapenem-resistant *K. pneumoniae* (CRKP) strains resulting in higher carbapenem resistance (Chen *et al.*, 2022).

A study investigated ceftazidime/avibactam (CAZ/AVI) activity against CRKP and its resistance mechanisms before it was released in the market. 50% of the CRKP isolates from hospitals in China were found to be metallo- β -lactamase-producing, 40% were carbapenemase producing and 6% produced both. *blaKPC-2* was present in Carbapenemase producing strains with high resistance to CAZ/AVI. While avibactam at a fixed concentration of 8 mg/L were able to restore susceptibility 90% of the isolates (Zhang *et al.*, 2020). A study was conducted in Ganzhou, China from hospital specimens to investigate ARGs and molecular characteristics for CRKP that causes nosocomial infections. All 42 CRKP isolates were MDR. 35 had *blaKPC-2* and 12 had *blaNDM-1* (12 isolates carried both). Detection rate of *blaIMP-4* and *blaOXA-48* was 2.4% (1/42), respectively. One of the isolates carried both *blaKPC-2* and *blaIMP-4* genes. And one other isolate carried *blaKPC-2*, *blaNDM-1* and *blaOXA-48*. And *blaSHV* and *blaCTX-M-1* was present in all of them. While *blaTEM* was found in 27 and *blaCTX-M-9* in 12. Consequently, the CRKP isolates exhibited severe multidrug resistance, primarily attributed to the production of *blaKPC-2*, which carried multiple ESBL genes concurrently. The predominant clonotype was identified as ST11-*blaKPC-2 K. pneumoniae* (Liao *et al.*, 2023).

According to research, a new NDM-23 allele emerged and was found in an MDR plasmid that spread throughout Spanish hospitals via the *K. pneumonia* ST437 clone. Using hybrid sequencing utilizing Illumina and Nanopore technologies, phylogenetic analysis, and antibiotic susceptibility testing, an epidemiological assessment for ESBL-producing *K. pneumonia* in Spain yielded a total of 1,972 isolates. Three CR strains with the bla NDM-23 gene (MICs of bla NDM-23 allele were identical to bla NDM-1 allele) were not identified by screening assays for CPE. A 97-kb Non mobilizable MDR plasmid with 19 resistance genes for 9 distinct antibiotic families included the bla NDM-23 allele. In the plasmid, the bla NDM-23 gene was located in the variable region of a complicated class 1 integron with a unique genetic environment. NDM-23 allele was transported by strain from Pakistan to Spain (García-González *et al.*, 2023).

In a study in Chile, 22 *K. pneumonia* strains were isolated from hospital patients which were found to be carbapenemase producing. 20 strains had blaKPC, 1 had NDM-1 and 1 had blaOXA-370. Moreover, blaTEM and blaSHV were present in every isolate. Moreover, 71% carried one qnr gene and 57% aac(6')Ib-cr. There were about ten distinct STs detected, the two most common ones being ST258 (found in five strains) and ST1161 (found in seven strains). Out of all the isolates, 82% were resistant to every β -lactam that was tested, 91% to CIP, 73% to colistin, 59% to gentamicin, 50% to amikacin, and just 9% to tigecycline. Ten isolates—four blaCTX-M-15 and six blaCTX-M-2—also had the blaCTX-M gene. Additionally, the MDR phenotype was linked to blaCTX-M-2 and class 1 integrons, but blaCTX-M-15, which was linked to other insertion sequences (ISCR1, ISECp1, and IS26), did not contribute to the MDR phenotype. (Carrasco-Anabalón *et al.*, 2018).

Prevalence and location of blaKPC-2 and blaNDM-1 genes in carbapenem-resistant *K. pneumoniae* were examined in a study using genomes from the National Center for Biotechnology Information (NCBI) database and hospital samples from China. Tests were conducted using 28 different antimicrobials against the bacteria. Each isolate included eight distinct ARGs and an MDR pattern. A phylogenetic study revealed that two lineages, consisting of ST11 and ST25, dominated all isolates. These lineages harboured the blaKPC-2 and blaNDM-1 genes, respectively. ISKpn7-dnaA/ISKpn27-blaKPC-2-ISkpn6 and blaNDM-1-ble-trpf-nagA may represent the core structure horizontal transmission of blaKPC-2 and blaNDM-1, respectively, according to genetic environment analysis. Additionally, blaNDM-1

expression and horizontal transfer may be facilitated by DNA transferase (hin). (Zhang *et al.*, 2020).

In Saudi Arabia a study analysed resistance pattern in MDR *K. pneumoniae*. Samples collected from a hospital. VITEK II and broth microdilution used for antimicrobial susceptibility testing. Resistance genes identified by Illumina whole-genome shotgun sequencing and PacBio SMRT sequencing protocols. The presence of *Klebsiella quasipneumoniae* subsp. CRE was detected, according to the results. It was discovered that the simlipneumoniae strain was a brand-new ST-3510 with the gene bla KPC-2 carbapenemase. Isolate NGKPC-421, was obtained from shotgun WGS and was obtained from septic patients and was mistook as *K. pneumoniae*. A putative 39.4 kb IncX6 plasmid containing a bla KPC-2 gene and transposable elements (ISKpn6-bla KPC-2-ISKpn27) were discovered during the sequencing of the NGKPC-421 genome. (Hala *et al.*, 2019).

The goal of this study was to understand the underlying molecular epidemiology and resistance mechanisms of carbapenem resistance in *Enterobacteriaceae* isolates from Heilongjiang Province in China. From a grade 3 hospital, a total of 53 isolates of *Enterobacteriaceae* (CNSE) that are not sensitive to carbapenem were obtained. These CNSE strains showed total resistance to a number of cephalosporins and enzyme inhibitors, as well as significant rates of resistance to other antibiotics. Resistance was verified by phenotypic testing, such as the modified Hodge test and modified carbapenem inactivation test. The results of molecular research indicated that blaKPC-2 was the most common carbapenemase gene, followed by blaIMP-4 and blaNDM variations. Multiple drug resistance genes were present in many isolates, including those for ESBLs including blaSHV, blaTEM, and blaCTX-M-15. Gene transferability was shown by conjugation studies. By using multilocus sequence typing, the dominant clone was shown to be ST76. This study uncovered an outbreak of blaKPC-2-positive *K. pneumoniae* ST76, underscoring the urgency of infection control measures to curb MDR bacteria dissemination (Gong *et al.*, 2018).

A study in Portugal aimed to update epidemiologic data of CR *K. pneumoniae*. blaKPC-3, blaOXA-181, and blaGES-5 carbapenemase genes were found present and there was also co-occurrence of blaKPC-3 and blaGES-5 in some isolates. GES-5-producing isolate co-produced the ESBL BEL-1; both corresponding genes were co-located on the same ColE1-like plasmid. blaOXA-181

gene was present on IncX3 plasmid while blaKPC-3 was carried on IncN, IncFII, IncFIB, and IncFIIA plasmid types. Isolates were of 13 pulsotypes and 9 STs. And all isolates were susceptible to CAZ/AVI but some had reduced antimicrobial susceptibility (MIC = 3 mg/L) (Aires-De-Sousa *et al.*, 2019). Two CAZ-AVI (CZA)-resistant KPC-producing *K. pneumoniae* strains (KP-14159 and KP-8788) that were isolated from infected individuals who were never treated with CZA were described in research. Both strains carried novel pKpQIL plasmid derivatives (pIT-14159 and pIT-8788, respectively) carrying two copies of the KPC-3-encoding transposon and altered outer membrane porins (a shortened OmpK35 and an Asp137Thr138 duplication in the L3 loop of OmpK36). WGS revealed that both strains belonged to the ST258 strain. pIT-8788 had a higher copy number than pIT-14159. DH10B when transferred to *E. coli*, increased CZA MIC by 32-fold. The result also suggested role of pKpQIL plasmid derivatives in increasing the blaKPC gene copy number and blaKPC-3 expression in bacteria. Non-transferable elements with multiple copies of Tn4401 could mediate CZA resistance upon mobilisation (Gene & Number, 2020).

Another study in Malaysia sequenced genomes of multiple carbapenem-resistant *K. pneumoniae* strains and identified the genetic basis of resistance, samples taken from hospital. Six different STs were identified. Carbapenem resistance in the strains was found to be due to presence of plasmid-localised blaNDM (blaNDM-1/blaNDM-5) or blaKPC (blaKPC-2/blaKPC-6). Majority of these genes were found to be flanked by transposase or integrase sequences suggesting their possible mobility. The investigation also revealed the assembly of the second *Klebsiella* contig and the first blaKPC-6-harboring plasmid contig for *K. pneumoniae*. The study urges for improved genomic surveillance of *K. pneumoniae* in Malaysia (Gan *et al.*, 2020).

In another study using *Klebsiella* isolates which exhibited the presence of the blaCTX-M gene, blaTEM, blaOXA, and blaSHV genes. Additionally, a range of other resistance genes, including blaLEN, aac (6')-Ib-cr, qnrA, qnrB, qnrS, oqxAB, aad, aph, dfr, sul1, sul2, fosA, and cat, were identified. Mutations in key genes such as parC, gyrAB, pmrAB, phoPQ, and kpnEF were associated with fluoroquinolone and colistin resistance. Four isolates carried the rarely described blaLEN gene. Several non-clone-specific virulence genes were also detected, and the study identified two novel integrons and multiple plasmid replicons (IncF, IncH and IncI) (Mbelle *et al.*, 2020).

Colistin-resistant *K. pneumonia* isolates that originated in chickens were gathered for research and subjected to antimicrobial susceptibility testing, PCR, conjugation assay, and S1-PFGE analysis. WGS was employed to comprehend the mechanism of colistin resistance. The strain's colistin resistance was shown to be caused by a unique mcr-8.2-bearing plasmid p2019036D-mcr8-345kb, 345655 bp in size, encoding for many resistance genes including floR, sul1, aadA16, aadA2, blaCTX-M-27, blaDHA-1, tet (D), dfrA12, and qnrB4. The plasmid had the same core conserved region carrying mcr-8.2, but it differed from other mcr-8.2 positive plasmids, according to the study. Additionally, it hinted that there may be a risk to people from this strain (Yang *et al.*, 2020). *E. coli* and *K.pneumoniae* have developed a new phenotype that is resistant to piperacillin/tazobactam (TZP) but susceptible to carbapenems and third generation cephalosporins due to hyperproduction of the TEM β -lactamase, although the specific mechanism underlying this hyperproduction via gene amplification is not well elucidated. A translocatable unit (TU) excising from a pseudo-compound transposon (PTn6762) flanked by IS26, containing the blaTEM-1B gene, is the mechanism revealed by research. Following its reinsertion into the chromosome, this TU forms a tandem array of TUs with IS26. This causes blaTEM-1B to multiply more often, which causes TEM-1B hyperproduction and TZP resistance. Interestingly, the TZP-resistant strain retains fitness similar to the TZP-susceptible progenitor despite the increased blaTEM-1B copy number. (Hubbard *et al.*, 2020).

2.3 PREVALENCE OF BLA GENE IN *P. aeruginosa*

P. aeruginosa is capable of developing antibiotic resistance and has a poor intrinsic sensitivity to antibiotics. It may also thrive in a variety of environments, including both natural niches and shifting inpatient habitats. The relationship between the emergence of antibiotic resistance and the availability of nutrients was examined in a research. *P. aeruginosa* antibiotic resistance has been seen to evolve differently in laboratory rich medium, urine, and synthetic sputum, with tendencies toward tobramycin, CAZ, and CAZ-AVI. The environment affects resistance level and fitness. In order to create evolution-based tactics to combat AR, it is crucial to anticipate the development of antibiotic resistance and the trade-offs that come with it, such as collateral sensitivity. *P.aeruginosa* were grown on different antibiotic containing media, urine and synthetic sputum, this showed that AR evolution varied

in different conditions suggesting environment affect the phenotype associated with AR mutations (Laborda *et al.*, 2022).

One significant factor in the transmission of antibiotic-resistant genes (ARGs) from *P. aeruginosa* to other bacteria is the integron, a mobile exogenous element. 100 clinical isolates of *P. aeruginosa* were used in an Iranian investigation; 41% of the isolates were MDR, 42% included class 1 integron, and 61.9% of the isolates with class 1 integron were MDR. 10 different gene cassettes were found of size range 0.6 to 3.5 kb. *aadB* gene cassette of size 0.75 kb is the most frequent resistant gene among all isolates. Other genes found were *aadA6-orfD*, *aacA4-blaOXA-10*, *aadB-aacA4-blaOXA-10*, *blaOXA-10-aacA4-VIM1*, *aacA4-cat B10*, *aacA5-aadA1-cmlA5*, *blaOXA31-aadA2*, and *aac(3)-Ic-aac A5-cmlA5*. Treatment of infections was majorly affected by prevalence of class 1 integron (Ahmadian *et al.*, 2020).

Research examined phenotypes of antibiotic resistance and performed molecular characterization of *P. aeruginosa* class 1 integron gene cassettes. 113 isolates were taken from aseptic locations or patient sputum specimens. The variable portions of the integrons were amplified and sequenced using PCR to screen them. The three drugs with the highest resistance were MEM (23%), imipenem (IPM) (23.89%), and CIP (26.55%). When it came to carbapenem-sensitive *P. aeruginosa*, CIP-levofloxacin was the most common multidrug resistance, but IPM-MEM was more common in carbapenem-resistant *P. aeruginosa*. In 14 isolates, the class 1 integron was detected. The most common gene cassette array among the six found was *aacA4-blaOXA101-aadA5*. A total of 17 gene cassettes were found. The most common ARGs were *blaOXA-1*, *blaOXA-101*, and *aacA4* (6.2%, 7/113). Additionally, ESBL genes were found. In *P. aeruginosa*, long-standing ARGs were shown to be being eliminated, while newly inserted integrons providing resistance to β -lactamases, fluoroquinolones, even carbapenems, were discovered (Liu *et al.*, 2020).

Because *P. aeruginosa* is becoming a worldwide health problem due to its carbapenem resistance, a study was done to find out how common MBL generating genes are in these bacteria. A bioinformatic study revealed that 37% of the clinically isolated *P. aeruginosa* had MBL producers and that 55% of the bacteria were resistant to IPM and MEM. The two most common genes that encode MBL are *bla VIM* and *bla IMP*. (Ghasemian *et al.*, 2018). In a study conducted in an Indian

hospital in around 60 clinical isolated of *P. aeruginosa*. Imipenem resistance was found in 75% of carbapenem-resistant isolates that are MBL producers. Among the strains, 100% sensitivity to Polymyxin-B, 44.4% sensitivity to Piperacillin/Tazobactam, 22.2% sensitivity to Amikacin and Tobramycin, and 11.1% sensitivity to CIP and Gentamicin were observed. (Radhika *et al.*, 2022) . In research on imipenem-resistant clinical *P. aeruginosa* isolates carried out in Pakistan, RAPD-PCR was used. 76% of the isolates produced MBL, while 68%, 48%, and 4% of the isolates had the blaIMP, blaNDM-1, and blaVIM genes, respectively. In *P. aeruginosa* isolates resistant to imipenem, a significant frequency of blaIMP and blaNDM-1 was found. (Ali *et al.*, 2021).

An investigation of the effects of MDR *P. aeruginosa* infections in individuals with acute pancreatitis was carried out. Acute pancreatitis patients with and without MDR-PA infections were the subjects of a retrospective case-control research comparing several MDR-PA resistance medications. The patients with MDR-PA infection had a greater death rate, according to the data. Compared to the carbapenem-sensitive *P. aeruginosa* group, the carbapenem-resistant *P. aeruginosa* group had a greater rate of multiple organ failure. It has been discovered that carbapenem-resistant *P. aeruginosa* infections are associated with inappropriate use of carbapenem drugs and multiple organ failure. (Wu *et al.*, 2023).

In another study, a total of 634 clinical *P. aeruginosa* isolates from diverse sources were analysed for their resistance profiles, focusing on carbapenems, the last-resort antibiotics for such infections. Among the isolates, 32 (5%) exhibited carbapenem resistance, primarily to imipenem and MEM. The predominant carbapenemase gene detected was blaVIM-1 (in 31 isolates), with a minority harboring blaNDM (in 4 isolates). Efflux pumps, particularly MexCD-OprJ and MexXY-OprM, were overexpressed in a significant portion of the resistant isolates. Strong correlations were observed between efflux pump expression and carbapenem resistance. RAPD-typing revealed four major clusters, indicating the potential for intra-hospital cross-infection. These findings emphasize the complexity of carbapenem resistance mechanisms in *P. aeruginosa* and underscore the critical need for stringent infection control measures (Hassuna *et al.*, 2020).

In a study using multiplex PCR, presence of beta lactamase genes encoding blaTem, blaOXA, blaCTX-M-15, blaVim, blaGes, blaVeb, blaDIM, AmpC, study is conducted with *P. aeruginosa*. For the study totally 200 sample isolates were taken.

From the results, the beta lactamases gene majorly bla TEM, OXA 35, VIM and many more subclasses of bla gene were identified in trace amounts. Upon phenotype analysis, it is found out that 29 to 38 out of 200 isolates were confirmed positive for ESBL production (Murugan *et al.*, 2018). UTIs are common in both genders and antibiotic resistance results in treatment failure. A another clinical diagnostics study on urinary tract infections in a hospital in Quetta, 16S rDNA gene approach identified isolates as *E. coli* and other bacterial species (*K. pneumonia*, *P. aeruginosa*, *P. mirabilis*) which were MDR to many antibiotics including carbapenems. PCR detected presence of blaNDM-1 and the strains were susceptible to only tigecycline and polymyxins suggesting XDR (Pirkani *et al.*, 2020).

A study in Egypt studied prevalence of virulence genes oprL, exoS, phzM, and toxA and AGRs bla TEM, tetA, and bla CTX-M in *P. aeruginosa*. Isolates were collected from 285 fish (*Oreochromis niloticus* and 120 *Clarias gariepinus*) from fish farms and examined for *P. aeruginosa*. The tested strains were resistance towards amoxicillin, cefotaxime, tetracycline, and gentamicin. PCR revealed all isolates carried oprL and toxA virulence genes and some also carried phzM gene. ARGs such as bla TEM, bla CTX-M, and tetA were present with high prevalence. It was seen that experimentally *P. aeruginosa* infected fishes showed high mortality and similar signs of septicemia as a naturally infected one. It was concluded that *P. aeruginosa* is a major pathogen of *O. niloticus* and *C. gariepinus* and oprL and toxA genes were the most prevalent virulent genes in *P. aeruginosa*. And blaCTX-M, blaTEM, and tetA were the prevalent MDR genes (Algammal *et al.*, 2020).

In a study in Egypt, 200 samples were collected from broiler chickens and investigated for oprL sequencing, antimicrobial resistance patterns, virulence-determinant Quorum sensing, and antibiotic resistance genes of the pathogen *P. aeruginosa*. 28.3% of the birds showed prevalence of *P. aeruginosa*. oprL gene sequence analysis showed the strain showed similarity to many other *P. aeruginosa* in different geographical regions like USA, India, China, Chile, and Ghana. PCR showed the presence of virulence genes like oprL and toxA (highest prevalence-100%), aprA (42.5%), phzM (33.3%), and exoS (25.9%). Quorum sensing genes present were: lasI, lasR, rhII, and rhIR in a prevalence of 85.2%, 85.2%, 81.5%, and 81.5%, respectively. And 40.7% of the isolated *P. aeruginosa* were XDR possessing sul1, blaTEM, tetA, blaCTX-M, blaOXA-1, and aadA1 genes – resistance to 7 antimicrobial classes (Algammal *et al.*, 2023). A study was conducted to identify the

imipenem resistance mechanism in *P. aeruginosa* (from dogs). The isolate (K19PSE24) was sensitive to the majority of the tested antibiotics but resistant to imipenem and piperacillin/tazobactam. According to DNA sequencing, K19PSE24 contained the genes *aph(3)-IIb*, *fosA*, *catB7*, *crpP*, and *blaOXA-913* and belonged to sequence type 313. Following co-treatment with imipenem (2 µg/mL) and either of the P-PNAs (12.5 µM or 25 µM), no viable colonies were discovered. The primary cause of imipenem resistance in K19PSE24 was the presence of the *blaOXA-913* gene. (Moon *et al.*, 2021).

2.4 PREVALENCE OF BLA GENES IN *ENTEROBACTER* Spp.:

The bloodstream isolate *E. cloacae* were characterized and its genetic environment and ARGs examined in a Chinese investigation. ST528's *E. cloacae* RJ702 had the *blaIMP-26*, *blaDHA-1*, *qnrB4*, and *fosA5* genes. It was resistant to nearly all β-lactam antibiotics, although it was vulnerable to tigecycline and quinolones. It was shown that the 329,420-bp IncHI2 conjugative plasmid pIMP26, which contained the operons for the *trhK/trhV*, *tra*, *parA*, and *stbA* families, carried transconjugant inherited MDR and resistance genes. Plasmid pIMP26 exhibited significant levels of flexibility, transferability, and phylogenetic mosaicism. (Wang *et al.*, 2019). In another study in India using blood stream isolates *Enterobacteriaceae* identified metallo-β-lactamases and PMQRs. Species present were *K. pneumonia*, *E. coli*, *Enterobacter cloacae* and *Enterobacter aerogens*. Isolates were resistant to CIP and MEM. NDM was the only metallo-β-lactamase found (NDM-1 was predominant and NDM-5, NDM-7, and NDM-15 were also present). PMQRS including *aac(6')-Ib-cr* was *qnrB1* and *qnrS1* were found to be present. Some isolates co-harboured PMQRs and *blaNDM* (IncFIK, IncA/C, and IncN plasmid types). *GyrA* and/or *ParC* mutations were present in a fraction of NDM positive isolates. Plasmid with only *blaNDM* was IncHIB-M type. In conclusion, spread of PMQRs was independent of the spread of NDM-1 (Mitra *et al.*, 2019).

A study conducted in Iran, characterised both ESBL and carbapenemase genes in *Enterobacter* from inpatients. The isolates were identified to be *E. gergoviae*, *E. aerogenes*, *E. cloacae*, *Cronobacter sakazakii*. Highest resistance was observed against AMP and lowest against imipenem in antibiotic susceptibility testing. High prevalence of MDR was detected and many were ESBL positive and non-susceptible to carbapenem including *blaCTX-M*, *blaTEM*, *blaSHV*, *blaIMP*, and

blaNDM (Khashei *et al.*, 2020). To comprehend the mechanism of colistin and carbapenem resistance in *E. cloacae* Complex (ECC) strains, a further study was carried out. The findings demonstrated that the mechanism of colistin resistance involves either the increased expression of *acrA* in the efflux pump AcrAB-TolC alone or in conjunction with a decrease in colistin-outer membrane affinity brought on by lipid A alteration. Furthermore, it was discovered that the ECC strain co-harbored blaNDM-1 and plasmid-mediated *mcr-4.3*. According to the findings, co-resistant ECC bacteria' resistance mechanisms to colistin and carbapenem do not overlap. However, it also suggested that because co-harboring plasmid mediated resistance genes are present, ECC may be a carrier for colistin and carbapenem resistance. (Liu *et al.*, 2021)

2.5 PRESENCE OF BLA GENES IN *A. baumannii*:

In a study in Thailand population, 50% of the clinical isolated showed MDR with commonly present with carbapenemase MDR-A gene blaIMP ,blaVIM ,bla OXA-40-like, and bla OXA-58-like were also commonly found in these *A. baumannii* MDR isolates that are commonly present in respiratory tract (Yungyuen *et al.*, 2021).

Endotracheal aspirate samples were utilized to examine ventilator-associated pneumonia (VAP), a nosocomial infection, using MALDI-TOF-MS and antibiotic susceptibility testing. *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and other carbapenem-resistant Gram-negative bacteria were among the isolates. blaNDM-1 and blaVIM were shown to be the main genes causing carbapenem resistance in VAP. (Kumari *et al.*, 2021). In South Africa, *A. baumannii* is a primary source of healthcare-associated infections and has the ability to acquire and spread antibiotic resistance. Using isolates of *A. baumannii*, a study was conducted to look at the genetic component of multidrug resistance in this organism. Out of the 100 isolates, 70 had blaOXA51gene positivity. The isolates had phenotypic resistance to practically every antibiotic examined, with the exception of one isolate that had intermediate tigecycline susceptibility, whereas all of them exhibited colistin susceptibility. In terms of oxacillinase, the most common gene was blaOXA-23 (few had blaOXA-40), whereas blaVIM was most common in terms of MBL (blaSIM-1 in few). A gene combination for an active efflux pump was present in certain isolates (56). Oxacillinases blaOXA-23 is a major genotypic determinant of drug resistances (Nogbou *et al.*, 2021).

A study reported emergence of VI (IC-6) clone of XDR *A. baumannii* in Brazilian Amazon region. A single XDR clone was identified as belonging to the high-risk pandemic IC-6 lineage from ST944Oxf/78Pas after carbapenem-resistant samples were gathered from Boa Vista City and characterized using PFGE and MLST. AadA5, aac(3'')-IIa, aph(3')-Ia, armA, aadB, msrE, blaTEM-1, IS15DIV–blaCTX-M-115–IS15DIV, blaOXA-90, ISAba1–blaADC-152, blaOXA-72, qacEΔ1, and sul1 were the components of the IC-6 resistome. On a 48 kb MDR genomic island within the chromosome, there were many ARGs. The Brazilian strains and IC-6/ST944Oxf/78Pas shared the blaOXA-72 and blaCTX-M-115, indicating an epidemiological connection. (Fonseca *et al.*, 2020).

A study in Taiwan investigated molecular epidemiology of carbapenem-resistant *A. baumannii* (CRAB) and resistance mechanism and spread in isolates with bla OXA-23-like or bla OXA-24-like genes and it was observed that CRAB carrying these genes increased 2008 onward. bla OXA-23-like gene was present on antibiotic resistance genomic island 1 (AbGRI1)-type structures present on plasmid or chromosome in isolates of different STs (CG92 and novel CG786), and blaOXA-24-like was present on plasmids in CRAB isolates of limited STs (CG92). Many aminoglycoside resistance genes coexisted in CRAB. Tn6180-borne armA was present in many CRAB isolates, among them some had Tn6179 upstream, constituting AbGRI3. blaTEM was found in many isolates and some carried it in AbGRI2-type structures. Many ampC genes had ISAba1 upstream (Kuo *et al.*, 2018).

A study analysed characteristics of NDM-1-producing *Acinetobacter haemolyticus* (sz1652) isolated from China. Antimicrobial susceptibility testing was used to see antibiotic spectrum and CDT confirmed production of MBLs. Transferability of CR tested using filter mating experiments and plasmid transformation assays and it was revealed to be unsuccessful. All antibiotics except amikacin, tigecycline, and colistin were ineffective against the strains, including carbapenems. A chromosome, two plasmids, and a 16 genomic island were found to make up the sz1652 genome, according to the WGS. Beta-lactamases such as blaNDM-1, blaOXA-214, and blaLRA-18, alterations linked to fluoroquinolone resistance (GyrA subunits, Ser81Ile, and ParC subunits, Ser84Tyr), and efflux pump genes linked to tetracycline and macrolide resistance were among the ARGs found. (Jiang *et al.*, 2020).

2.6 PRESENCE OF BLA GENES IN *Salmonella* spp.

MDR strains of *Salmonella* spp. are becoming common in both industrialized and developing nations. Multiple drug resistance in *Salmonella typhi* strains that originated in south-east Asia and the Indian subcontinent is now the norm. Numerous epidemics have been caused by these MDR bacteria, and some of them have been linked to tainted water sources (Threlfall, 2002). *Salmonella enteritidis* was shown to have drug-resistant genes *sul2*, *sul3*, *blaTEM-1*-like, *tet(A)*, and *tet(G)* in a recent study. The strains exhibited extremely high resistance to ampicillin, Cefoperazone, nalidixic acid, and streptomycin. A few strains (AMP-CFP-STR-NA-TE, AMP-STR-NA-TE, and AMP-CFP-STR-NA) showed mixed drug resistance spectrum (Dai *et al.*, 2021). It is rare for *Salmonella paratyphi A* to exhibit drug resistance. A instance of ceftriaxone-induced paratyphoid fever was documented in Pakistan (often used for paratyphoid therapy). The isolate exhibited resistance to AMP, CIP, cefixime, CTX, and certain fluoroquinolones. (Irfan *et al.*, 2023).

A further investigation was conducted to examine the resistance pattern of *S. enterica* isolates derived from food towards antimicrobial preparations. Among the 11 serovars identified, *Salmonella infantis* was the most prevalent at 28%, followed by *Salmonella enteritidis* at 19% and *Salmonella Typhimurium* at 13.4%. These isolates exhibited a high level of resistance towards trimethoprim/sulfamethoxazole, cefazolin, tetracycline, and amikacin. Additionally, a subset of strains demonstrated resistance towards more than six different antibiotics. (Rakitin *et al.*, 2022). A *S. enterica* serovar Senftenberg strain P5558, isolated from a patient with a stump infection. This isolate was found to carry various resistance genes, including *blaTEM-1*, *blaOXA-9*, *blaCMY-2*, *blaNDM-1*, and aminoglycoside modifying enzymes. Fluoroquinolone resistance was attributed to mutations in *gyrA* and *parC* genes. Plasmid analysis revealed four resistance plasmids, with p5558-NDM (IncA/C) harboring key resistance genes including NDM-1, CMY 2. The genome also contained major virulence factors and *Salmonella* pathogenicity islands, indicating a concerning combination of virulence and antibiotic resistance in this strain (Veeraraghavan *et al.*, 2019).

A study was done to look at the relationship between integrons from clinical isolates of *Salmonella* and antibiotic resistance (human fecal samples). *S. typhimurium* ST34 and ST19 were the predominant subtypes among the 52 strains,

which were further classified into 11 serotypes and 11 sequence types. Class II and III integrons were missing, but class I integrons were found at a rate of 38.46% (20/52). Nine gene cassettes, dihydrofolate reductase A12, open reading frame F, aminoglycoside-adenylyltransferase (aad)A2, aadA22, aadA23, aadA1, cadmium-translocating P-type ATPase 2, lincosamide, and linF, were linked to drug resistance, indicating that class I integrons are significant factors underlying drug resistance in *Salmonella*. Variable regions of three of the twenty class I integrons were found to be amplified. (Xu *et al.*, 2022). Another study investigated relation of intl gene in *S. typhi* leading to MDR. 30 blood samples from patients under suspicion of typhoid were taken. *S. typhi* isolates were found to be MDR and intl gene was detected in the isolates by PCR (Jassim *et al.*, 2022).

In this comprehensive study on 2019, spanning 17 poultry breeding farms across nine Chinese provinces. Among the *Salmonella* isolates, alarmingly, 70.53% of the isolates exhibited multi-drug resistance. PCR analysis unveiled the presence of β -lactam, aminoglycoside, and sulphonamide resistance genes, with a 100% detection rate for blaTEM and sul3, and rates of 52.63% and 23.16% for sul2 and aaC4, respectively (Song *et al.*, 2020). In another study, in which samples were collected from meat (poultry and pork), showed that 53.84% of the bacterial isolates were MDR. ARGs like blaSHV, blaPSE- 1, blaTEM, but not blaCTX-M were found. Several genes showed resistance to aminoglycosides, cephalosporins, fluoroquinolones, sulfonamides, and tetracyclines (Pławińska- czarnak *et al.*, 2022). *S. Typhimurium* SH160 strain, isolated from supermarket pork meat in China, exhibited resistance to a broad spectrum of antibiotics. Whole-genome sequencing revealed the isolate belonged to the prevalent ST34 lineage and harbored a transferable IncX3 plasmid. This plasmid encoded the blaNDM-5 gene, conferring carbapenem resistance. Additionally, the isolate possessed a distinct IncHI2 plasmid carrying genes for resistance against various other antibiotics, including aadA1, aadA3, aph(3')-Ia, sul1-3, floR, cmlA, and dfrA12. (Gao *et al.*, 2020).

Salmonella isolate SG17-135 from Australian silver gull chicks was found to be resistant to twelve different antibiotics from nine different classes, including aminoglycosides, sulphonamides, monobactams, macrolides, fluoroquinolones, dihydrofolate reductase inhibitors (trimethoprim), penicillin, and cephalosporins. *S. enterica* serovar *Salmonella agona* was discovered by WGS, and its chromosome included the IncX1 (pSG17-135-X) and IncHI2 ST2 (pSG17-135-HI2) plasmids. A

complex resistance area including many resistance genes, including bla CTX-M-55, was found in pSG17-135-HI2, suggesting that wild birds are important for the transmission of resistance genes. (Cummins *et al.*, 2020). A study was conducted in Egypt (2020-2022) to investigate prevalence of *Salmonella* infections in broiler chicken in farms. Serotypes found were *S. enteritidis*, *Salmonella shangani*, *Salmonella gueuletapee*, *S. II (salamae)*. Compared to six-week-old and uninfected flocks, *Salmonella* infection was more prevalent in one-week-old chicks. All isolates were resistant to ampicillin and erythromycin and sensitive towards CIP, chloramphenicol, and levofloxacin. MDR genes found were blaTEM, blaSHV, ermB, ereA, mphA, and ermB (Shalaby *et al.*, 2022).

In a study in Tehran, blaTEM gene was present in ESBL resistant *Salmonella* strains especially in chicken isolates. Prevalence of class 1 and 2 integrons was found responsible for resistance to trimethoprim/sulfamethoxazole (TS) and chloramphenicol. It was concluded that there was lack of association between isolates from chicken and human samples due to discrepancy between serogroups and resistance phenotypes (Besharati *et al.*, 2020). *Salmonella* isolates obtained from chicken and hog production chains, as well as ill hens, were used in a serotyping investigation. The predominant serotypes, which corresponded to ST11, ST17, and ST40 clones, were *S. Enteritidis*, *Salmonella indiana*, and *Salmonella derby*. The majority of isolates from ST40-*S. Derby* clones and ST11-*S. Enteritidis* were resistant to around 4–8 antimicrobial drugs. 9–16 antimicrobial agents were XDR to ST17 *S. Indiana*. The ST11 and ST17 clones have the blaTEM-1-like gene, which conferred AMP resistance (Cao *et al.*, 2018). In a recent study using mutants, antimicrobial resistance was observed to be caused by transposable elements in infected chicken in Korea, which resulted in emergence of aadA1 resistance gene against sulphonamides in *Salmonella gallinarium* (Tran *et al.*, 2023).

CHAPTER 3

AIM AND OBJECTIVES

3.1. AIM

- ❖ To develop a multiplex PCR assay for detection of MDR genes from bacterial pathogens

3.2. OBJECTIVES

- ❖ Standardisation of PCR assay conditions with singleplex PCR.
- ❖ Standardisation PCR protocol for multiplex with five most prevalent multidrug resistance encoding genes.
- ❖ Analysis of clinical and environmental bacterial pathogens for confirmation of working of multiplex PCR assay protocol.
- ❖ Validation of the multiplex PCR assay using 20 clinical isolates thereby confirming the prevalence of the MDR genes.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS:

EMB and nutrient agar and LB broth from HiMEDIA was used for culturing bacterial samples. PCR machine from Eppendorf was used. Glass petridishes was used for plating of culture. Sterile cotton swabs were purchased commercially for collection of samples. Primers for running singleplex and multiplex assay were designed using bioinformatics tools and then purchased from “bioserve”. And mastermix was purchased commercially.

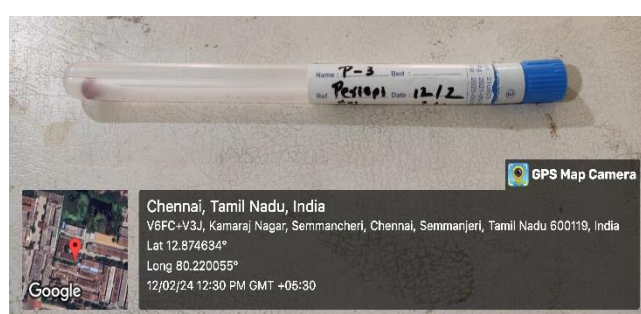


Fig 4.1 :Sterile swabs used for collection of oral swabs



Fig 4.2 : Primers

4.2 METHODOLOGY:

4.2.1 Clinical specimens:

About 20 oral swabs were collected from Sathyabama Dental Hospital. The study was approved by the Institutional Human Ethics Committee (IHEC) of Sathyabama Institute of Science and Technology, ref no: 320/IRB-IBSEC/SIST. The oral swabs were collected by rubbing around the infected area of oral region of the patients who were diagnosed with periapical abscess, gingivitis, mild to moderate periodontitis and pus. The samples were collected using sterile cotton swabs which were purchased commercially. The swabs were taken directly to the laboratory for the isolation and culturing of bacteria, which were then used as templates in both singleplex and multiplex PCR assays.



Fig 4.3: Collection of samples from Sathyabama Dental Hospital

4.2.2 Bacterial strains and culture methods:

For this study, *E. Coli* strains were isolated from the clinical isolates. The clinical samples were cultured in fresh EMB agar (acquired from HiMEDIA PVT LTD) plates by the use of streak plate procedure. Specifically, *E. coli* Were isolated for the PCR primers set. After isolating individual colonies from the plates, they were subcultured in LB broth at 37°C in an incubator.



Fig 4.4: Streak plate for patient samples

4.2.3 Bacterial sample procedure before PCR:

After extracting a specific colony from agar plate, was dissolved in 200µl of sterile water and stored in -20°C for use during the experiment. And 2ml of the liquid broth culture of the same sample cultured overnight was dissolved in 15% of glycerol and stored as a stock sample in -20 °C for future use.

4.2.4 Bacterial DNA extraction:

Plasmid DNA was also isolated from the cultured patient samples and used as template in the PCR along with direct colony samples to help with optimisation of the process. The plasmid DNA isolation was carried out by alkaline lysis method. The isolated plasmid DNA pellet was then dissolved in 150 µl of sterile water and then stored at -20°C.

4.2.5 Selection of *E. coli* specific primers:

After extensive literature survey, 5 MDR genes from class ESBL of β lactamase with high prevalence were selected. The primers were selected with the help of NCBI database. Genes used includes blaOXA-48, blaCTX-M1, blaNDM-1, blaKPC2 and qnrB. The primer set was finalized using PrimerBlast. To reduce variations in annealing temperature, the length and Guanine+Cytosine content of the primers was kept as constant for feasibility. The primer sets were designed in a way that the amplicon length of the PCR product will only vary at most by 50 bp from

each other. The forward and reverse primer sequence for all the primers along with amplicon length is displayed in (table 4.1).

Table 4.1: Primer set list

S. No	Gene name	Primer sequence	Amplicon Length
1.	blaOXA-48 Forward	GCCTGTTTATCAAGAATTTGCC	277
	blaOXA-48 Reverse	CGAGTATCCCGTTTTAGCCC	
2.	blaCTXM-1 Forward	CGTTAAACACCGCCATTCC	102
	blaCTXM-1 Reverse	TGTCGCCCAATGCTTTACC	
3.	blaNDM-1 Forward	GCAAATGGAACTGGCGAC	139
	blaNDM-1 Reverse	GCCATCCCTGACGATCAAAC	
4.	blaKPC-2 Forward	TGCAATACAGTGATAACGCC	250
	blaKPC-2 Reverse	CAATCAACAACTGCTGCC	
5.	qnrB Forward	CGGATTTTCGCAATGCCAG	204
	qnrB Reverse	TCCAACGGTTTTCCACAG	

4.2.6 Optimization of PCR protocol:

PCR protocol was optimized for each pair of primers in a heated lid gradient thermocycler (Eppendorf, Germany) using *E. coli* (sourced from environmental samples). Colony, liquid culture pellet and plasmid of the organism was used as template. Appropriate positive and negative controls were selected.

For the positive control for presence of *E. coli*, housekeeping gene *gyrA* was used. The PCR conditions were set according to the manual of the primers set. To deduce an optimum annealing temperature for the PCR assay, gradient PCR was performed with all the primers taking culture pellet as template. The gradient was set for the temperature range of 51.1°C to 57.9°C. The annealing temperature for individual primer set was also calculated arithmetically.



Fig 4.5 : Gradient PCR for standardisation of annealing temperature of primers

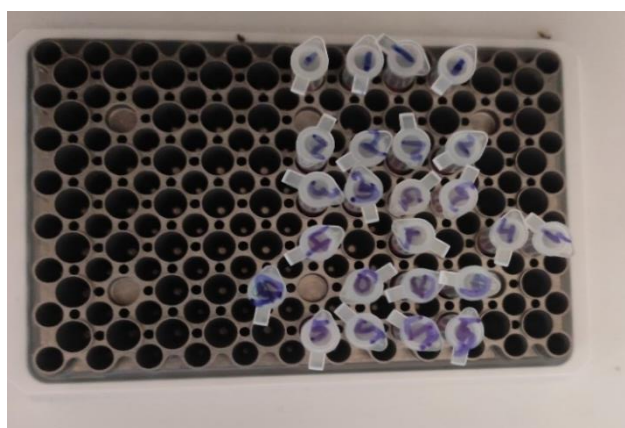


Fig 4.6: Gradient PCR

Each PCR vial consisted of mastermix, one primer set, sterile water and a particular template.

Information regarding melting temperature of the primers and PCR amplicons along with GC content is shown below in (table 4.2).

Table 4.2: Melting and annealing temperature, amplicon length and GC content of the forward and reverse primers

S. No	Gene name	Primer sequence	Tm of primers (°C)	Amplicon length (bp)	Amplicon melting temperature (°C)	GC content	Ta (°C)
1F	blaOXA-48 Forward	GCCTGTTTATCAAGAATTTGCC	51	277	77	47.7	54.2
1R	blaOXA-48 Reverse	CGAGTATCCCGTTTTAGCCC	54	277	77	47.7	54.2
2F	blaCTXM-1 Forward	CGTTAAACACCGCCATTCC	51	102	77	57.8	54.5
2R	blaCTXM-1 Reverse	TGTCGCCCAATGCTTTACC	51	102	77	57.8	54.5
3F	blaNDM-1 Forward	GCAAATGGAACTGGCGAC	51	139	78.6	57.6	55.8
3R	blaNDM-1 Reverse	GCCATCCCTGACGATCAAAC	54	139	78.6	57.6	55.8
4F	blaKPC-2 Forward	TGCAATACAGTGATAACGCC	50	250	82.6	62	57.9
4R	blaKPC-2 Reverse	CAATCAACAACTGCTGCC	49	250	82.6	62	57.9
5F	qnrB Forward	CGGATTTTCGCAATGCCAG	51	204	77.1	50	54.7
5R	qnrB Reverse	TCCAACGGTTTTCCACAG	51	204	77.1	50	54.7

*Tm: melting temperature and Ta: annealing temperature

Table 4.3: Primers and calculated annealing temperature

Gene name	Calculated annealing temperature (°C)
blaOXA-48 Forward	54.3
blaOXA-48 Reverse	55.2
blaCTXM-1 Forward	54.3
blaCTXM-1 Reverse	54.3
blaNDM-1 Forward	55.4
blaNDM-1 Reverse	56.32
blaKPC-2 Forward	57.92
blaKPC-2 Reverse	57.62
qnrB Forward	54.37
qnrB Reverse	54.37

By using the formula below, individual annealing temperature of the primers set were arithmetically calculated. Table (4.3) discusses about the individual annealing temperature calculated using the formula. And according to that the gradient PCR was performed.

Formula: $Ta_{Opt} = 0.3 \times (Tm \text{ of primer}) + 0.7 \times (Tm \text{ of product}) - 14.9$

4.2.7 Evaluation of the PCR protocol:

Using the above PCR conditions singleplex PCR was performed in duplicates and triplicates. It was done so to standardize the PCR and to find the best fit assay combination. In singleplex PCR, each patient sample was run with all five different primers at the same, thus targeting different genes in the same sample separately. The results were evaluated for the targeted outcome. The PCR products were qualified by performing Agarose Gel Electrophoresis (BIORAD). And the outcome was evaluated to know the presence and absence of genes of interest. And also to evaluate the working of the protocol.

4.2.8 Optimization of Multiplex PCR protocol:

Once the standardization of the primers and the PCR was completed, it was then followed by development of the multiplex PCR assay. The multiplex PCR vial consisted of the primers set, required amount of master mix, sterile water and the template similar to standard protocols for multiplex. For running a multiplex assay, a primer cocktail was prepared where the compatible primers were mixed together in a particular concentration.

While preparing this mix, multiple experiments were conducted to arrive at the right combination of the primers so that the primers with similar annealing temperature are in one primer cocktail, also the primers were chosen in such a way that the amplicon size has a difference of at least 50bp to avoid overlaying of bands during AGE. As we had a total of 5 sets of primers, primer cocktail was prepared accordingly. The primer-cocktail-mix-1 consisted of blaOXA-48, blaNDM-1 and blaKPC-2. These cocktail mixes were then used for the multiplex PCR. The total amount of the PCR vial was kept at 50 μ l to avoid the over-crowding of the primers as there is usually a high possibility for formation of primer dimers. Same as in singleplex PCR, in multiplex also duplicates, triplicates were performed for single patient for keeping a parameter static and another in variable.

4.2.9 Evaluation of Multiplex PCR Protocol:

The multiplex PCR product was then evaluated by performing AGE. Using Gel Doc XR (BIORAD) gel image analyser the gel was then visualized under trans-UV.

4.2.10 Agarose Gel Electrophoresis:

The AGE was performed for the quantification and interpretation of the PCR products. Visualisation of the AGE results was done with Bio-Rad Gel Doc XR imaging system. Optimum concentration of 1.7% (1.7 g agarose in 100ml of 1X TBE). The same 1X TBE was used as tank buffer and pH was set to 8.3. The parameters were set to 100V and 100mA and AGE was run for about one and half hour till the bromophenol blue dye passed 3/4th of the gel. A 100bp DNA ladder was used in the study. Bromophenol blue was added as an indicator to know how far the contents have travelled and for the visualization purpose.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CULTURING OF THE SAMPLES

After 24 hours of culturing the samples on EMB agar (specific for *E. coli*) colonies of bacteria were observed. Metallic green sheen was visible indicating presence of *E. coli*.



Fig 5.1: Broth culture



Fig 5.2: Streak plate after 24 hrs

From the 20 clinical isolates collected from Sathyabama Dental Hospital, only 8 samples showed positive for the presence of *E. coli* by exhibiting metallic green sheen. Only those 8 samples were taken for subculturing and plasmid DNA isolation and also preserved for future use.

5.2 EVALUATION OF PCR PROTOCOL

5.2.1 Optimisation of PCR Conditions by Running a Gradient PCR

Agarose gel was prepared According to the gradient temperature order, each well was loaded with a PCR product containing different primer set numbered 1 to 5. For instance, the first well is loaded with a 1 kb DNA ladder, and the next four wells are loaded with the primer set blaOXA 48 at four different temperatures. The additional primer sets were also loaded. The BIORAD Gel Doc Gel image analyzer is used to view the GEL. It is evident from the figure above that no DNA amplification using the primers qnrB and blaCTX M1 has occurred at any of the gradient temperatures. Additionally, the band with the highest intensity within its gradient is chosen in order to determine the ideal temperature that has the most DNA amplification. As 56.8 °C as indicated in this instance, the third band or well exhibits strong intensity. In order to achieve an efficient DNA amplification, the annealing

temperature for the next singleplex PCR and multiplex PCR is set at 56.8°C. The Agarose Gel Electrophoresis picture for the Gradient PCR Product is displayed in figure 5.3.

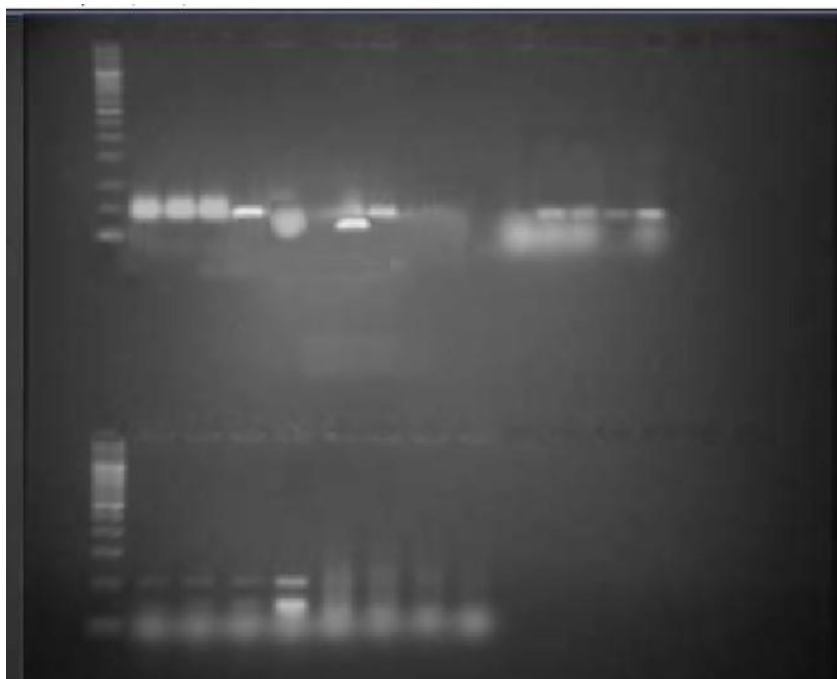


Fig 5.3: Gradient PCR result

5.2.2 Singleplex PCR

From the 8 pateint sample infected with *E.coli* only 5 sample were given positive result for the MDR gene detection in singleplex PCR. So, for further study those 5 samples were used.

5.2.2.1 Singleplex PCR Trial-1

Numerous singleplex PCRs have been conducted in order to develop an efficient methodology. Using plasmid and colony DNA as each patient's DNA template is one such efficient experiment. A singleplex PCR is carried out for patients whose E. Coli infection has been confirmed. Single-plex PCR using all primer sets in colony sample and plasmid DNA is performed on those patients.

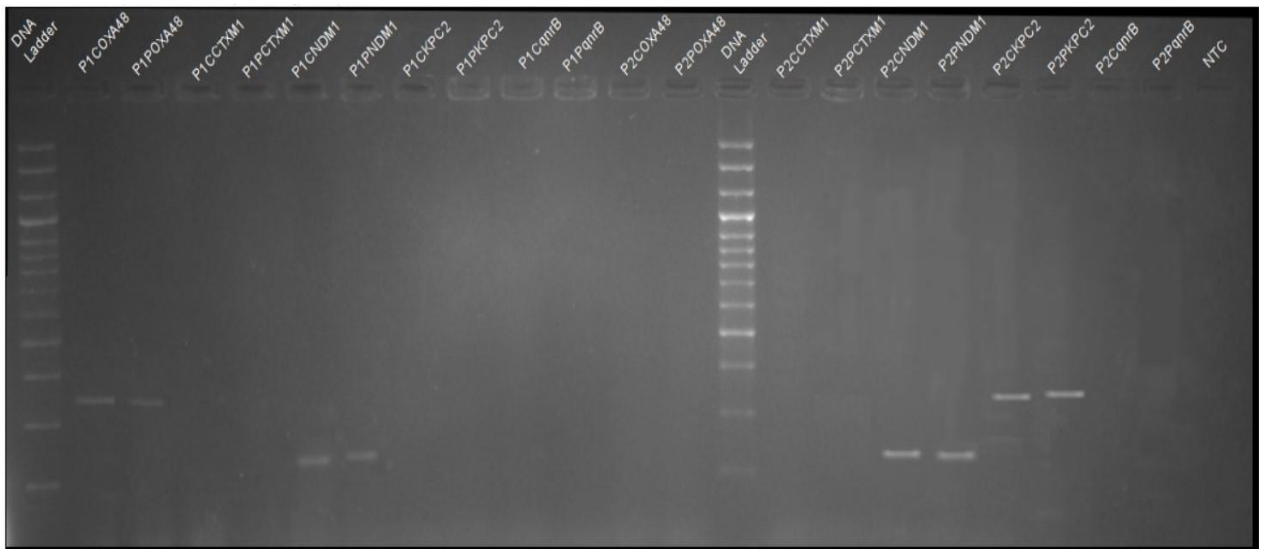


Fig 5.4: Singleplex PCR result for patient 1 and 2 with colony and plasmid samples- Trial 1

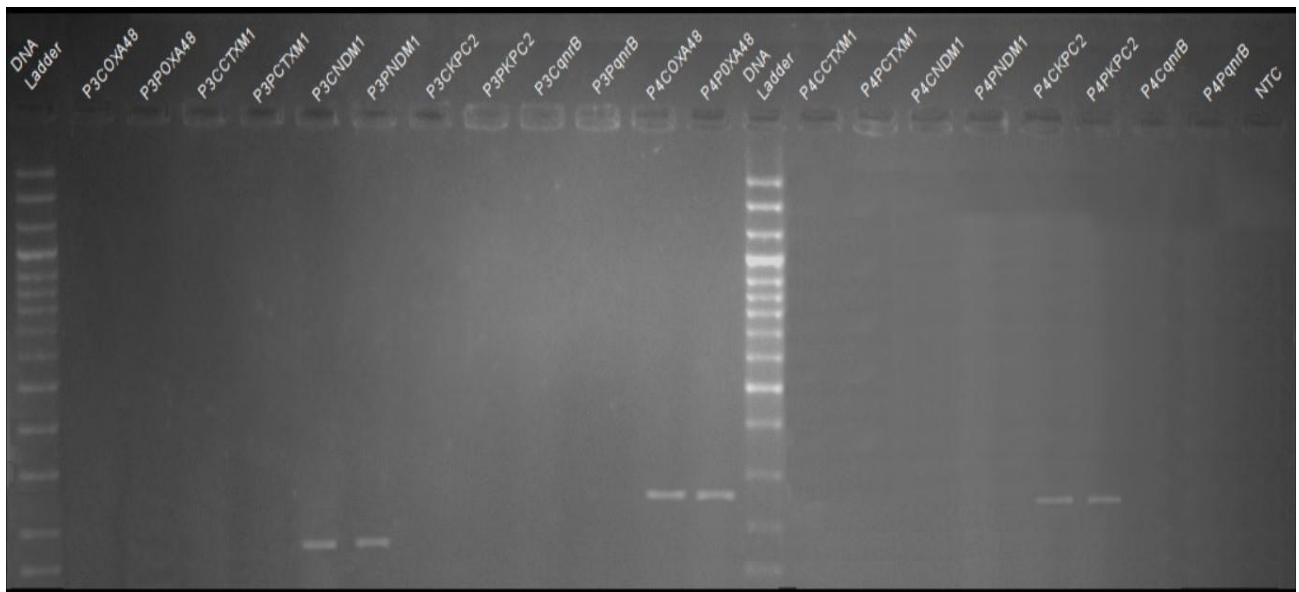


Fig 5.5: Singleplex PCR result for patient 3 and 4 with colony and plasmid samples- Trial 1

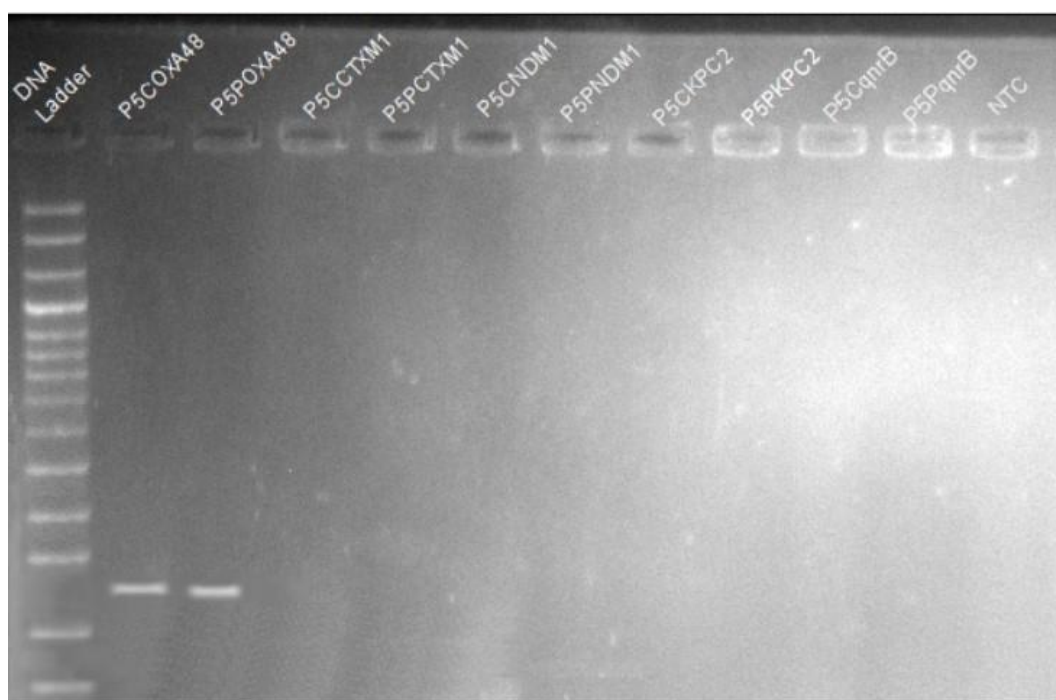


Fig 5.6: Singleplex PCR result for patient 5 with colony and plasmid samples- Trial 1

The above agarose gel pictures (fig:5.4,5.5 and 5.6) display the results for the singleplex PCR with all five primer sets. Templates taken involved both colony sample and plasmid DNA. The PCR was run at an annealing temperature of 56.8°C. The labels in the pictures for example P1COXA-48 and P1POXA-48, can be interpreted as where (P1) as in patient 1,, C or P for the type of template taken either colony sample or plasmid DNA, and the gene name comes after, in this case blaOXA-48.

It was discovered using singleplex PCR that Patient 1 contains the blaOXA 48 and blaNDM1 MDR genes. The second patient's sample has the MDR genes blaNDM 1 and blaKPC 2. Amplification of blaNDM 1 MDR gene is the sole gene detected in patient 3 sample. While Patient 5 only carries the blaOXA 48 MDR gene, Patient 4's sample also contains the blaKPC2 and blaOXA 48 genes.

As was seen from several trial runs, the genes qnrB and blaCTXM-1 was absent in all patients. And also plasmid DNA and colony as templates gave similar results indicating both are potent as template. Hence the duplicate and triplicate of the experiment was run with only colony as the template.

5.2.2.2 Singleplex PCR Trial-2

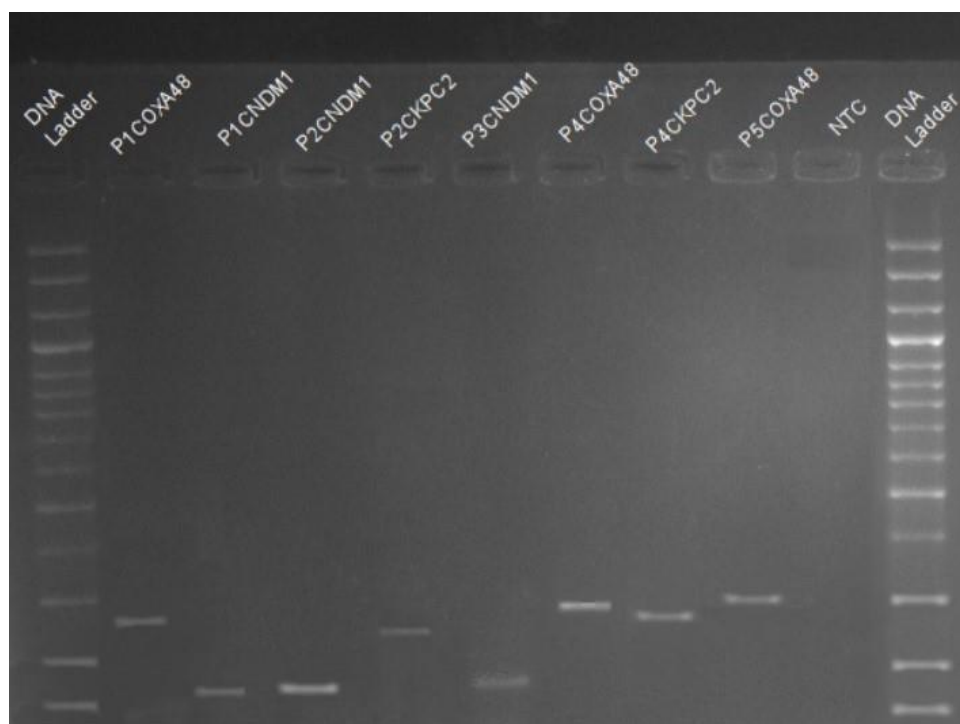


Fig 5.7: Singleplex PCR result for all 5 patients with colony and plasmid samples- Trial 2

Figure 5.7 displays the agarose gel image of a singleplex PCR that was run again as a duplicate to validate the singleplex PCR and to corroborate the singleplex results obtained earlier. The outcome of the two experiments were found to be almost identical as shown in the previous figures, which indicates a replicability in the results for the singleplex PCR assay.

5.2.2.3 Analysis of the results

Table 5.1: Intensity of gene blaOXA-48 in singleplex

Patient No.	Sample type	Trial No	Intensity
1	Colony	1	50.491
	Plasmid	1	49.509
	Colony	2	54.559
4	Colony	1	45.205
	Plasmid	1	54.019
	Colony	2	52.504
5	Colony	1	53.647
	Plasmid	1	46.353
	Colony	2	59

Intensity of blaOXA 48 in Singleplex PCR for P1,4,& 5

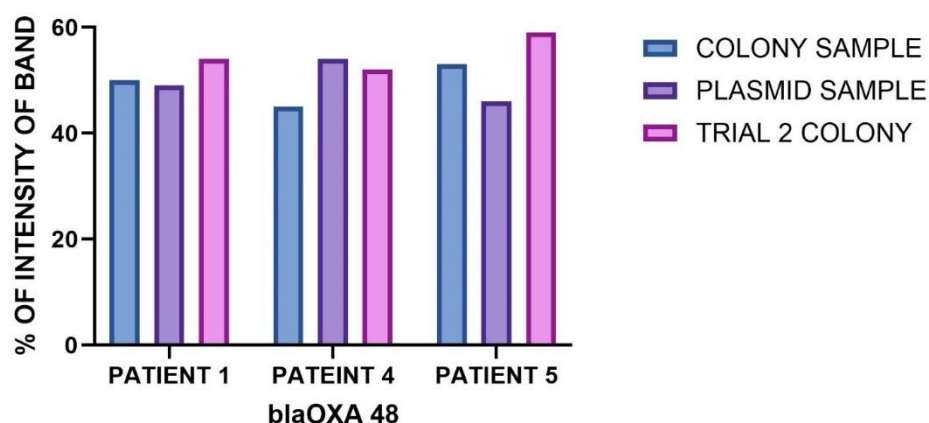


Fig 5.8: Intensity of gene blaOXA-48 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

The intensity of gene expression for blaOXA-48 gene in patient 1, 4, and 5 is shown graphically in fig. 5.8 above. The graph displays the percentage of band intensity for each sample type as well as for the duplicate experiment, Trial 2. The colony and plasmid DNA sample give the almost the same amount of intensity, according to the graph above. The percentage of intensity of two trials was comparable, but in some cases the intensity of gene expression in trial-2 was higher than that of Trial 1 when the same experiment was conducted by using colony sample as the DNA template. The intensity of gene expression for colony and plasmid DNA in trial-1, and colony in trial-2 for patient 1 was found to be 50%, 49%, and 54%, respectively. Similarly for patient 4 it was 45%, 54% and 52% respectively and 53%, 46%, and 59% 5.

Intensity of blaOXA 48 in Singleplex PCR for P1,4,& 5

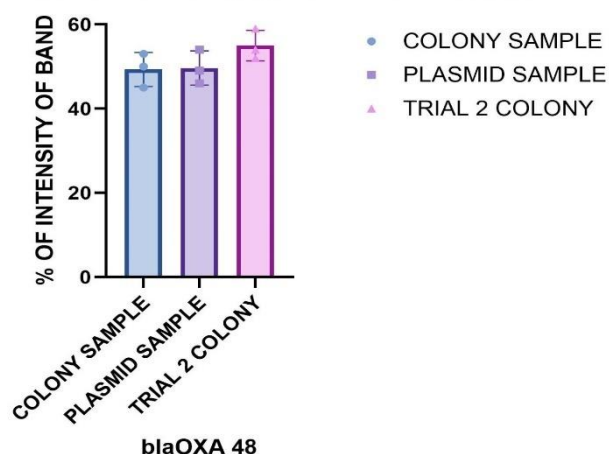


Fig 5.9: Mean Intensity of gene blaOXA-48 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

Figure 5.9 shows a graphical representation of the mean of the blaOXA-48 gene expression among the colony, plasmid sample, and Trial 2. In comparison to the conventional plasmid DNA sample, this indicates that the approach was effective in experiment 2 with the highest gene expression.

Table 5.2: Intensity of gene blaNDM-1 in singleplex

Patient No.	Sample type	Trial No	Intensity
1	Colony	1	51.31
	Plasmid	1	55.69
	Colony	2	47.441
2	Colony	1	52.644
	Plasmid	1	55.356
	Colony	2	54
3	Colony	1	55.088
	Plasmid	1	52.912
	Colony	2	57

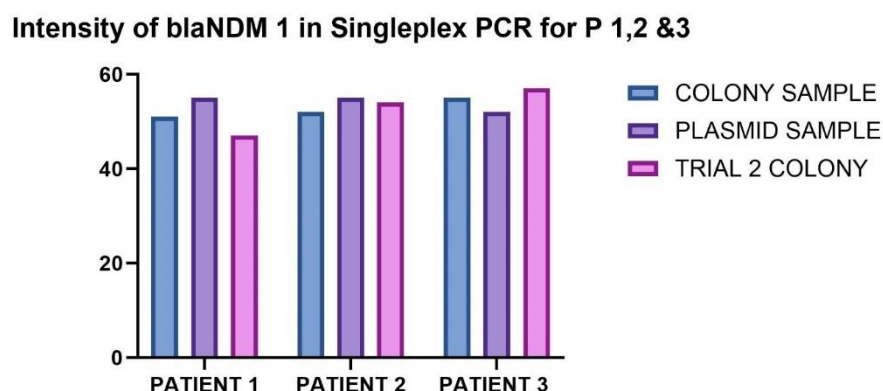


Fig 5.10: Intensity of gene blaNDM-1 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

The percentage of blaNDM 1 MDR gene intensity among patients 1, 2, and 3 in each of their three distinct samples (trial 1 colony sample and plasmid DNA, and trial 2 colony sample) is graphically represented in Fig. 5.10. It can be deduced from the results that, as MDR genes are usually present in plasmid, it is a direct target for the primers, and hence there is a higher gene expression when plasmid is used as template in comparison to colony for PCR, and hence higher intensity of the bands in AGE. However, it was found that the results didn't vary by a large sum, and when a single colony was likewise exposed to the same methodology, the outcomes were comparable, and the range of intensities was also very close. The intensities of the patients 1, 2 & 3 are given in (table 5.5).

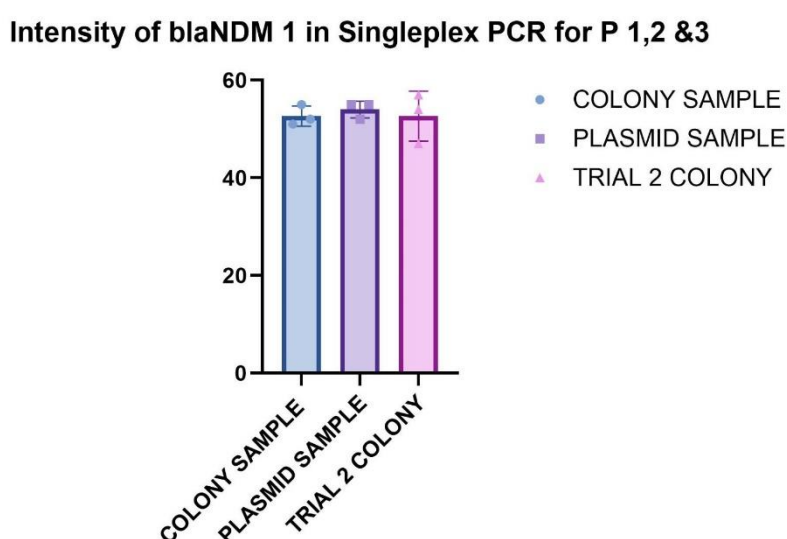


Fig 5.11: Mean Intensity of gene blaNDM-1 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

Figure 5.11 displays the mean intensity of the gene expression for patients 1, 2, and 3. The mean expression of the plasmid DNA gene is somewhat higher than the colony sample in trial 1 and 2.

Table 5.3: Intensity of gene blaKPC-2 in singleplex

Patient No.	Sample type	Trial No	Intensity
2	Colony	1	60.527
	Plasmid	1	66.473
	Colony	2	64
4	Colony	1	46.205
	Plasmid	1	53.795
	Colony	2	47.496

Intensity of blaKPC 2 in Singleplex PCR for P 2 & 4

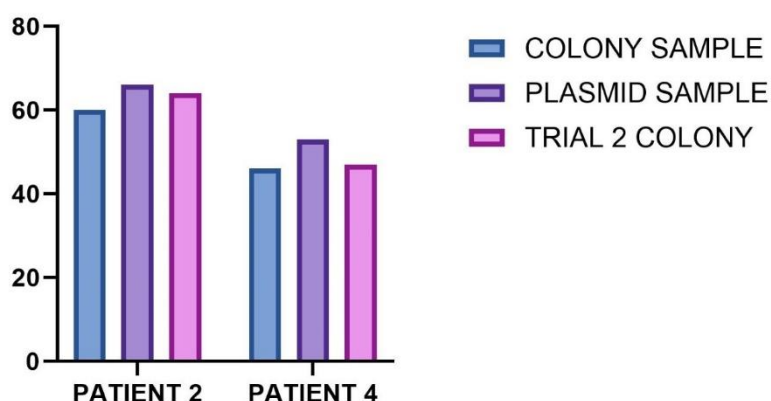


Fig 5.12: Intensity of gene blaKPC-2 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

The intensities of the gene expression for blaKPC 2 in singleplex PCR for the patient 2 & 4 is given in fig.5.12 as a graphical representation. In the first trial the intensity of colony sample seems to be somewhat lesser compared to the plasmid sample. So, in trial 2 the troubleshoots were rectified to make the protocol

as effective as possible. The values of intensity of gene expression have been shown in (table 5.6).

Intensity of blaKPC 2 in Singleplex PCR for P 2 & 4

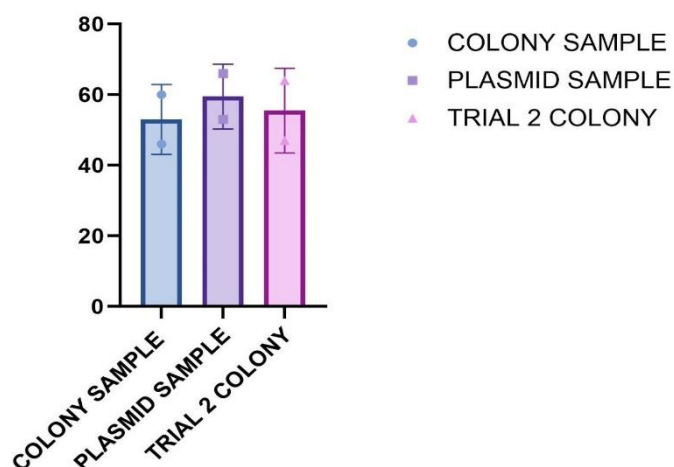


Fig 5.13: Mean Intensity of gene blaKPC-2 in singleplex PCR among patients in trial 1 and 2 with colony and plasmid sample

The mean intensity of the three separate samples of patients 2 and 4 is displayed in fig. 5.13. The plasmid DNA sample had slightly higher gene expression, as shown in the graph, than the colony samples.

% of Intensity of Trial 2 Singleplex PCR for all Patients

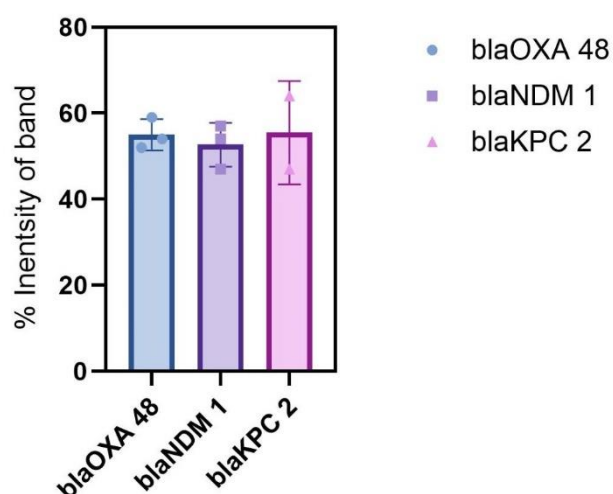


Fig 5.14: Mean Intensity of all genes in singleplex PCR among all patients in trial 2 with colony and plasmid sample

Figure 5.14 displays the mean intensity of the gene expression for all patients for all the genes.

The protocol was made effective in the Trial 2 on a whole. So, the best fit protocol for running the singleplex PCR was formulated to have a total volume of 25 μ l. The contents added to make up the volume includes 12.5 μ l of the PCR master mix, 2 μ l of forward primer, 2 μ l of reverse primer, 2 μ l of template (specific colony dissolved in sterile water) and remaining sterile water to make up the volume of 25 μ l and the PCR was run at an optimum annealing temperature of 56.8°C as obtained from the gradient PCR.

5.2.3 Multiplex PCR

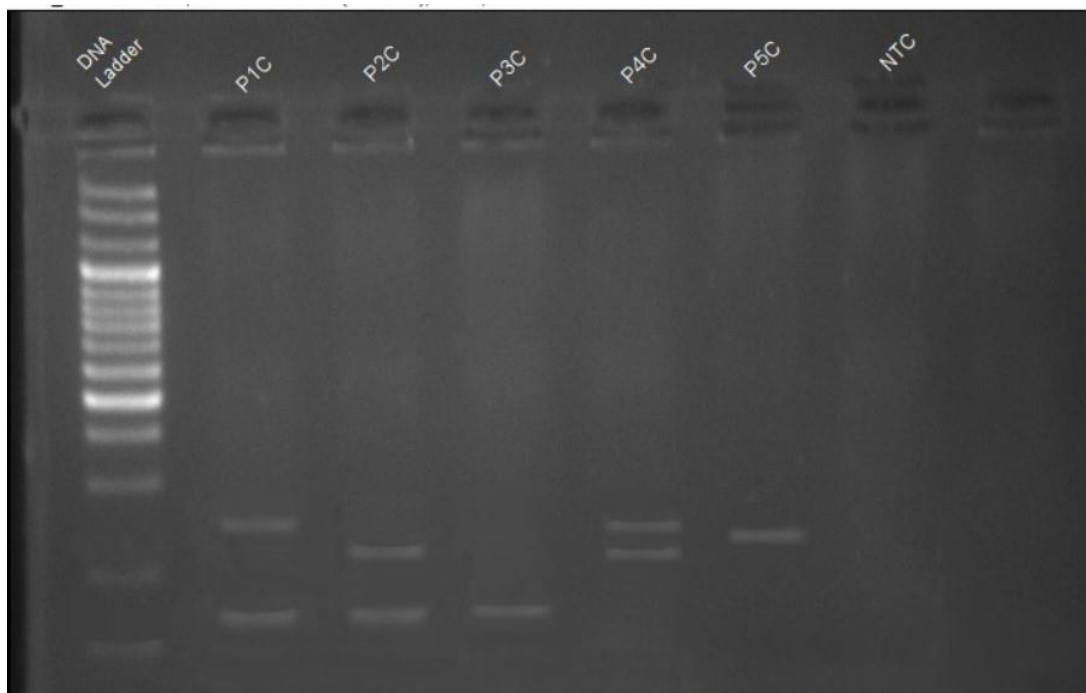


Fig 5.15: Multiplex PCR for all 5 patients with viable primers-Trial 1

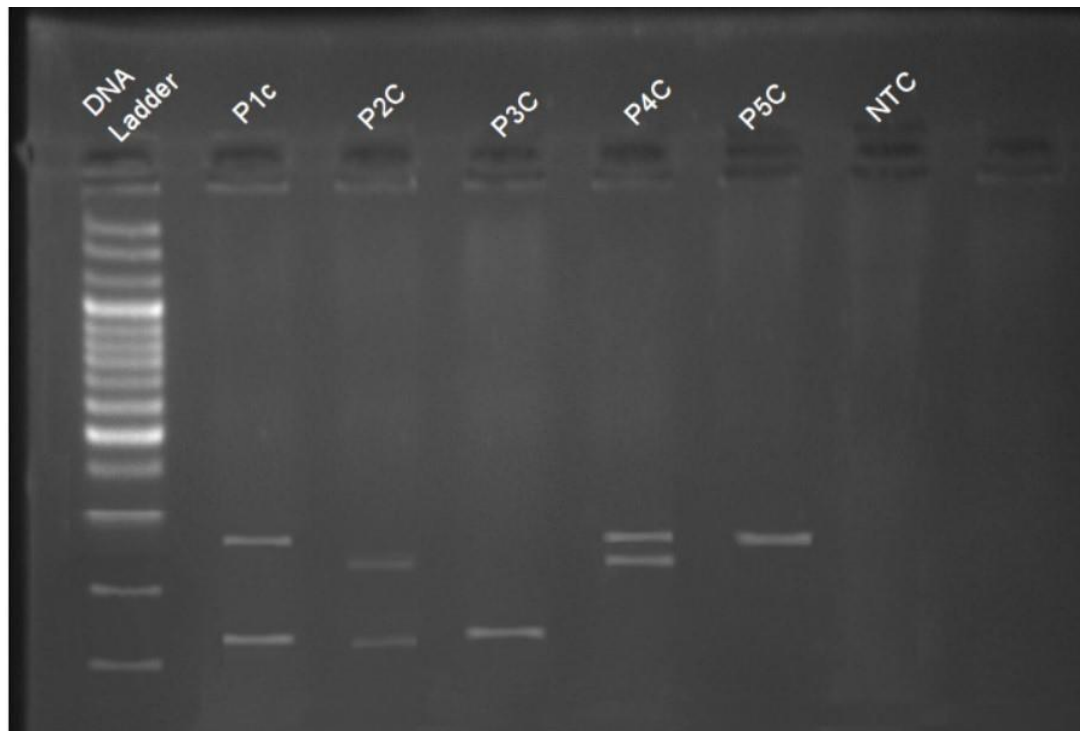


Fig 5.16: Multiplex PCR for all 5 patients with viable primers-Trial 2

In order to identify MDR genes in bacterial pathogens (*E. Coli*) from oral swabs of individuals with oral infections, a multiplex PCR test was attempted. After conducting a number of trials and standardizing the PCR process with gradient and singleplex PCR, multiplex PCR was attempted. A standard methodology was established following several studies that had both favorable and unfavorable outcomes. The outcome of the most successful procedure for finding MDR genes in the clinical isolates is displayed in Fig. 5.16. Patients 1, 2, and 4 exhibit the presence of two MDR genes by producing two different bands, whereas the remaining patients indicate the presence of a single MDR gene by forming a single band. The primers are engineered so that, in the event that several MDR genes are present, the band formation will be different and observable, with a minimum of 50 bp amplicon length difference. The amplicon lengths of blaOXA 48 and blaNDM1, which are present in patient 1, are 277 bp and 139 bp, respectively. Two bands were detected in patient 2 for the genes blaNDM 1 and blaKPC 2, whose amplicon lengths are 250 bp and 139 bp, respectively. Furthermore, patient 4 demonstrated the presence of two MDR genes, blaOXA 48 and blaKPC 2, with amplicon lengths of 277 bp and 250 bp, respectively. Among the five patients, patient 3 and patient 5 carried only one MDR gene which was blaNDM1 and blaOXA 48.

To ensure that the results are accurate or not, the experiment was repeated again. Fig. 5.16 depicts the preceding experiment's replica. The results of Trial 2 further verified the existence of the identical MDR gene as was seen in fig. 5.15. The two tests were verified by looking at the gel with a gel analyzer.

The multiplex PCR protocol involves adding 25 µl of PCR master mix, then primer cocktail (2 µl of forward primer of each of blaOXA 48, blaNDM1 and blaKPC 2 + 2 µl of reverse primer of blaOXA 48, blaNDM 1 and blaKPC 2) + 4 µl of DNA template (individual colony dissolved in sterile water), and finally filling the vial to 50 µl with sterile water.

Table 5.4: Intensity of genes in trial 1 and 2 of multiplex

Patient No.	Gene	Trial	Intensity
1	blaOXA-48	1	12.6633
		2	13.484
	blaNDM-1	1	14.95
		2	15.542
2	blaNDM-1	1	13.227
		2	11.603
	blaKPC-2	1	17.563
		2	15.568
3	blaNDM-1	1	13.368
		2	15.864
4	blaOXA-48	1	9.267
		2	12.034
	blaKPC-2	1	8.821
		2	11.379
5	blaOXA-48	1	9.319
		2	10.348

% Intensity of blaOXA 48 and blaNDM 1 in Multiplex PCR of Patient 1

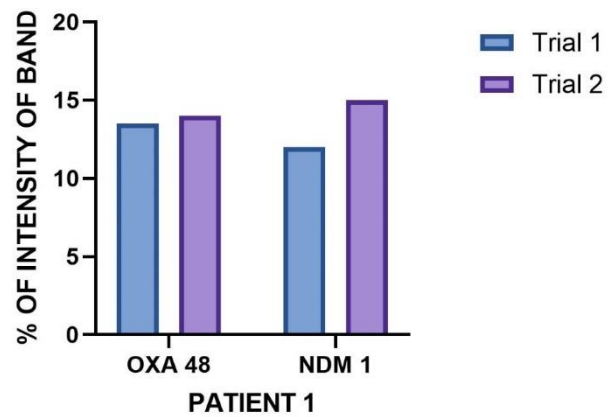


Fig 5.17: Intensity of blaOXA-48 and blaNDM-1 for patient 1 in multiplex PCR in trial 1 and 2

% Intensity of blaNDM 1 and blaKPC 2 in Multiplex PCR in Patient 2

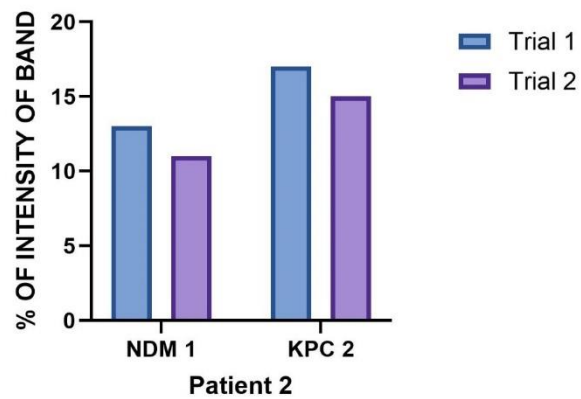


Fig 5.18: Intensity of blaNDM-1 and blaKPC-2 for patient 2 in multiplex PCR trial 1 and 2

% Intensity of blaNDM 1 in Multiplex PCR for Patient 3

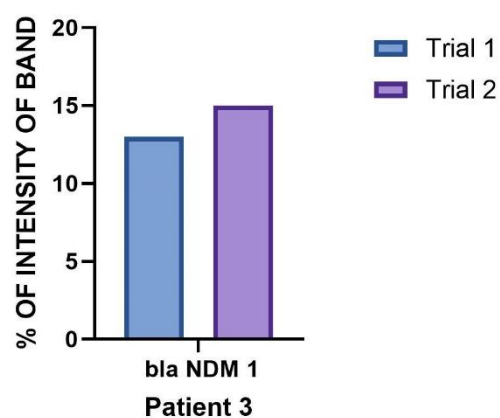


Fig 5.19: Intensity of blaNDM-1 for patient 3 in multiplex PCR trial 1 and 2

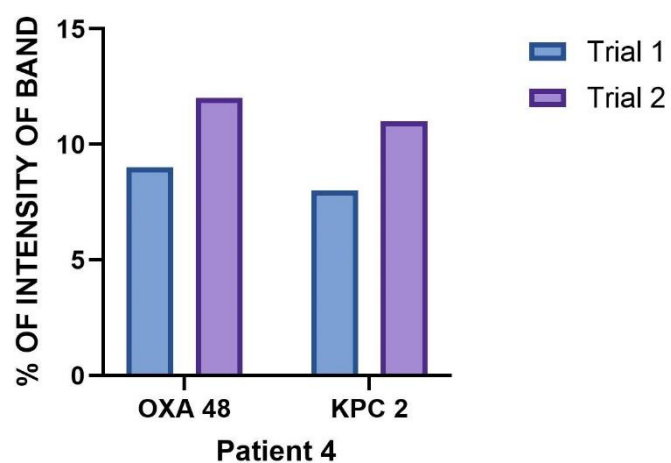


Fig 5.20: Intensity of blaOXA-48 and blaKPC-2 for patient 4 in multiplex PCR trial 1 and 2

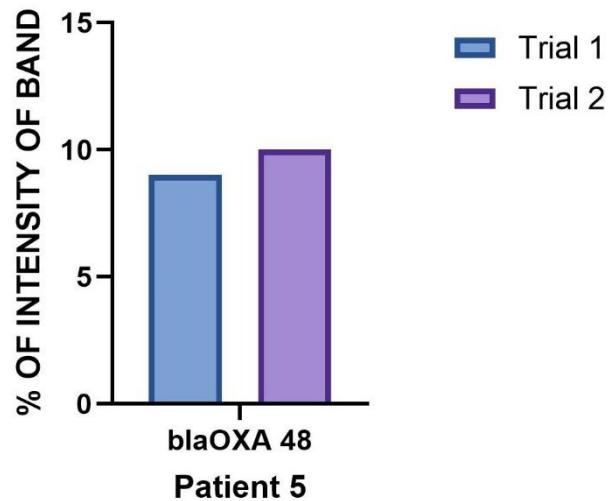


Fig 5.21: Intensity of blaOXA-48 for patient 5 in multiplex PCR trial 1 and 2

The graphs above show the percentage of band intensity in the gel picture as seen through a gel image analyzer. With the use of gel image analysis tools, imageJ, the bands were quantified. The gene expression or DNA amplification in PCR is correlated with the intensity of the bands. The intensity of gene expression in patients 1 through 5 in trial 1 is compared with the intensity in the Trial 2 experiment as shown in Figures 5.17 to 5.21. Following trial and error, it was concluded that trial 2 is more precise and transparent sampling approach yields the higher intensities. Table 5.7 contains the intensities of the bands in trial 1 and 2.

It may be inferred from the aforementioned experiment and research that, in addition to the traditional technique of employing plasmid DNA as the DNA template for multiplex development, particular colonies can also be utilised as the template, and this method has been shown to be effective. This study attempts to cut down on the amount of time needed to isolate the plasmid DNA, which necessitates the use of an additional technique, by using a colony sample as the temple. Duplicates of the same process have been used to validate the developed test. The characteristics of the bandwidth and the peaks in the intensities have also been determined using the image analysis programme.

It can be inferred from the above combination that patient 1 with blaOXA 48 is resistant to medicines that contain carbapenem and belong to the penicillin family of medications. blaNDM 1 belongs to the drug classes of sulphamethoxazole,

macrolides, and aminoglycosides. Aztreonam discs, ticarcillin/tazobactam, and cefepime are the drug classes to which blaKPC 2 is resistant.

In these situations, doctors may administer drugs from the fluoroquinolone, bedaquiline, and linezolid groups to the patients.

5.3 DISCUSSION

MDR genes in bacterial strains. In this study, a strategic decision was made to prioritize colony samples over plasmids as templates for PCR, despite the widespread use of plasmids in previous research studies due to their direct linkage with MDR genes (Patil *et al.*, 2019; Abraham *et al.*, 2018; Yuan *et al.*, 2021). The rationale behind this decision stemmed from the time-intensive nature of plasmid isolation procedures. Given the study's objective to streamline the MDR gene detection process, colony samples emerged as a more practical and time-efficient alternative. Remarkably, the choice of colony templates yielded results comparable to those obtained using plasmid templates, as evidenced by numerous trials. This convergence in outcomes between colony and plasmid templates highlights the efficacy of colony-based PCR for MDR gene detection. The findings from this study are further corroborated by similar research conducted by (Salem *et al.*, 2024; Normura *et al.*, 2018), which also employed colony-based approaches and reported favorable outcomes.

By leveraging colony samples as templates, this study not only expedites the MDR gene detection process but also underscores the viability and potency of colony-based PCR methodologies. These findings contribute significantly to the optimization of MDR gene detection protocols, offering a promising avenue for accelerating research and clinical interventions aimed at combating antibiotic resistance.

The study leverages technical expertise to address the pressing issue of high pathogen prevalence leading to multidrug resistance. Through a comprehensive review of pertinent literature, the problem statement was meticulously scrutinized, providing a solid foundation for the research endeavor. Employing multiplex PCR as a primary method, the study aimed to detect MDR genes effectively to ensure accuracy and reliability in assessing the results, advanced software tools such as ImageJ and BIORAD gel analyzer were employed. These tools facilitated the qualitative and quantitative analysis of the obtained data, enhancing the precision

of the findings. Also, the initiative of the study also aligns with the principles of United Nations Sustainable Development Goal 3, which advocates for universal access to healthcare and the prevention and treatment of diseases.

CHAPTER 6

SUMMARY AND CONCLUSION

The primary aim of this experiment was to develop a multiplex PCR assay tailored for the detection of MDR genes in clinical isolates. Among the MDR genes under investigation, namely blaOXA-48, blaNDM-1, and blaKPC-2, prevalent occurrences were observed. Notably, among the five patients sampled, three were found to harbor blaOXA-48 and blaNDM-1, respectively, while two exhibited the presence of blaKPC-2. Conversely, genes qnrB and blaCTXM-1 were absent across all patient samples. The results, coupled with visual observations, strongly support the conclusion that utilizing specific colonies as DNA templates for PCR yields comparable outcomes to employing plasmid DNA. Traditionally, researchers have favored plasmid DNA or genomic DNA as templates for MDR gene detection due to their direct association with these genes.

However, the research findings demonstrate that colony PCR can be equally effective. By opting for specific colonies as DNA templates, the time-consuming step of isolating plasmid or genomic DNA can be circumvented. Comparative analysis between the two sample types—plasmid DNA and colony PCR—revealed similar levels of gene expression intensity, underscoring the reliability of colony PCR as a viable alternative. Furthermore, the prevalence of *E. coli* among the 20 isolates was determined to be 40%, with blaOXA-48, blaNDM-1, and blaKPC-2 occurring at rates of 15%, 15%, and 10%, respectively, as MDR genes. Overall, these findings represent a significant advancement in the field, particularly in terms of expediting the detection of MDR genes. By streamlining the PCR process through the utilization of colony PCR, our study has demonstrated promising outcomes in reducing the time required for MDR gene detection, thus contributing to more efficient clinical and research practices.

REFERENCES

- Abbasi, E., Mondanizadeh, M., Belkum, A. van, & Ghaznavi-Rad, E. Multi-drug-resistant diarrheagenic escherichia coli pathotypes in pediatric patients with gastroenteritis from central Iran. *Infection and Drug Resistance*, 13, 2020,1387–1396.
- Abraham, S., Kirkwood, R. N., Laird, T., Saputra, S., Mitchell, T., Singh, M., Linn, B., Abraham, R. J., Pang, S., Gordon, D. M., Trott, D. J., & O'Dea, M. Dissemination and persistence of extended-spectrum cephalosporin-resistance encoding IncI1-bla CTXM-1 plasmid among Escherichia coli in pigs. *ISME Journal*, 12(10), 2018, 2352–2362.
- Abrar, S., Ain, N. U., Liaqat, H., Hussain, S., Rasheed, F., & Riaz, S. Distribution of bla CTX-M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum- β -lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. *Antimicrobial Resistance and Infection Control*, 8(1), 2019.
- Ahmadian, L., Haghshenas, M. R., Mirzaei, B., Bazgir, Z. N., & Goli, H. R. Distribution and molecular characterization of resistance gene cassettes containing class 1 integrons in multi-drug resistant (Mdr) clinical isolates of pseudomonas aeruginosa. *Infection and Drug Resistance*, 13, 2020, 2773–2781.
- Aires-De-Sousa, M., De La Rosa, J. M. O., Gonçalves, M. L., Pereira, A. L., Nordmann, P., & Poirel, L. Epidemiology of carbapenemase-producing Klebsiella pneumoniae in a hospital, Portugal. *Emerging Infectious Diseases*, 25(9), 2019, 1632–1638.
- Al Azad, M. A. R., Rahman, M. M., Amin, R., Begum, M. I. A., Fries, R., Husna, A., Khairalla, A. S., Badruzzaman, A. T. M., El Zowalaty, M. E., Lampang, K. N., Ashour, H. M., & Hafez, H. M. Susceptibility and multidrug resistance patterns of Escherichia coli isolated from cloacal swabs of live broiler chickens in Bangladesh. *Pathogens*, 8(3), 2019, 1–9.
- Algammal, A. M., Eidaroos, N. H., Alfifi, K. J., Alatawy, M., Al-Harbi, A. I., Alanazi, Y. F., Ghobashy, M. O. I., Khafagy, A. R., Esawy, A. M., El-Sadda, S. S., Hetta, H. F., & El-Tarabili, R. M. (2023). oprL Gene Sequencing, Resistance Patterns,

Virulence Genes, Quorum Sensing and Antibiotic Resistance Genes of XDR *Pseudomonas aeruginosa* Isolated from Broiler Chickens. *Infection and Drug Resistance*, 16, 853–867.

Algammal, A. M., Mabrok, M., Sivaramasamy, E., Youssef, F. M., Atwa, M. H., El-kholy, A. W., Hetta, H. F., & Hozzein, W. N. Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor oprL and toxA virulence genes and bla TEM, bla CTX-M, and tetA antibiotic-resistance genes. *Scientific Reports*, 10(1), 2020, 1–12.

Ali, A., Ahmad, K., Rahat, S., & Ahmad, I. Genetic diversity and molecular analysis of metallo beta lactamases among imipenem resistant clinical isolates of *pseudomonas aeruginosa* from Peshawar, Pakistan. *Pakistan Journal of Medical Sciences*, 37(7), 2021, 1865–1870.

Amanati, A., Sajedianfard, S., Khajeh, S., Ghasempour, S., Mehrangiz, S., Nematollahi, S., & Shahhosein, Z. Bloodstream infections in adult patients with malignancy, epidemiology, microbiology, and risk factors associated with mortality and multi-drug resistance. *BMC Infectious Diseases*, 21(1), 2021.

Ballén, V., Gabasa, Y., Ratia, C., Ortega, R., Tejero, M., & Soto, S. Antibiotic Resistance and Virulence Profiles of *Klebsiella pneumoniae* Strains Isolated From Different Clinical Sources. *Frontiers in Cellular and Infection Microbiology*, 11, 2021.

Bandy, A., Wani, F. A., Mohammed, A. H., Dar, U. F., Mallick, A., Dar, M. R., & Tantry, B. A. Bacteriological profile of wound infections and antimicrobial resistance in selected gram-negative bacteria. *African Health Sciences*, 22(4), 2022, 576–586.

Besharati, S., Sadeghi, A., Ahmadi, F., Tajeddin, E., Mohammad Salehi, R., Fani, F., Pouladfar, G., Nikmanesh, B., Majidpour, A., Soleymanzadeh Moghadam, S., Mirab Samiee, S., Rahnamaye Farzami, M., Rahbar, M., Eslami, P., Rakhshani, N., Eshrati, B., Gouya, M. M., Fallah, F., Karimi, A., ... Alebouyeh, M. Serogroups, and drug resistance of nontyphoidal *Salmonella* in symptomatic patients with community-acquired diarrhea and chicken meat samples in Tehran. *Iranian Journal of Veterinary Research*, 21(4), 2020, 269–278.

Bhattacharya, S., Junghare, V., Pandey, N. K., Baidya, S., Agarwal, H., Das, N., Banerjee, A., Ghosh, D., Roy, P., Patra, H. K., & Hazra, S. Variations in the SDN Loop of Class A Beta-Lactamases: A Study of the Molecular Mechanism of BlaC (*Mycobacterium tuberculosis*) to Alter the Stability and Catalytic Activity Towards Antibiotic Resistance of MBIs. *Frontiers in Microbiology*, 12, 2021.

Bradford, P. A. Extended-spectrum β -lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. In *Clinical Microbiology Reviews*, 14 (4), 2001, 933–951.

Bush, K. (2018). Past and present perspectives on β -lactamases. In *Antimicrobial Agents and Chemotherapy* (Vol. 62, Issue 10). American Society for Microbiology.

Bush, K., & Bradford, P. A. Epidemiology of β -lactamase-producing pathogens. In *Clinical Microbiology Reviews*, American Society for Microbiology, 33(2), 2020.

Cao, Y., Shen, Y., Cheng, L., Zhang, X., Wang, C., Wang, Y., Zhou, X., Chao, G., & Wu, Y. Combination of multilocus sequence typing and pulsed-field gel electrophoresis reveals an association of molecular clonality with the emergence of extensive-drug resistance (XDR) in *Salmonella*. *Microbiological Research*, 207(November 2017), 2018, 170–176.

Carrasco-Anabalón, S., Vera-Leiva, A., Quezada-Aguiluz, M., Morales-Rivera, M. F., Lima, C. A., Fernández, J., Ulloa, S., Domínguez, M., González-Rocha, G., & Bello-Toledo, H. Genetic platforms of blaCTX-Min carbapenemase-producing strains of *K. pneumoniae* isolated in Chile. *Frontiers in Microbiology*, 9(MAR), 2018, 1–8.

Chen, H., Li, N., Wang, F., Wang, L., & Liang, W. Carbapenem antibiotic stress increases blaKPC-2 gene relative copy number and bacterial resistance levels of *Klebsiella pneumoniae*. *Journal of Clinical Laboratory Analysis*, 36(7), 2022.

Chinemerem Nwobodo, D., Ugwu, M. C., Oliseloke Anie, C., Al-Ouqaili, M. T. S., Chinedu Ikem, J., Victor Chigozie, U., & Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. In *Journal of Clinical Laboratory Analysis* (Vol. 36, Issue 9). John Wiley and Sons Inc.

Chowdhury, G., Ramamurthy, T., Das, B., Ghosh, D., Okamoto, K., Miyoshi, S. I., Dutta, S., & Mukhopadhyay, A. K. (2022). Characterization of NDM-5 Carbapenemase-Encoding Gene (blaNDM-5) – Positive Multidrug Resistant Commensal *Escherichia coli* from Diarrheal Patients. *Infection and Drug Resistance*, 15, 3631–3642.

Cummins, M. L., Sanderson-Smith, M., Newton, P., Carlile, N., Phalen, D. N., Maute, K., Monahan, L. G., Hoyer, B. J., & Djordjevic, S. P. (2020). Whole-Genome Sequence Analysis of an Extensively Drug-Resistant *Salmonella enterica* Seroovar Agona Isolate from an Australian Silver Gull (*Chroicocephalus novaehollandiae*) Reveals the Acquisition of Multidrug Resistance Plasmids. *MSphere*, 5(6).

Dai, W., Zhang, Y., Zhang, J., Xue, C., Yan, J., Li, X., Zheng, X., Dong, R., Bai, J., Su, Y., Xie, P., Zhong, W., Zhang, H., Yan, Z., Zhong, W., & Song, Y. Analysis of antibiotic-induced drug resistance of *Salmonella enteritidis* and its biofilm formation mechanism. *Bioengineered*, 12(2), 2021, 10254–10263.

Dandachi, I., Sokhn, E. S., Dahdouh, E. A., Azar, E., El-Bazzal, B., Rolain, J. M., & Daoud, Z. Prevalence and characterization of multi-drug-resistant gram-negative bacilli isolated from Lebanese poultry: A nationwide study. *Frontiers in Microbiology*, 9(MAR), 2018, 1–11.

Davies, J. (1996). Origins and evolution of antibiotic resistance. In *Microbiología (Madrid, Spain)*, 12 (1), 1996, 9–16.

de Kraker, M. E. A., Stewardson, A. J., & Harbarth, S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? *PLoS Medicine*, 13(11), 2016.

Ding, Y., Wang, H., Pu, S., Huang, S., & Niu, S. Resistance trends of *Klebsiella pneumoniae* causing urinary tract infections in Chongqing, 2011–2019. *Infection and Drug Resistance*, 14, 2021, 475–481.

Fonseca, É. L., Caldart, R. V., Freitas, F. S., Morgado, S. M., Rocha, L. T., dos Santos, R. C., & Vicente, A. C. P. Emergence of extensively drug-resistant international clone IC-6 *Acinetobacter baumannii* carrying blaOXA-72 and

blaCTX-M-115 in the Brazilian Amazon region. *Journal of Global Antimicrobial Resistance*, 20, 2020, 18–21.

Gama, J. A., Kloos, J., Johnsen, P. J., & Samuelsen, Ø. Host dependent maintenance of a bla NDM-1-encoding plasmid in clinical *Escherichia coli* isolates. *Scientific Reports*, 10(1), 2020, 1–7.

Gan, H. M., Eng, W. W. H., & Dhanoa, A. First genomic insights into carbapenem-resistant *Klebsiella pneumoniae* from Malaysia. *Journal of Global Antimicrobial Resistance*, 20, 2020, 153–159.

Gao, Y., Wen, J., Wang, S., Xu, X., Zhan, Z., Chen, Z., Bai, J., Qu, X., Zhang, H., Zhang, J., & Liao, M. Plasmid-encoded bla_{NDM-5} gene that confers high-level carbapenem resistance in *Salmonella typhimurium* of pork origin. *Infection and Drug Resistance*, 13, 2020, 1485–1490.

García-González, N., Beamud, B., Fuster, B., Giner, S., Domínguez, M. V., Sánchez, A., Sevilla, J., Coque, T. M., Gimeno, C., González-Candelas, F., García-González, N., González-Candelas, F., Fuster, B., Tormo, N., Salvador, C., Gimeno, C., Domínguez, V., Giner, S., Colomina, J., ... Sánchez-Hellín, V. Tracking the Emergence and Dissemination of a bla NDM-23 Gene in a Multidrug Resistance Plasmid of *Klebsiella pneumoniae*. *Microbiology Spectrum*, 11(2), 2023.

Gene, K.-, & Number, C. *crossm Ceftazidime-Avibactam Resistance Associated with Increased Derivatives in Sequence Type 258 Klebsiella pneumoniae*. January, 2020, 1–8.

Ghasemian, A., Rizi, K. S., Vardanjani, H. R., & Nojoomi, F. Prevalence of clinically isolated metallo-beta-lactamase-producing *Pseudomonas aeruginosa*, coding genes, and possible risk factors in Iran. *Iranian Journal of Pathology*, 13(1), 2018, 1–9.

Gomi, R., Yamamoto, M., Tanaka, M., & Matsumura, Y. Chromosomal integration of blaCTX-M genes in diverse *Escherichia coli* isolates recovered from river water in Japan. *Current Research in Microbial Sciences*, 3, 2022.

Gong X., Zhang, J., Su, S., Fu, Y., Bao, M., Wang, Y., & Zhang, X. Molecular characterization and epidemiology of carbapenem non-susceptible Enterobacteriaceae isolated from the Eastern region of Heilongjiang Province, China. *BMC Infectious Diseases*, 18(1), 2018, 1–10.

Guyomard-Rabenirina, S., Reynaud, Y., Pot, M., Albina, E., Couvin, D., Ducat, C., Gruel, G., Ferdinand, S., Legreneur, P., Le Hello, S., Malpote, E., Sadikalay, S., Talarmin, A., & Breurec, S. Antimicrobial Resistance in Wildlife in Guadeloupe (French West Indies): Distribution of a Single blaCTX-M-1/IncI1/ST3 Plasmid Among Humans and Wild Animals. *Frontiers in Microbiology*, 11(July), 2020, 1–11.

Hala S., Antony, C. P., Alshehri, M., Althaqafi, A. O., Alsaedi, A., Mufti, A., Kaaki, M., Alhaj-Hussein, B. T., Zowawi, H. M., Al-Amri, A., & Pain, A. First report of Klebsiella quasipneumoniae harboring bla KPC-2 in Saudi Arabia. *Antimicrobial Resistance and Infection Control*, 8(1), 2019, 1–8.

Halat D. H., & Moubareck, C. A. The Intriguing Carbapenemases of Pseudomonas aeruginosa: Current Status, Genetic Profile, and Global Epidemiology. In *Yale Journal of Biology and Medicine*, 95, 2022.

Hamza D., Dorgham, S., Ismael, E., El-Moez, S. I. A., Elhariri, M., Elhelw, R., & Hamza, E. Emergence of β -lactamase- And carbapenemase-producing Enterobacteriaceae at integrated fish farms. *Antimicrobial Resistance and Infection Control*, 9(1), 2020, 1–12.

Harboring M., Gene, O.-C., Desmet, S., Dijn, M. Van, Ranst, M. Van, Chlebowicz, M. A., & Rossen, J. W. (2018). *crosssm Antibiotic Resistance Plasmids Cointegrated into a*. 1–6.

Hassuna N. A., Darwish, M. K., Sayed, M., & Ibrahim, R. A. Molecular epidemiology and mechanisms of high-level resistance to meropenem and imipenem in pseudomonas aeruginosa. *Infection and Drug Resistance*, 13, 2020, 285–293.

Heinz E., Ejaz, H., Bartholdson Scott, J., Wang, N., Gujran, S., Pickard, D., Wilksch, J., Cao, H., Haq, I. ul, Dougan, G., & Strugnell, R. A. Resistance

mechanisms and population structure of highly drug resistant *Klebsiella* in Pakistan during the introduction of the carbapenemase NDM-1. *Scientific Reports*, 9(1), 2019, 1–13.

Hubbard A. T. M., Mason, J., Roberts, P., Parry, C. M., Corless, C., van Aartsen, J., Howard, A., Bulgacim, I., Fraser, A. J., Adams, E. R., Roberts, A. P., & Edwards, T. Piperacillin/tazobactam resistance in a clinical isolate of *Escherichia coli* due to IS26-mediated amplification of bla TEM-1B. *Nature Communications*, 11(1), 2020, 1–9.

Ikuta K. S., Swetschinski, L. R., Robles Aguilar, G., Sharara, F., Mestrovic, T., Gray, A. P., Davis Weaver, N., Wool, E. E., Han, C., Gershberg Hayoon, A., Aali, A., Abate, S. M., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abd-Elsalam, S., Abebe, G., Abedi, A., Abhari, A. P., Abidi, H., ... Naghavi, M. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, 400(10369), 2022, 2221–2248.

Irfan S., Hasan, Z., Qamar, F., Ghanchi, N., Ashraf, J., Kanji, A., Razzak, S., Greig, D., Nair, S., & Hasan, R. Ceftriaxone resistant *Salmonella enterica* serovar Paratyphi A identified in a case of enteric fever: first case report from Pakistan. *BMC Infectious Diseases*, 23(1), 2023.

Jacopin E., Lehtinen, S., Débarre, F., & Blanquart, F. Factors favouring the evolution of multidrug resistance in bacteria. *Journal of the Royal Society Interface*, 17(168), 2020.

Jassim M. Z., Obeid, M. F., & Neama, S. Integron-Associated Antibiotic Resistance in *Salmonella typhi*. *Archives of Razi Institute*, 77(2), 2022, 771–777.

Jiang L., Yu, Y., Zeng, W., Guo, J., Lv, F., Wang, X., Liu, X., & Zhao, Z. (2020). Whole-genome analysis of New Delhi Metallo-Beta-Lactamase-1-producing *Acinetobacter baumannii* from China. *Journal of Global Antimicrobial Resistance*, 20, 2020,

Jo J. H., Harkins, C. P., Schwardt, N. H., Portillo, J. A., Zimmerman, M. D., Carter, C. L., Hossen, M. A., Peer, C. J., Polley, E. C., Dartois, V., Figg, W. D.,

- Moutsopoulos, N. M., Segre, J. A., & Kong, H. H. Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics. *Science Translational Medicine*, 13(625), 2021.
- Kaderabkova N., Bharathwaj, M., Furniss, R. C. D., Gonzalez, D., Palmer, T., & Mavridou, D. A. I. (2022). The biogenesis of β -lactamase enzymes. In *Microbiology (United Kingdom)*, Microbiology Society, 168 (8), 2022.
- Khashei R., Sarvestani, F. E., Malekzadegan, Y., & Motamedifar, M. The first report of *Enterobacter gergoviae* carrying blaNDM-1 in Iran. *Iranian Journal of Basic Medical Sciences*, 23(9), 2020, 1184–1190.
- Kim B., Kim, J. H., & Lee, Y. (2021). Virulence factors associated with *Escherichia coli* bacteremia and urinary tract infection. *Annals of Laboratory Medicine*, 42(2), 2021, 203–212.
- Kumari M., Verma, S., Venkatesh, V., Gupta, P., Tripathi, P., Agarwal, A., Siddiqui, S. S., Arshad, Z., & Prakash, V. (2021). Emergence of blaNDM-1 and blaVIM producing Gram-negative bacilli in ventilator-associated pneumonia at AMR Surveillance Regional Reference Laboratory in India. *PLoS ONE*, 16(9 September).
- Kuo S. C., Huang, W. C., Huang, T. W., Wang, H. Y., Lai, J. F., Chen, T. L., & Lauderdale, T. L. (2018). Molecular epidemiology of emerging bla OXA-23-Like - and bla OXA-24-Like -carrying *acinetobacter baumannii* in Taiwan. *Antimicrobial Agents and Chemotherapy*, 62(3), 1–9.
- Laborda P., Martínez, J. L., & Hernando-Amado, S. Evolution of Habitat-Dependent Antibiotic Resistance in *Pseudomonas aeruginosa*. *Microbiology Spectrum*, 10(4), 2022.
- Li J., Zhang, H., Ning, J., Sajid, A., Cheng, G., Yuan, Z., & Hao, H. The nature and epidemiology of OqxAB, a multidrug efflux pump. *Antimicrobial Resistance and Infection Control*, 8(1), 2019, 1–13.
- Liao Y., Gong, J., Yuan, X., Lu, H., & Jiang, L. Drug Resistance Genes and Molecular Epidemiological Characteristics of Carbapenem-Resistant *Klebsiella pneumoniae*. *Infection and Drug Resistance*, 16, 2023, 1511–1519.

- Liu M., Ma, J., Jia, W., & Li, W. Antimicrobial Resistance and Molecular Characterization of Gene Cassettes from Class 1 Integrons in *Pseudomonas aeruginosa* Strains. *Microbial Drug Resistance*, 26(6), 2020, 670–676.
- Liu S., Fang, R., Zhang, Y., Chen, L., Huang, N., Yu, K., Zhou, C., Cao, J., & Zhou, T. Characterization of resistance mechanisms of *Enterobacter cloacae* Complex co-resistant to carbapenem and colistin. *BMC Microbiology*, 21(1), 2021.
- Livermore D. M. Lactamases in Laboratory and Clinical Resistance. In *Clinical Microbiology Reviews*, 8(4), 1995.
- Manandhar S., Zellweger, R. M., Maharjan, N., Dongol, S., Prajapati, K. G., Thwaites, G., Basnyat, B., Dixit, S. M., Baker, S., & Karkey, A. A high prevalence of multi-drug resistant Gram-negative bacilli in a Nepali tertiary care hospital and associated widespread distribution of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-encoding genes. *Annals of Clinical Microbiology and Antimicrobials*, 19(1), 2020.
- Mbelle N. M., Feldman, C., Sekyere, J. O., Maningi, N. E., Modipane, L., & Essack, S. Y. Pathogenomics and Evolutionary Epidemiology of Multi-Drug Resistant Clinical *Klebsiella pneumoniae* Isolated from Pretoria, South Africa. *Scientific Reports*, 10(1), 2020, 1–17.
- Mitra S., Mukherjee, S., Naha, S., Chattopadhyay, P., Dutta, S., & Basu, S. Evaluation of co-transfer of plasmid-mediated fluoroquinolone resistance genes and bla NDM gene in Enterobacteriaceae causing neonatal septicemia. *Antimicrobial Resistance and Infection Control*, 8(1), 2019, 1–15.
- Moon D. C., Mechesso, A. F., Kang, H. Y., Kim, S. J., Choi, J. H., Song, H. J., Yoon, S. S., & Lim, S. K. Imipenem resistance mediated by bla_{oxa}-913 gene in *pseudomonas aeruginosa*. *Antibiotics*, 10(10), 2021.
- Murray C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., ... Naghavi, M. Global burden of

bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325), 2022, 629–655.

Murugan N., Malathi, J., Therese, K. L., & Madhavan, H. N. R. Application of six multiplex PCR's among 200 clinical isolates of *Pseudomonas aeruginosa* for the detection of 20 drug resistance encoding genes. *Kaohsiung Journal of Medical Sciences*, 34(2), 2018, 79–88.

Naziri Z., Derakhshandeh, A., Borchaloei, A. S., Poormaleknia, M., & Azimzadeh, N. Treatment failure in urinary tract infections: A warning witness for virulent multi-drug resistant ESBL-producing *Escherichia coli*. *Infection and Drug Resistance*, 13, 2020, 1839–1850.

Nogbou N. D., Phofa, D. T., Nchabeleng, M., & Musyoki, A. M. (2021). Investigating multi-drug resistant *Acinetobacter baumannii* isolates at a tertiary hospital in Pretoria, South Africa. *Indian Journal of Medical Microbiology*, 39(2), 2021, 218–223.

Nomura T, Hashimoto Y, Kurushima J, Hirakawa H, Tanimoto K, Zheng B, Ruan G, Xue F, Liu J, Hisatsune J, Sugai M. New colony multiplex PCR assays for the detection and discrimination of vancomycin-resistant enterococcal species. *Journal of Microbiological Methods*. 145, 2018, 69-72.

Ortega-Paredes D., de Janon, S., Villavicencio, F., Ruales, K. J., De La Torre, K., Villacís, J. E., Wagenaar, J. A., Matheu, J., Bravo-Vallejo, C., Fernández-Moreira, E., & Vinueza-Burgos, C. Broiler Farms and Carcasses Are an Important Reservoir of Multi-Drug Resistant *Escherichia coli* in Ecuador. *Frontiers in Veterinary Science*, 7(November), 2020, 1–8.

Paskeh M. D. A., Moghaddam, M. J. M., & Salehi, Z. Objective(s): Resistance to carbapenems as the last line for controlling resistant bacteria is increasing due to production of carbapenemase. The aim of this study was to detect the plasmid-encoded carbapenemases using phenotypic methods and multiplex PCR. *Iranian Journal of Basic Medical Sciences*, 23(5), 2020, 586–593.

Patil S., Chen, H., Zhang, X., Lian, M., Ren, P. G., & Wen, F. Antimicrobial resistance and resistance determinant insights into multi-drug resistant gram-

negative bacteria isolates from paediatric patients in China. *Infection and Drug Resistance*, 12, 2019, 3625–3634.

Patil S., Chen, X., Lian, M., & Wen, F. Phenotypic and genotypic characterization of multi-drug-resistant *Escherichia coli* isolates harboring blaCTX-M group extended-spectrum β -lactamases recovered from pediatric patients in Shenzhen, southern China. *Infection and Drug Resistance*, 12, 2019, 1325–1332.

Patil S., Chen, X., & Wen, F. Exploring the phenotype and genotype of multi-drug resistant *Klebsiella pneumoniae* harbouring bla CTX-M group extended-spectrum β -lactamases recovered from paediatric clinical cases in Shenzhen, China. *Annals of Clinical Microbiology and Antimicrobials*, 18(1), 2019.

Philippon A., Slama, P., Dény, P., & Labia, R. (2016). A structure-based classification of class A β -Lactamases, a broadly diverse family of enzymes. In *Clinical Microbiology Reviews*, American Society for Microbiology, Vol. 29(1), 2016, 29–57.

Pirkani G. S., Awan, M. A., Abbas, F., & Din, M. Culture and PCR based detection of bacteria causing urinary tract infection in urine specimen. *Pakistan Journal of Medical Sciences*, 36(3), 2020.

Plawińska-czarnak J., Wódz, K., Kizerwetter-świda, M., Bogdan, J., Kwieciński, P., Nowak, T., Strzałkowska, Z., & Anusz, K. Multi-Drug Resistance to *Salmonella* spp. When Isolated from Raw Meat Products. *Antibiotics*, 11(7), 2022.

Prestinaci F., Pezzotti, P., & Pantosti, A. Antimicrobial resistance: A global multifaceted phenomenon. In *Pathogens and Global Health*, Maney Publishing. 109(7), 2015, 309–318.

Rada A. M., Correa, A., Restrepo, E., & Capataz, C. *Escherichia coli* ST471 Producing VIM-4 Metallo- β -Lactamase in Colombia. *Microbial Drug Resistance*, 28(3), 2022, 288–292.

Radhika A., Lakshmi, J. T., Ariyanachi, K., & Sakthivadivel, V. Detection of Metallo Beta-Lactamase (MBL) Producing *Pseudomonas aeruginosa* in a Tertiary Care Hospital, Ghanpur, Medchal, India. *Maedica*, 17(1), 2022, 134–142.

Rakitin A. L., Yushina, Y. K., Zaiko, E. V., Bataeva, D. S., Kuznetsova, O. A., Semenova, A. A., Ermolaeva, S. A., Beletskiy, A. V., Kolganova, T. V., Mardanov, A. V., Shapovalov, S. O., & Tkachik, T. E. Evaluation of antibiotic resistance of salmonella serotypes and whole-genome sequencing of multiresistant strains isolated from food products in Russia. *Antibiotics*, 11(1), 2022.

Ramsamy Y., Mlisana, K. P., Allam, M., Amoako, D. G., Abia, A. L. K., Ismail, A., Singh, R., Kisten, T., Han, K. S., Muckart, D. J. J., Hardcastle, T., Suleman, M., & Essack, S. Y. Genomic analysis of carbapenemase-producing extensively drug-resistant *Klebsiella pneumonia* isolates reveals the horizontal spread of p18-43_01 plasmid encoding bla_{NDM-1} in South Africa. *Microorganisms*, 8(1), 2020.

Sacha P., Wieczorek, P., Hauschild, T., Zórawski, M., Olszańska, D., & Tryniszewska, E. Metallo- β -lactamases of *Pseudomonas aeruginosa* - A novel mechanism resistance to β -lactam antibiotics. In *Folia Histochemica et Cytobiologica*, Polish Histochemical and Cytochemical Society. 46 (2), 2008, 137–142).

Sakamoto N., Akeda, Y., Sugawara, Y., Matsumoto, Y., Motooka, D., Iida, T., & Hamada, S. Role of Chromosome- and/or Plasmid-Located bla_{NDM} on the Carbapenem Resistance and the Gene Stability in *Escherichia coli*. *Microbiology Spectrum*, 10(4), 2022.

Salam M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. A. (2023). Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. In *Healthcare (Switzerland)*, Multidisciplinary Digital Publishing Institute (MDPI), 11 (13), 2023.

Salem M, Younis G, Sadat A, Nouh NA, Binjawhar DN, Abdel-Daim MM, Elbadawy M, Awad A. Dissemination of mcr-1 and β -lactamase genes among *Pseudomonas aeruginosa*: molecular characterization of MDR strains in broiler chicks and dead-in-shell chicks infections. *Annals of Clinical Microbiology and Antimicrobials*, (1), 2024, 9.

Salimraj R., Zhang, L., Hinchliffe, P., Wellington, E. M. H., Brem, J., Schofield, C. J., Gaze, W. H., & Spencer, J. Structural and biochemical characterization of

Rm3, a subclass B3 metallo- β -lactamase identified from a functional metagenomic study. *Antimicrobial Agents and Chemotherapy*, 60(10), 2016, 5828–5840.

Scavuzzi A. M. L., Firmo, E. F., de Oliveira, É. M., & Lopes, A. C. de S. (2019). Emergence of blaNDM-1 associated with the aac(6')-ib-cr, acrB, cps, and mrkD genes in a clinical isolate of multi-drug resistant *Klebsiella pneumoniae* from Recife-PE, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, 52, 0–3.

Sewunet T., Asrat, D., Woldeamanuel, Y., Ny, S., Westerlund, F., Aseffa, A., & Giske, C. G. Polyclonal spread of blaCTX-M-15 through high-risk clones of *Escherichia coli* at a tertiary hospital in Ethiopia. *Journal of Global Antimicrobial Resistance*, 29, 2022, 405–412.

Shaikh S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences*, 22(1), 2015, 90–101.

Shalaby A., Ismail, M. M., & El-Sharkawy, H. Isolation, Identification, and Genetic Characterization of Antibiotic Resistance of *Salmonella* Species Isolated from Chicken Farms. *Journal of Tropical Medicine*, 2022.

Shoaib M., He, Z., Geng, X., Tang, M., Hao, R., Wang, S., Shang, R., Wang, X., Zhang, H., & Pu, W. The emergence of multi-drug resistant and virulence gene carrying *Escherichia coli* strains in the dairy environment: a rising threat to the environment, animal, and public health. *Frontiers in Microbiology*, 14, 2023.

Song W., Kim, J., Bae, I. K., Jeong, S. H., Seo, Y. H., Shin, J. H., Jang, S. J., Uh, Y., Shin, J. H., Lee, M. K., & Lee, K. Chromosome-encoded AmpC and CTX-M extended-spectrum β -lactamases in clinical isolates of *Proteus mirabilis* from Korea. *Antimicrobial Agents and Chemotherapy*, 55(4), 2011, 1414–1419.

Song Y., Wang, F., Liu, Y., Song, Y., Zhang, L., Zhang, F., Gu, X., & Sun, S. Occurrence and Characterization of *Salmonella* Isolated From Chicken Breeder Flocks in Nine Chinese Provinces. *Frontiers in Veterinary Science*, 7(August), 2020, 1–11.

- Stewart A., Harris, P., Henderson, A., & Paterson, D. Treatment of infections by OXA-48-producing Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, 62(11), 2018.
- Threlfall E. J. Antimicrobial drug resistance in Salmonella : problems and perspectives in food- and water-borne infections. *FEMS Microbiology Reviews*, 26(2), 2002, 141–148.
- Tian D., Wang, B., Zhang, H., Pan, F., Wang, C., & Shi, Y. *Clinical Science and Epidemiology*. September 2019, 2020, 1–10.
- Tran T. Q., Park, M., Lee, J. E., Kim, S. H., Jeong, J. H., & Choy, H. E. Analysis of antibiotic resistance gene cassettes in a newly identified Salmonella enterica serovar Gallinarum strain in Korea. *Mobile DNA*, 14(1), 2023.
- Veeraraghavan B., Jacob, J. J., Prakash, J. A. J., Pragasam, A. K., Neeravi, A., Narasimman, V., & Anandan, S. Extensive drug resistant Salmonella enterica serovar Senftenberg carrying bla NDM encoding plasmid p5558 (IncA/C) from India. *Pathogens and Global Health*, 113(1), 2019, 20–26.
- Wang C. H., Ma, L., Huang, L. Y., Yeh, K. M., Lin, J. C., Siu, L. K., & Chang, F. Y. Molecular epidemiology and resistance patterns of blaOXA-48 Klebsiella pneumoniae and Escherichia coli: A nationwide multicenter study in Taiwan. *Journal of Microbiology, Immunology and Infection*, 54(4), 2021, 665–672.
- Wang S., Zhou, K., Xiao, S., Xie, L., Gu, F., Li, X., Ni, Y., Sun, J., & Han, L. A Multidrug Resistance Plasmid pIMP26, Carrying bla IMP-26, fosA5, bla DHA-1, and qnrB4 in Enterobacter cloacae. *Scientific Reports*, 9(1), 2019, 1–7.
- Wilson H., & Török, M. E. Extended-spectrum β -lactamase-producing and carbapenemase-producing Enterobacteriaceae. In *Microbial Genomics* Microbiology Society, 4(7), 2018.
- Wozniak A., Figueroa, C., Moya-Flores, F., Guggiana, P., Castillo, C., Rivas, L., Munita, J. M., & García, P. C. A multispecies outbreak of carbapenem-resistant bacteria harboring the bla KPC gene in a non-classical transposon element. *BMC Microbiology*, 21(1), 2021.

Wu D., Lu, W., Huang, Y., Qin, G., liu, H., Xiao, J., & Peng, J. The impact of multi-drug resistant *Pseudomonas aeruginosa* infections on acute pancreatitis patients. *BMC Infectious Diseases*, 23(1), 2023.

Xiang T., Chen, C., Wen, J., Liu, Y., Zhang, Q., Cheng, N., Wu, X., & Zhang, W. Resistance of *Klebsiella pneumoniae* Strains Carrying blaNDM-1 Gene and the Genetic Environment of blaNDM-1. *Frontiers in Microbiology*, 11(April), 2020, 1–9.

Xihui Z., Yanlan, L., Zhiwei, W., Zheyu, P., Zhenshu, S., Cheng, L., Jianbiao, L., Shengliang, C., Lanying, P., & Yubao, L. Antibiotic resistance of *Riemerella anatipestifer* and comparative analysis of antibiotic-resistance gene detection methods. *Poultry Science*, 102(3), 2023.

Xu L., He, Q., Tang, Y., Wen, W., Chen, L., Li, Y., Yi, C., & Fu, B. Multi-locus sequence and drug resistance analysis of *Salmonella* infection in children with diarrhea in Guangdong to identify the dominant ST and cause of antibiotic-resistance. *Experimental and Therapeutic Medicine*, 24(5), 2022.

Yang J.-T., Zhang, L.-J., Lu, Y., Zhang, R.-M., & Jiang, H.-X. Genomic Insights into Global bla CTX-M-55 -Positive *Escherichia coli* Epidemiology and Transmission Characteristics . *Microbiology Spectrum*, 11(4), 2023.

Yang X., Peng, K., Zhang, Y., Liu, L., & Li, R. Characterization of a novel mcr-8.2-bearing plasmid in ST395 *Klebsiella pneumoniae* of chicken origin. *Infection and Drug Resistance*, 13, 2020, 1781–1784.

Yuan J., Wang, X., Shi, D., Ge, Q., Song, X., Hu, W., Wei, D., Ge, C., Li, X., & Hu, C. Extensive antimicrobial resistance and plasmid-carrying resistance genes in mcr-1-positive *E. coli* sampled in swine, in Guangxi, South China. *BMC Veterinary Research*, 17(1), 2021.

Yungyuen T., Chatsuwan, T., Plongla, R., Kanthawong, S., Yordpratum, U., Voravuthikunchai, S. P., Chusri, S., Saeloh, D., Samosornsuk, W., Suwantararat, N., Chaiwarith, R., Wannalerdsakun, S., Rotjanapan, P., Chantharit, P., Tulyaprawat, O., Thaipisuttikul, I., & Kiratisin, P. Nationwide Surveillance and Molecular

Characterization of Critically Drug-Resistant Gram-Negative Bacteria: Results of the Research University Network Thailand Study Epidemiology And Surveillance. 2021.

Zeng Z., Lei, L., Li, L., Hua, S., Li, W., Zhang, L., Lin, Q., Zheng, Z., Yang, J., Dou, X., Li, L., & Li, X. In silico characterization of blaNDM-harboring plasmids in *Klebsiella pneumoniae*. *Frontiers in Microbiology*, 13, 2022.

Zhang C., Fu, X., Liu, Y., Zhao, H., & Wang, G. Burden of infectious diseases and bacterial antimicrobial resistance in China: a systematic analysis for the global burden of disease study 2019. *The Lancet Regional Health - Western Pacific*, 43, 2024.

Zhang P., Shi, Q., Hu, H., Hong, B., Wu, X., Du, X., Akova, M., & Yu, Y. Emergence of ceftazidime/avibactam resistance in carbapenem-resistant *Klebsiella pneumoniae* in China. *Clinical Microbiology and Infection*, 26(1), 124.e1-124.e4.

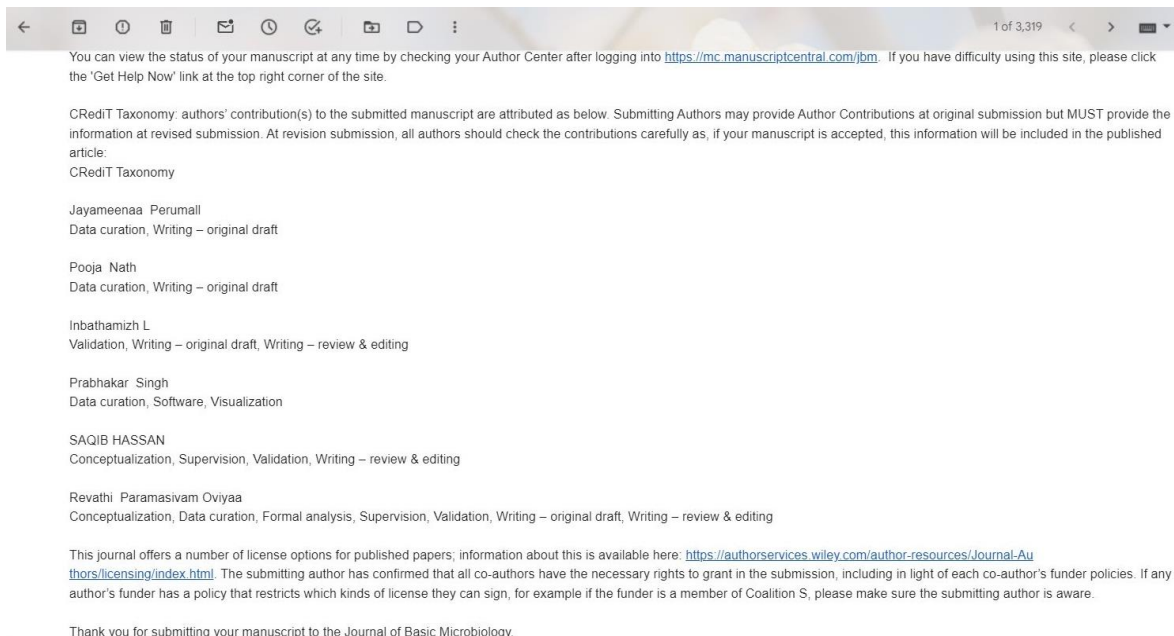
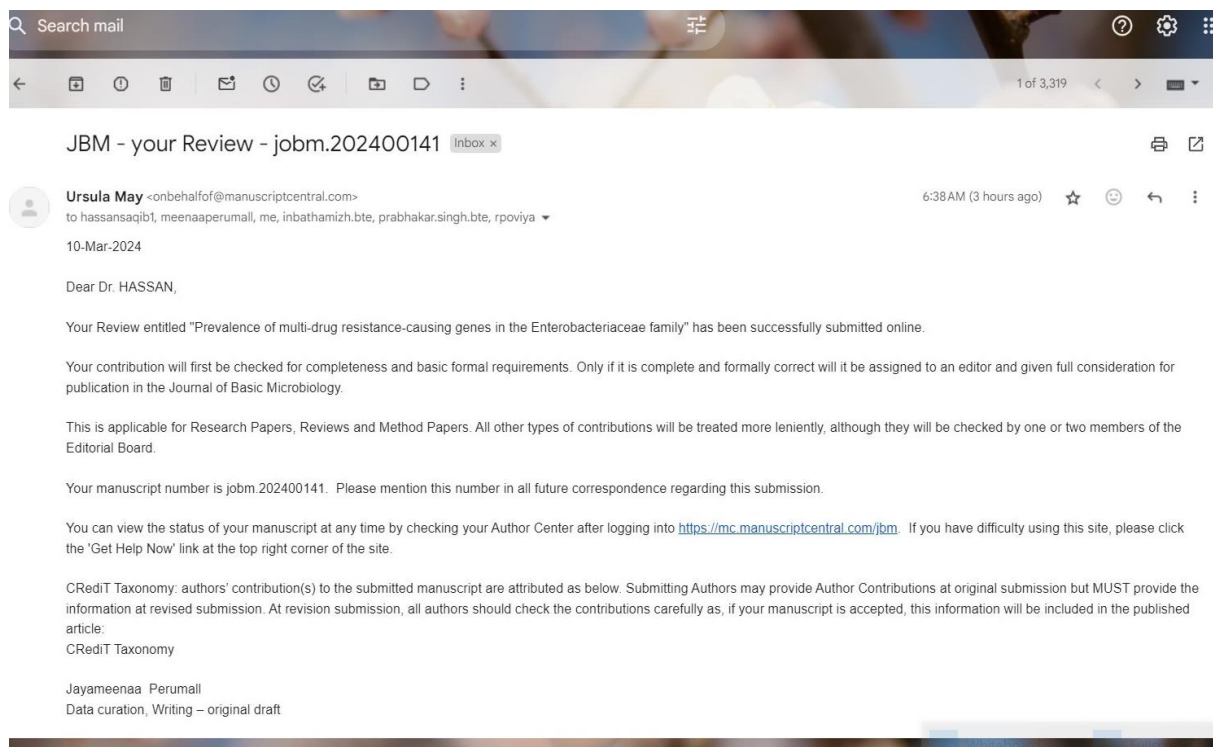
Zhang X., Feng, Y., Zhou, W., McNally, A., & Zong, Z. Cryptic transmission of ST405 *Escherichia coli* carrying blaNDM-4 in hospital. *Scientific Reports*, 8(1), 2018, 8–11.

Zhang X., Li, F., Cui, S., Mao, L., Li, X., Awan, F., Lv, W., & Zeng, Z. Prevalence and distribution characteristics of bla_{kpc}-2 and bla_{ndm}-1 genes in *klebsiella pneumoniae*. *Infection and Drug Resistance*, 13, 2020, 2901–2910.

Zhao Q., Sha, L., Wu, Z., Meng, L., Yang, F., Wu, L., Yu, C., Zhang, H., Yu, J., & Jin, Z. Evolution of carbapenem resistance in *klebsiella pneumoniae* and *escherichia coli* carrying bla NDM-1 gene: imipenem exposure results in sustained resistance memory of strains in vitro. *Annals of Clinical Microbiology and Antimicrobials*, 22(1), 2023, 1–12.

ANNEXURE

Review article submission mail



Ethical clearance certificate



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Dr. Sivakumar MD (Siddha)

National Institute of Siddha,

Tambaram Sanatorium

Chair Person,

IHEC, Sathyabama

EC/NEW/INST/2020/1397

30th October 2023

Ref: 320/IRB-IBSEC/SIST Dated 18th October 2023

To,

Dr. R.P Oviya,

Assistant Professor,

Department of Biotechnology

Sathyabama Institute of Science & Technology

Dr.T.Sasipraba

Vice chancellor

Co Chair Person

IHEC, Sathyabama

This is to inform you that the Institutional Human Ethical Committee has approved your study proposal entitled "**Development of Multiplex PCR for detection of Multi Drug Resistance in Bacterial Pathogen**", based on the presentation of the proposal made and recommendations of the Institutional Biosafety & Ethical Committee meeting held on 18th October 2023.

The validity of the Ethical clearance is for one year and you are expected to submit six-month progress and the final report of the study to IHEC/IRB

Dr. Y.Swarnalatha

Dean Higher studies

Secretary & Convener

IHEC, Sathyabama


Dr. Y.Swarnalatha
Secretary & Convener
IBEC, SIST


Dr. T.Sasipraba
Co Chair Person
IBEC, SIST

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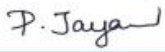
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GATE Admit Card

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Name of the Candidate JAYAMEENAA P	
Registration Number	BT24S57406067
Paper Code/Name	BT : Biotechnology
Date	10th February 2024 (Saturday)
Time	9:30 AM to 12:30 PM
Session	Forenoon : S5
Photo ID Submitted	Aadhaar ID (Number : 867600457473)
Examination Centre Code: 7406 Address of the Centre: Mohammed Sathak College of Engineering, No.34,Rajiv Gandhi Road OMR, IT Highway, Siruseri, Inside Sipcot IT Park, Chennai, Tamilnadu, PIN : 603103, India.	
 Prof. Chandra Sekhar Seelamantula Organising Chairperson, GATE 2024 (on behalf of NCB-GATE, for MoE)	   T206S83 AB1DE267D34B07E8750E5F1E3640E02A
INSTRUCTIONS TO CANDIDATES	
1. An electronic copy of the Admit Card is NOT acceptable. A printed copy of the Admit Card must be presented for verification along with the original valid photo identification document submitted at the time of application. In case the quality of the photograph on the identification document is poor, the candidate must bring an additional recent valid photo ID (Aadhaar Card, Driving License, PAN Card, Passport and Voter ID).	invigilator. A scribble pad once surrendered will not be returned to the candidate. At the end of the examination, the scribble pad in possession of the candidate must be returned to the invigilator.
2. To facilitate the identity verification by the Examination Centre personnel, candidates are advised to report to the examination venue at least 90 minutes before the scheduled commencement of the examination. Candidates will NOT BE ALLOWED to login 30 minutes after the start of the exam.	7. Candidates must bring their own pen and pencil. Candidates are also allowed to bring a transparent water bottle.
3. Candidates will be permitted to occupy their seats before the scheduled start of the examination. Candidates can login and start reading the instructions 20 minutes before the start of the examination.	8. Candidates are not allowed to bring any items other than those mentioned above. Examination Centre personnel are not responsible for the safety of the candidate's personal belongings. Candidates in possession of prohibited items will be deregistered, debarred from the examination, and may also be banned from appearing in future GATE examinations.
4. During the examination, a virtual scientific calculator will be	9. Candidates will not be allowed to leave the examination hall before the end of the examination.
	10. Impersonation and violation of the above guidelines or the Code of Conduct as given in the Information Brochure will lead to cancellation of candidature and attract penal action.

Offer Letter



OFFER LETTER

OL-2023: 0220

Dear **Jayameenaa Perumall,**

Congratulations!

You have been selected to join Zifo through the Campus Recruitment process held at your College / University.

We are pleased to offer you the position of **Associate Analyst** with a consolidated pay of **Rs. 476,700/- p.a. (Rupees Four Lakhs Seventy Six Thousand Seven Hundred only per annum)**. This consolidated pay includes your Basic Salary, Gratuity, Medical & Health Insurance.

Probation

You will be on probation for a period of 12 months from the date of joining. You would receive a **Confirmation letter** at the end of the probation period.

Insurance Coverage

The insurance coverage extends to yourself, your spouse and two of your children.

Leaves

You would be entitled to 12 Government-mandated public holidays per year. The office will be closed for 10 business days (which includes 5 Government mandated holidays) in a year and an additional 2 days can be chosen as optional holidays from the marked list of holidays in the calendar year.

In addition to this, you can avail leave for **18 days** in your first year as part of the earned leave component.

Maternity and Paternity Policy

At Zifo, we are committed to ensuring a family-friendly environment and a culture that builds a great work-life balance. Women will be entitled to maternity leave with full pay for a period of 26 weeks, in line with latest Maternity Benefit (Amendment Act) 2017. Men will be entitled to paternity leave with full pay for a period of 14 continuous working days.

Working Hours

Your working hours will be from **8:45 am to 5:45 pm** from Monday to Friday.





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Name of the student : Jayameenaa P

Register Number : 40230014

Title of the Thesis : DEVELOPMENT OF MULTIPLEX PCR ASSAY TO
DETECT THE MULTIDRUG RESISTANCE IN BACTERIAL
PATHOGENS

Internal Guide Name : Dr. Saqib Hassan

External Guide Name : Dr. R. P. Oviya

PO / PSO / PEO / SDG	Justification
PEO 3	There was good communication and understanding among the team member. Each step was taken after thoughtful consideration and proper discussion to avoid conflicts. Exhibited good team work with shared responsibilities.
PEO 4	This project was related to microbiology and Multidrug resistance pathogens, strict measures were taken during the project for the safety of project student, fellow people and the environment. For example class 2 Biosafety cabinet, wearing mask, gloves and lab coat. Properly discarding the waste. Obtained ethical clearance certificate for the collection of oral swabs from patients.
PO 2	It was seen after an extensive literature survey that, multidrug resistance among environmental and disease causing bacterial pathogens is accelerating over the years, this is caused by mutation in these bacteria leading to development of MDR genes. This is making it difficult for healthcare employees to treat patients with bacterial infections as they often have little to no knowledge about what drug resistance might be exhibited by the infecting bacterial pathogens this could lead to delayed treatment as it will take a longer period of time to find the right antibiotic. The project was attempted with the idea to reduce this time as the aim of the project was to design a multiplex assay that will detect the MDR genes. As knowing the genes it can help easily deduce the classes of drugs an individual might have resistance towards.
PO3	An appropriate study design was planned from development of the multiplex assay to its validation. Precautions were taken to prevent any harm to the environment or fellow project students.



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PO 5	Utilized modern technologies such as Gel Doc XR imaging system to visualize the agarose gel, Image J for quantification of the gel image and graphpad Prism for statistical analysis.
PO 6	The project attempts to combat health issue related to multidrug resistant bacterial infections. The objective of the project is to reduce the time period of treatment and prevent the usage of ineffective drugs.
PO 8	The samples were collected from patients of Sathyabama Dental Hospital; it was approved by Institutional Human Ethics Committee of Sathyabama Institute of Science and Technology. Ethical regulations were strictly throughout the entirety of the project.
PO 9	The project was carried out as a team project, with proper communication and understanding between the members and the guides and Without any conflicts
PSO 1	The project aimed to develop multiplex PCR to detect MDR gene in bacterial pathogen from both environment and clinical sample. To develop a multiplex PCR assay for easy detection.
UNSDG 3	The project aligns with United Nations Sustainable Development Goal 3 that is good health and wellbeing.