Figure 4.1 Growth of *P. aeruginosa* isolates on different culture media: 

A. Nutrient agar - smooth, large, translucent colonies with diffused greenish blue pigment

B. MacConky agar - non-lactose fermenting colonies

C. Blood agar - clear zone of β-hemolysis

D. Pseudomonas isolation agar - blue-green colonies with diffused pigment are visible
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Figure 4.2  Microscopic examination of Gram stained preparations of *P. aeruginosa* isolate (Gram negative rods are visible)

Figure 4.3  Demonstration of citrate utilization test by the isolate Palg99 as evidenced by the change of colour to blue (tube A). The tube B contains *E. coli* which doesn’t utilize citrate
Figure 4.4 The test isolate PaIg97 is positive for nitrate reduction (tube B) as the solution in this tube does not turn red on addition of zinc dust. The tube A contains uninoculated control

Figure 4.5 Demonstration of positive catalase test by the isolate PA2 was evidenced by effervescence (spot B) on the glass slide in contrast to uninoculated control (spot A)
Figure 4.6  Demonstration of positive oxidase test by isolate PA143 as evidenced by the appearance of purple colouration of the disc B against uninoculated control disc A

Figure 4.7  Demonstration of motility of the isolate PaIg95 is evidenced by the presence of growth around the stab line (tube B) whereas no growth is visible in the negative control, *K. pneumoniae* (tube A)
Figure 4.8 The isolate PaIg83 is negative for urease as no colour change was observed in the culture medium (tube B) while the colour changed to pink in positive control, *K. pneumoniae* strain (tube A).

Figure 4.9 The isolate PA103 didn’t produce rosindole after adding Kovac’s reagent (tube A) in indole test while cherry red coloured ring is visible in the positive control tube B inoculated with *E. coli*.
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Figure 4.10 Demonstration of negativity of methyl red test by isolate PA14 (tube B) while *E. coli* represents positive control (tube A)

Figure 4.11 The isolate PaIg86 exhibiting negative Voges - Prauskauer test (tube A) whereas the positive control (*K. pneumoniae*) produced pink colour which is indicative of positive VP test (tube B)
Figure 4.12  Demonstration of glucose fermentation test by isolate Palg90 which didn’t ferment glucose as there is no colour change (tube A) whereas the control strain ferment glucose with the production of acid only (tube B)

Figure 4.13  The isolate Palg82 neither produced acid nor gas in lactose fermentation test (tube A) while there is colour change to pink in case of positive control, E. coli (tube B)
Figure 4.14  Antibiotic cultural sensitivity assay. The isolate PA79 showing sensitivity to gentamicin, amikacin and resistance to most antibiotics

Figure 4.15  Percentage of MDR, XDR and PDR among *P. aeruginosa* isolates examined
Figure 4.16 Resistance of *P. aeruginosa* isolates towards third generation cephalosporins presented as percentage of isolates resistant to a particular cephalosporin (CTX - cefotaxime; CTR - ceftriaxone; CPD - cefpodoxime; CAZ - ceftazidime; AT - aztreonam; CXM - cefuroxime)
Figure 4.17 A. Isolate PaIg11 showing resistance to all third generation cephalosporins tested. 
B. Double disc diffusion test: Isolate PaIg76 shows increase of zone of inhibition to agumentin disc. 
C. E - Test: Ellipse formation by isolate PaIg09
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Figure 4.18 Controls used in the phenotypic E–Test: A. *Klebsiella pneumoniae* ATCC 700603 was used as positive control B. *Escherichia coli* ATCC 25922 as negative control

Figure 4.19 Agarose gel electrophoresis of genomic DNA of *P. aeruginosa* isolates
Figure 4.20 Agarose gel electrophoresis of PCR products of \textit{bla}_{TEM} amplicons of isolates Palg53 and Palg76 (lane 4 and 5). Lane 3 was loaded with 100bp DNA marker. Lane 1 was loaded with positive control (in house strain, PA38) and lane 2 was loaded with negative control (\textit{E. coli} ATCC 25922)
Figure 4.21 Agarose gel electrophoresis of PCR product of bla<sub>SHV</sub> amplicon of isolate PaIg48 (Lane 4). A band of ~ 530bp is visible in the lane 4. Amplification was, however not observed in lane 5 (PaIg47) and lane 6 (PA51). Lane 3 was loaded with 100bp DNA marker. Lane 1 contains amplicon of positive control (K. pneumoniae ATCC 700603) and amplicon of E. coli ATCC 25922 (negative control) (lane 2)
Figure 4.22 Agarose gel electrophoresis of PCR products of amplicon of gene $bla_{PER}$. No amplification was observed in case of isolates PA41, PA47, PA60, PaIg27 (lane 3 - 6). Lane 2 was loaded with 100bp DNA marker, positive control (in house strain, PA1013) (lane 7) and negative control ($E. coli$ ATCC 25922) (lane 1)
Figure 4.23 Agarose gel electrophoresis of PCR products of $bla_{CTX-M}$ amplicons of isolates PA137 and PaIg29 (lane 4 and 5). Amplification was not observed in case of PA122 (lane 6), lane 3 was loaded with 100bp DNA marker. Amplicon of positive control (in house strain, PaIg27) was loaded in lane 1 and negative control (*E. coli* ATCC 25922) (lane 2)
Nucleotide sequencing of \( \text{bla}_{\text{TEM}} \) gene amplicon of PA38 isolate

AGAGCACTCGGTGCCCACATACACTATTTCAGAGTGTGGGATCTC
ACCAGTCACAGAAAGCATCTTACGGGATGGCATGACAGTAAAGGAATTATGC
AGTGCGTCCATAACCAGTAGTGATAACAGCTGCTGCAAACCTTTCTCGACAAC
GATCGGAGGACCAGAGGCTAACCACGCTTGGGATGGCCAAACTTCTTGCAACT
GTAACGTGACACCACGTGCAGACTGCAATGGAAGCCATACCGAACAG
ACGAGCGTGACACCACGATGCCTGCAATGGCAACACGACTGGGA
ATTACTGGCAGAATCTTTCTCTAGGTTCTTCCGCAACATTGAGACTGGA
TGGAGGCGGATAA

*represents nucleotide sequence homology between isolates

**Figure 4.24** CLUSTAL OMEGA multiple sequence alignment of \( \text{bla}_{\text{TEM}} \) gene amplicon of PA38 isolate of *P. aeruginosa* with *Klebsiella pneumonia* (KP853090.1), *Klebsiella pneumonia* (KP853089.1), *Escherichia coli* (KP853092.1) and *Escherichia coli* (KP853091.1)
Figure 4.25 Jal view of multiple sequence alignment of \textit{bla}_{TEM} gene amplicon of PA38 isolate of \textit{P. aeruginosa} with \textit{Klebsiella pneumonia} (KP853090.1), \textit{Klebsiella pneumonia} (KP853089.1), \textit{Escherichia coli} (KP853092.1) and \textit{Escherichia coli} (KP853091.1)
Nucleotide sequencing of $bla_{TEM}$ gene amplicon of PaIg53 isolate

CGGGCAGAGCACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAT
GTACTCAACAGTCACAGAAAGCATCTTACGGGATGGCATGACAGTAAAGGAA
TTATGCAATGCTGCCATAACCAGATGATGATAACACTGCGCCAACTTACTTCT
GACAAACGATCGAGGGAACTGGGAGCTAAACGGCCTTTTGTGACAACATGGGG
GATCATGTAATCTGCCTTTGATCTGTTGGGAACCCGAAGCTGAATGAAAGGAC
CAAACGACGAGCCTGACACCAGCTGCTCAGCAATGGCAACAACGTTGCCG
AAAACGTATTAACCTAGCGAAACTAATCTACTACTCTAGCTTCGCCGCAAATAATAG
ACTGGATGGAGGGCGGATAAA

*represents nucleotide sequence homology between isolates

Figure 4.26 CLUSTAL OMEGA multiple sequence alignment of $bla_{TEM}$ gene amplicon of Palg53 isolate of $P. aeruginosa$ with $bla_{TEM-1}$ gene variant of Escherichia coli strain RIGLD-1B9-F1 (KP308219.1) and Klebsiella pneumoniae strain T3 (KR303752.1)
Figure 4.27 Jal view of multiple sequence alignment of \( \text{bla}_{TEM} \) gene amplicon of PaIg53 isolate of \( P. \ aeruginosa \) with \( \text{bla}_{TEM-1} \) gene variant of \( E. \ coli \) strain RIGLD-1B9-F1 (KP308219.1) and \( K. \ pneumoniae \) strain T3 (KR303752.1)

Nucleotide sequencing of \( \text{bla}_{TEM} \) gene amplicon of PaIg76 isolate
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CCAAAAGAGCTGGGACAGGACTCGGTCGCCGCATACACTATTCTCAGAATGA
CTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACA
GAAGCCATAACAAACGAGCAGCGCTGACACCCGATGCCTGTAGCAATGGCA
ACAACGTTGCGCAAATATTCAGTTGGAAGACTTTACTCTGAGCTTCGCGCA
ACAATTAATAGACTCGGATGGAGGAGCTTCCCGGCA

*represents nucleotide sequence homology between isolates

Figure 4.28 CLUSTAL OMEGA multiple sequence alignment of blaTEM gene amplicon of Palg76 isolate of P. aeruginosa with blaTEM-1 gene variant of uncultured soil bacterium clone M5 (EF514086.1), Escherichia coli strain RIGLD-1B9-F1 (KP308219.1) and Klebsiella pneumoniae strain T3 (KR303752.1)
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Figure 4.29 Jal view of multiple sequence alignment of \( \text{bla}_{\text{TEM}} \) gene amplicon of PaIg76 isolate of \( P. \text{aeruginosa} \) with \( \text{bla}_{\text{TEM-1}} \) gene variant of uncultured soil bacterium clone M5 (EF514086.1), \( E. \text{coli} \) strain RIGLD-1B9-F1 (KP308219.1) and \( K. \text{pneumoniae} \) strain T3 (KR303752.1).
Figure 4.30 CLUSTAL OMEGA multiple sequence alignment of \( \text{bla}_{\text{TEM}} \) gene amplicons of PA38, Pa1g53 and Pa1g76 isolates of \( P. \) aeruginosa

*represents nucleotide sequence homology between isolates
Figure 4.31 Jal view of multiple sequence alignment of $bla_{TEM}$ gene amplicons of PA38, Palg53 and Palg76 isolates of $P.\ aeruginosa$
Nucleotide sequencing of $\text{bla}_{\text{SHV}}$ gene amplicon of PaIg48 isolate

ATGCGTTATATTCCGCTTGTTATATTCTCCCCCTGTAGCAGCAAAATAACAAAGCAAGACATGGAGCGCGCCCGGCTGGCCTGAGCAGCCCGCAGCCGCTGGCCTGGAGCAAATTAAACAAAGCGAAAGCCAGCTGTCGGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGCCTGGCGCGCCGATGACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGCGGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATCCACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTGCCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGACGATAACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCTTTTTTGCGCCAGATCGGCGACAACGTCACCGCCTTGACCGCTGGGAAACGGAACTGAATGAGGCGCTTCCCGGCGACGCCGCGACACCAC

Cont……
Figure 4.32 CLUSTAL OMEGA multiple sequence alignment of \textit{bla}_{\textit{SHV}} \text{ gene amplicon} of \textit{P. aeruginosa} with \textit{bla}_{\textit{SHV-12}} \text{ gene variant of \textit{Acinetobacter} sp. SVU/ SVIMS1 (KJ083256.1), \textit{Escherichia coli} strain H59L (KM011336.1), \textit{Citrobacter freundii} (AY940490.1) and \textit{Klebsiella pneumoniae} strain (KF585138.1)}
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Figure 4.33 Jal view of multiple sequence alignment of \textit{bla}_{SHV} gene amplicon of PaIg48 of \textit{P. aeruginosa} with \textit{bla}_{SHV-12} gene of \textit{Escherichia coli} strain H59L (KM011336.1), \textit{Citrobacter freundii} (AY940490.1), \textit{Klebsiella pneumoniae} strain (KF585138.1) and \textit{Acinetobacter} sp. SVU/ SVIMS1 (KJ083256.1)
Nucleotide sequencing of \textit{bla}_{CTX-M} \ gene amplicon of PaIg27 isolate

AAACTTGGCAATTAAGCAGCCAGTGGGAGGCAGACTGGGTGTGGCATTGA
TTTACACAGCAGATTATTCGCAAATACTTCTGCTGCAGATGAGCTTGAGAAAT
CTGGTCATATTTCCAGGCTGGGAAAAACGGCGATGGGACATGATGGAAGGTC
GCTGAGCTTACGCGGCAGCGCTCAGATACAGCGATACAGCGATACAGCGAT
GGCGGAAATCTGCAAATCTGCAAATAGCGTTGAGATGTCACTGGCTGAGC

\textit{Continued…}
*represents nucleotide sequence homology between isolates

**Figure 4.34** CLUSTAL OMEGA multiple sequence alignment of $\text{bla}_{\text{CTX-M}}$ gene amplicon of PaIg27 isolate of *P. aeruginosa* with $\text{bla}_{\text{CTX-M-15}}$ gene variant of *Escherichia coli* strain: V512 (LC095574.1), *Escherichia coli* strain: V508 (LC095573.1) *Escherichia coli* strain: V501 (LC095572.1) and *Escherichia coli* strain: V496 (LC095571.1)
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Cont……
Figure 4.35 Jal view of multiple sequence alignment of $bla_{CTX-M}$ gene amplicon of Palg27 isolate of $P. aeruginosa$ with $bla_{CTX-M-15}$ gene variant of $Escherichia coli$ strain: V512 (LC095574.1), $Escherichia coli$ strain: V508 (LC095573.1) $Escherichia coli$ strain: V501 (LC095572.1) and $Escherichia coli$ strain: V496 (LC095571.1)
Nucleotide sequencing of \textit{bla}$_{\text{CTX-M}}$ gene amplicon of PaIlg29 isolate

CTAGTCGCGATACTGCGATGATAAGTGGCATGACATGGTGAAGCTGATTGCTACCGGTGAATCCCTCGGAAATCTCGCAGGTGCTAAGCTGGCGGCCCGG
CTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCTCGGACAGCCAACCCGCGGCAAGCCTGGGACG
CCGTACCGAGCCGACGTTAAACACCGCCATTCCGGCGATCCGCGTGATACCTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAG
CATTGGGCGACAGCCAACGGGGGAGCTGTTGACATGGTGAAGCTGATTGCTACCGGTGAATCCCTCGGAAATCTCGCAGGTGCTAAGCTGGCGGCCCGG

**\textit{bla}$_{\text{CTX-M}}$ gene amplicon of PaIlg29 isolate**

**Cont.……**
*represents nucleotide sequence homology between isolates

**Figure 4.36** CLUSTAL OMEGA multiple sequence alignment of $bla_{CTX-M}$ gene amplicon of Palg29 isolate of *P. aeruginosa* with $bla_{CTX-M-15}$ gene variant of *Escherichia coli* strain NK-73 (KP849465.1), *Klebsiella pneumoniae* strain 628 (KP987217.1), *Escherichia coli* strain NK-76 (KP849464.1) and *Klebsiella pneumoniae* strain KP80 (KP698226.1)
Cont......
Figure 4.37 Jal view of multiple sequence alignment of \textit{bla\textsubscript{CTX-M}} gene amplicon of Palg29 isolate of \textit{P. aeruginosa} with \textit{bla\textsubscript{CTX-M-15}} gene variant of \textit{Escherichia coli} strain NK-73 (KP849465.1), \textit{Klebsiella pneumoniae} strain 628 (KP987217.1), \textit{Escherichia coli} strain NK-76 (KP849464.1) and \textit{Klebsiella pneumoniae} strain KP80 (KP698226.1)
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Nucleotide sequencing of \( \text{bla}_{\text{CTX-M}} \) gene amplicon of PA137 isolate

GGGCAAGATTAGCAGTACGTGGGCGATAGTAAAGCTGATTGCCTACGCTTGCGG
CCCGGTGATGCGTCGTCGCGGCGGCGAAGCTGATTGCCTACGCTTGCGG
CTGACGCTGCGGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGG
ATACGATTGCTGACGCTGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGG
TAAAGCTGATTGCTGACGCTGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGG
CAATACGATTGCTGACGCTGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGG
GCTGATCTGCGGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGGCGGCGGCGG
CTGACGCTGCGGCGGCGGCGGCGAAGCTGATTGCCTACGCTTGCGGCGGCGG

Cont......
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*represents nucleotide sequence homology between isolates

**Figure 4.38** CLUSTAL OMEGA multiple sequence alignment of \( \text{bla}_{\text{CTX-M}} \) gene amplicon of PA137 isolate of \( P. \text{aeruginosa} \) with \( \text{bla}_{\text{CTX-M-15}} \) gene variant of \( \text{Klebsiella pneumoniae} \) strain 628 (KP987217.1), \( Escherichia coli \) strain NK-73 (KP849465.1), \( Escherichia coli \) strain NK-76 (KP849464.1) and \( \text{Klebsiella pneumoniae} \) strain KP30 (KP698224.1)
Figure 4.39 Jal view of multiple sequence alignment of $bla_{CTX-M}$ gene amplicon of PA137 isolate of *P. aeruginosa* with $bla_{CTX-M-15}$ gene variant of *Klebsiella pneumoniae* strain 628 (KP987217.1), *Escherichia coli* strain NK-73 (KP849465.1), *Escherichia coli* strain NK-76 (KP849464.1) and *Klebsiella pneumoniae* strain KP30 (KP698224.1)
*represents nucleotide sequence homology between isolates

**Figure 4.40** CLUSTAL OMEGA multiple sequence alignment of \( \text{bla}_{\text{CTX-M}} \) gene amplicons of Palg27, Palg29 and PA137 isolates of \( P. \) aeruginosa
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Figure 4.41 Jal view of multiple sequence alignment of \textit{bla}_{CTX-M} gene amplicons of Palg27, Palg29 and PA137 isolates of \textit{P. aeruginosa}