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SHEET 1

PCR controls for ESBL & pAmpC

Escherichia coli **(Biosafety Level: 2)**

Pseudomonas aeruginosa **(Biosafety Level: 2)**

These multidrug-resistant isolates were chosen to represent different variants of Class A (Extended Spectrum β-lactamase, ESBL) & Class C β-lactamases (Plasmid-mediated AmpC, pAmpC) available at this repository.

Revival of Culture from Stab:

1. Open the stab culture tube under a Laminar Air Flow (LAF) cabinet.
2. Using a sterile needle (straight or loop type - either disposable plastic or sterilized reusable metal), stab into the existing line of growth within the medium.
3. Once growth material adheres to the needle, inoculate it into a general or enriched broth medium (e.g., LB or TSB).
4. Incubate the broth culture at 37°C overnight (O/N).
5. On the next day, using a sterile loop, streak the broth culture onto a non-selective agar plate to obtain isolated colonies.
6. Incubate the plate at 37°C overnight to obtain single colonies.

Preparation of 20% Glycerol Stock (for Long-term Preservation):

1. Prepare 50% glycerol by mixing sterile distilled water and glycerol in a 1:1 ratio in a conical flask.
2. Autoclave the mixture at 121°C for 15 minutes and allow it to cool.
3. Dispense 400 µL of the sterile 50% glycerol into each sterile cryovial.
4. Pick colonies from a fresh nutrient agar plate and inoculate into TSB broth to prepare a dense bacterial suspension.
5. Incubate the broth culture at 37°C in a shaker incubator overnight.
6. After incubation, mix 600 µL of the overnight TSB culture with 400 µL of 50% glycerol to obtain a final glycerol concentration of 20%.
7. Mix thoroughly and store at -80°C for long-term preservation.

LAB ID	Organism	Class A β-lactamases (ESBL)	Susceptibility (MIC)	Accession number
AMRIR00300	*Klebsiella pneumoniae*	*bla*$_{\text{CTX-M-15}}$ *bla*$_{\text{SHV-182}}$ *bla*$_{\text{TEM-1B}}$	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (2 mg/L, I) Meropenem (>=16 mg/L, R) Amikacin (<=2 mg/L, S) Gentamicin (>=16 mg/L, R) Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Colistin (<=0.125 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JAWVAF000000000

AMRIR00496	*Klebsiella pneumoniae*	*bla*$_{\text{CTX-M-15}}$ *bla*$_{\text{SHV-106, 28}}$ *bla*$_{\text{TEM-1A}}$	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (2 mg/L, R) Imipenem (<=0.25 mg/L, S) Meropenem (<=0.25 mg/L, S) Amikacin (32 mg/L, I) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Fosfomycin (32 mg/L, S) Colistin (2 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JAWVAK000000000
AMRIR00637	*Pseudomonas aeruginosa*	*bla*$_{\text{VEB-5}}$	Piperacillin/Tazobactam (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Aztreonam (>=64 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	JBLAVS000000000
AMRIR00636	*Pseudomonas aeruginosa*	*bla*$_{\text{VEB-1}}$	Piperacillin/Tazobactam (64 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=32 mg/L, R)	JBLAVR000000000

			Aztreonam (>=64 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	
LAB ID	**Organism**	**Class C β-lactamases (pAmpC)**	**Susceptibility (MIC)**	**Accession number**
AMRIR00274	*Escherichia coli*	*bla*$_{\text{DHA-1}}$	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (16 mg/L, I) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (32 mg/L, R) Ertapenem (<=0.5 mg/L, S) Imipenem (<=0.25 mg/L, S) Meropenem (4 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>4 mg/L, R) Nitrofurantoin (<=16 mg/L, S) Colistin (0.5 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JBLFCW000000000
AMRIR00482	*Escherichia coli*	*bla*$_{\text{DHA-1}}$	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (<=4 mg/L, S) Cefuroxime (16 mg/L, I)	JBAGCE000000000

			Ceftriaxone (<=0.25 mg/L, S) Cefepime (<=0.12 mg/L, S) Ertapenem (<=0.12 mg/L, S) Imipenem (<=0.25 mg/L, S) Meropenem (<=0.25 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Ciprofloxacin (0.5 mg/L, I) Fosfomycin (<=16 mg/L, S) Colistin (0.25 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00460	*Escherichia coli*	$bla_{\text{CMY-42}}$	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (0.25 mg/L, S) Imipenem (0.5 mg/L, S) Meropenem (<=0.25 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Ciprofloxacin (>=4 mg/L, R) Fosfomycin (<=16 mg/L, S) Colistin (0.5 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JAZEUD000000000
AMRIR00966	*Escherichia coli*	$bla_{\text{CMY-2}}$	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R)	JBLFDE000000000

			Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (4 mg/L, R) Imipenem (1 mg/L, S) Meropenem (0.5 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Ciprofloxacin (>=4 mg/L, R) Fosfomycin (<=16 mg/L, S) Colistin (0.5 mg/L, I) Trimethoprim/Sulfamethoxazole (<=20 mg/L, S)	

SHEET 2

PCR controls for PMQR genes

Escherichia coli **(Biosafety Level: 2)**

Klebsiella pneumoniae **(Biosafety Level: 2)**

These isolates were chosen to represent different variants of Plasmid-mediated Quinolone Resistance Determinants (PMQR) available at the repository.

Revival of Culture from Stab:

8. Open the stab culture tube under a Laminar Air Flow (LAF) cabinet.
9. Using a sterile needle (straight or loop type - either disposable plastic or sterilized reusable metal), stab into the existing line of growth within the medium.
10. Once growth material adheres to the needle, inoculate it into a general or enriched broth medium (e.g., LB or TSB).
11. Incubate the broth culture at 37°C overnight (O/N).
12. On the next day, using a sterile loop, streak the broth culture onto a non-selective agar plate to obtain isolated colonies.
13. Incubate the plate at 37°C overnight to obtain single colonies.

Preparation of 20% Glycerol Stock (for Long-term Preservation):

14. Prepare 50% glycerol by mixing sterile distilled water and glycerol in a 1:1 ratio in a conical flask.
15. Autoclave the mixture at 121°C for 15 minutes and allow it to cool.
16. Dispense 400 µL of the sterile 50% glycerol into each sterile cryovial.
17. Pick colonies from a fresh nutrient agar plate and inoculate into TSB broth to prepare a dense bacterial suspension.
18. Incubate the broth culture at 37°C in a shaker incubator overnight.
19. After incubation, mix 600 µL of the overnight TSB culture with 400 µL of 50% glycerol to obtain a final glycerol concentration of 20%.
20. Mix thoroughly and store at -80°C for long-term preservation.

LAB ID	Organism	Plasmid-mediated Quinolone Resistance Determinants (PMQR)	Susceptibility (MIC)	Accession numbers
AMRIR00460	*Escherichia coli*	*aac(6')-Ib-cr*	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (0.25 mg/L, S) Imipenem (0.5 mg/L, S) Meropenem (<=0.25 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Ciprofloxacin (>=4 mg/L, R) Fosfomycin (<=16 mg/L, S)	JAZEUD000000000

			Colistin (0.5 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00300	*Klebsiella pneumoniae*	*aac(6')-Ib-cr*	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (2 mg/L, I) Meropenem (>=16 mg/L, R) Amikacin (<=2 mg/L, S) Gentamicin (>=16 mg/L, R) Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Colistin (<=0.125 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JAWVAF000000000
AMRIR00300	*Klebsiella pneumoniae*	*qnrB1*	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (2 mg/L, I) Meropenem (>=16 mg/L, R) Amikacin (<=2 mg/L, S) Gentamicin (>=16 mg/L, R)	JAWVAF000000000

			Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Colistin (<=0.125 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00482	*Escherichia coli*	*qnrB4*	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (<=4 mg/L, S) Cefuroxime (16 mg/L, I) Ceftriaxone (<=0.25 mg/L, S) Cefepime (<=0.12 mg/L, S) Ertapenem (<=0.12 mg/L, S) Imipenem (<=0.25 mg/L, S) Meropenem (<=0.25 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Ciprofloxacin (0.5 mg/L, I) Fosfomycin (<=16 mg/L, S) Colistin (0.25 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JBAGCE000000000
AMRIR00300	*Klebsiella pneumoniae*	*qnrS1*	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (2 mg/L, I) Meropenem (>=16 mg/L, R) Amikacin (<=2 mg/L, S)	JAWVAF000000000

			Gentamicin (>=16 mg/L, R) Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Colistin (<=0.125 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00499	*Klebsiella pneumoniae*	*qnrS1*	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (4 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (32 mg/L, I) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Fosfomycin (32 mg/L, S) Colistin (2 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	JAWVAN00000000

SHEET 3

PCR controls for carbapenemase

Escherichia coli **(Biosafety Level: 2)**

Klebsiella pneumoniae **(Biosafety Level: 2)**

Pseudomonas aeruginosa **(Biosafety Level: 2)**

Acinetobacter baumannii **(Biosafety Level: 2)**

These multidrug-resistant isolates were chosen to represent different variants of carbapenemases available at this repository.

Revival of Culture from Stab:

21. Open the stab culture tube under a Laminar Air Flow (LAF) cabinet.
22. Using a sterile needle (straight or loop type - either disposable plastic or sterilized reusable metal), stab into the existing line of growth within the medium.
23. Once growth material adheres to the needle, inoculate it into a general or enriched broth medium (e.g., LB or TSB).
24. Incubate the broth culture at 37°C overnight (O/N).
25. On the next day, using a sterile loop, streak the broth culture onto a non-selective agar plate to obtain isolated colonies.
26. Incubate the plate at 37°C overnight to obtain single colonies.

Preparation of 20% Glycerol Stock (for Long-term Preservation):

27. Prepare 50% glycerol by mixing sterile distilled water and glycerol in a 1:1 ratio in a conical flask.
28. Autoclave the mixture at 121°C for 15 minutes and allow it to cool.
29. Dispense 400 µL of the sterile 50% glycerol into each sterile cryovial.
30. Pick colonies from a fresh nutrient agar plate and inoculate into TSB broth to prepare a dense bacterial suspension.
31. Incubate the broth culture at 37°C in a shaker incubator overnight.
32. After incubation, mix 600 µL of the overnight TSB culture with 400 µL of 50% glycerol to obtain a final glycerol concentration of 20%.
33. Mix thoroughly and store at -80°C for long-term preservation.

LAB ID	Organism	Class B carbapenemase (Metallo-β-lactamase, MBL)	Susceptibility (MIC)	Accession number
AMRIR00274	*Escherichia coli*	$bla_{\text{NDM-1}}$	Ampicillin (>=32 mg/L, R) Amoxicillin/Clavulanic acid (16 mg/L, I) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (32 mg/L, R) Ertapenem (<=0.5 mg/L, S) Imipenem (<=0.25 mg/L, S) Meropenem (4 mg/L, S) Amikacin (4 mg/L, S) Gentamicin (<=1 mg/L, S) Nalidixic acid (>=32 mg/L, R) Ciprofloxacin (>4 mg/L, R)	JBLFCW000000000

			Nitrofurantoin (<=16 mg/L, S) Colistin (0.5 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00139	*Pseudomonas aeruginosa*	$bla_{\text{NDM-5}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (2 mg/L, I)	JBBBSP000000000
AMRIR00139	*Pseudomonas aeruginosa*	$bla_{\text{VIM-5}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (2 mg/L, I)	JBBBSP000000000
AMRIR00154	*Pseudomonas*	$bla_{\text{VIM-2}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R)	JBBBJZ000000000

	aeruginosa		Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (16 mg/L, I) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	
AMRIR00144	*Pseudomonas aeruginosa*	$bla_{\text{IMP-1}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R) Piperacillin/Tazobactam (32mg/L, I) Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (32 mg/L, I) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	Sanger sequencing
AMRIR00159	*Pseudomonas aeruginosa*	$bla_{\text{IMP-1}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R) Piperacillin/Tazobactam (64mg/L, I) Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R)	Sanger sequencing

			Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (32 mg/L, I) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	
AMRIR00111	*Pseudomonas aeruginosa*	*bla*$_{\text{DIM-1}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (2 mg/L, I)	JBBBSN000000000
LAB ID	**Organism**	**Class A carbapenemase**	**Susceptibility (MIC)**	**Accession number**
AMRIR00485	*Klebsiella pneumoniae*	*bla*$_{\text{KPC-2}}$	Amoxicillin/Clavulanic acid (>=32 mg/L, R) Piperacillin/Tazobactam (>=128 mg/L, R) Cefuroxime (>=64 mg/L, R) Ceftriaxone (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Ertapenem (>=8 mg/L, R) Imipenem (4 mg/L, R) Meropenem (>=16 mg/L, R)	JAWVAI000000000

			Amikacin (32 mg/L, I) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>4 mg/L, R) Fosfomycin (<=16 mg/L, S) Colistin (1 mg/L, I) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	
AMRIR00637	*Pseudomonas aeruginosa*	$bla_{\text{KPC-2}}$	Piperacillin/Tazobactam (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Aztreonam (>=64 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (1 mg/L, I)	JBLAVS000000000
AMRIR001225	*Acinetobacter baumannii*	$bla_{\text{GES-5}}$	Piperacillin/Tazobactam (>=128 mg/L, R) Ceftazidime (>=64 mg/L, R) Cefepime (>=32 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>4 mg/L, R) Colistin (16 mg/L, R) Trimethoprim/Sulfamethoxazole (>=320 mg/L, R)	Sanger sequencing
AMRIR00139	*Pseudomonas*	$bla_{\text{GES-9}}$	Ticarcillin/Clavulanic acid (>=128 mg/L, R)	JBBBSP000000000

	aeruginosa		Ceftazidime (>=64 mg/L, R) Cefepime (>=64 mg/L, R) Aztreonam (>=64 mg/L, R) Doripenem (>=8 mg/L, R) Imipenem (>=16 mg/L, R) Meropenem (>=16 mg/L, R) Amikacin (>=64 mg/L, R) Gentamicin (>=16 mg/L, R) Ciprofloxacin (>=4 mg/L, R) Levofloxacin (>=8 mg/L, R) Colistin (2 mg/L, I)	