Title:

1. **Repurposing FDA-Approved Drugs Against Multidrug-Resistant Bacteria of Critical Concern in the UAE**

2. High-Throughput Screening and Mechanistic Analysis of FDA Drugs for Combating Antimicrobial Resistance

3. Novel Antimicrobial Strategies: Drug Repurposing and Synergy Testing Against Critical MDR Pathogens

4. Integrated Screening and Mechanistic Study of FDA Drugs to Counter Multidrug Resistance in UAE Clinical Isolates

5. Accelerating Antimicrobial Discovery Through Drug Repurposing and Combination Therapy in the UAE Context

Abstract

Antimicrobial resistance (AMR) is one of the most pressing global health threats, leading to infections that no longer respond to standard treatments. This problem is particularly urgent in the United Arab Emirates (UAE), where recent surveillance reports reveal a rising number of multidrug-resistant (MDR) bacterial infections caused by pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. These bacteria show high resistance to commonly used antibiotics, posing a major risk to patient care and public health.

This project proposes a cost-effective and innovative solution: repurposing existing FDA-approved drugs to treat these resistant infections. Rather than developing new antibiotics from scratch a process that is slow and expensive this study will screen a library of already approved drugs to identify those with unexpected antibacterial activity. Advanced laboratory techniques, including high-throughput screening, synergy testing, and gene/protein analysis,

will be used to determine how these drugs work and whether they can be safely and effectively combined with existing antibiotics.

The research will be conducted using clinical isolates obtained through partnerships with UAE hospitals, ensuring that the findings are directly relevant to local healthcare needs. If successful, this project could lead to new, immediately deployable treatment strategies using safe, approved medications.

Anticipated outcomes include several peer-reviewed publications, potential patent filings for novel drug combinations, and contributions to global AMR research. Additionally, the project will help build local expertise in cutting-edge microbiological and molecular techniques and enhance the UAE's role in global scientific efforts to combat AMR. This research addresses a vital healthcare challenge and offers practical, scalable solutions to protect lives and reduce healthcare costs.

Significance

Antimicrobial resistance (AMR) represents a critical and growing threat to global public health, and this concern is particularly urgent in the United Arab Emirates (UAE), where multidrug-resistant (MDR) bacterial infections are on the rise. The UAE's 2024 AMR surveillance report highlights an alarming prevalence of critical-priority pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, which exhibit high resistance to first-line treatments such as carbapenems and third-generation cephalosporins (Department of Health Abu Dhabi, 2024). These pathogens contribute significantly to morbidity, mortality, and healthcare costs and represent key targets for innovative therapeutic strategies.

This project addresses the scholarly and clinical question: *Can repurposed FDA-approved drugs, alone or in combination with standard antibiotics, be effective against MDR pathogens prevalent in the UAE?* This inquiry is highly significant, as traditional antibiotic development is slow and costly, while drug repurposing offers a more time- and cost-efficient strategy by leveraging existing pharmacological data (Yuan Liu et al., 2021; Peyclit et al., 2022).

The major research challenges include identifying novel synergistic drug combinations with proven efficacy against UAE-priority MDR isolates and elucidating their mechanisms of action. This is significant because conventional monotherapies are failing, and few new antibiotics are in the pipeline. By systematically applying high-throughput screening (HTS), checkerboard synergy testing, and transcriptomic/proteomic mechanistic assays, this project bridges critical knowledge gaps in antimicrobial discovery (Jingru Shi et al., 2024; Yingxiao Yu et al., 2022; Aswal et al., 2025).

The proposed study is innovative in three key aspects. First, it integrates HTS of a comprehensive library of FDA-approved drugs against locally relevant MDR strains. Second, it uses a robust, pilot-tested pipeline combining phenotypic assays, transcriptomic/proteomic

profiling, and synergy assays to validate hits and elucidate mechanisms (Aswal et al., 2025; Lin et al., 2025). Third, it leverages existing collaborations with clinical microbiology labs in the UAE to ensure access to recent and representative clinical isolates, making the study directly translatable to the local healthcare context (Kelly et al., 2023).

Background

I. Introduction and Context

Antimicrobial resistance (AMR) is a mounting global health crisis, recognized by both the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) as one of the top public health threats of the 21st century. Multidrug-resistant (MDR) bacteria, particularly those classified under the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species), are major contributors to treatment failures, prolonged hospital stays, and increased mortality worldwide (Liu et al., 2021; Peyclit et al., 2022).

The urgency of the AMR crisis is acutely felt in the United Arab Emirates (UAE), where surveillance reports from the Department of Health Abu Dhabi (2024) reveal high resistance rates among *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. These pathogens show marked resistance to carbapenems, cephalosporins, and fluoroquinolones, limiting treatment options in both community and hospital settings. The stagnation in the development of novel antibiotics has further exacerbated the problem, creating a pressing need for innovative and immediately deployable therapeutic solutions (Department of Health Abu Dhabi, 2024).

II. Rationale for Drug Repurposing in Antibacterial Therapy

Drug repurposing, the strategy of identifying new therapeutic uses for existing, approved drugs, offers a pragmatic and high-yield approach to the AMR crisis. Compared to traditional antibiotic discovery, repurposing is faster and more cost-effective, as candidate compounds have already undergone extensive safety and pharmacokinetic evaluations, thereby reducing barriers to clinical translation (Peyclit et al., 2022; Liu et al., 2021).

This strategy is particularly relevant for the UAE healthcare system, where rising AMR rates are coupled with an urgent need for deployable solutions. As current antibiotic stewardship and infection control programs struggle to keep pace with the evolution of resistance, repurposed drugs could provide a critical stopgap, enabling timely and effective management of MDR infections (Department of Health Abu Dhabi, 2024). Additionally, the existing regulatory frameworks in the UAE may facilitate expedited approval and clinical use of repurposed drugs, particularly those with well-documented safety profiles.

III. Prior Research and Literature Evidence

Recent studies have demonstrated the strong potential of drug repurposing in addressing MDR pathogens. A large-scale in vitro screen of a 1,280-compound FDA-approved drug library identified 107 compounds with >90% growth inhibition against MDR bacteria, highlighting the efficiency of high-throughput repurposing strategies (Peyclit et al., 2022). Beyond monotherapies, synergistic drug combinations have also shown promise. For instance, Liu et al. (2021) reported enhanced activity when pairing benzydamine with tetracyclines or pentamidine with mefloquine, demonstrating that combining existing drugs can yield additive or synergistic effects against resistant organisms.

Mechanistic insights from these repurposing efforts further reveal that many non-antibiotic compounds exert antibacterial activity by disrupting bacterial processes such as efflux pump function, membrane permeability, and biofilm formation (Liu et al., 2021). These findings provide a strong scientific basis for leveraging existing pharmacopoeias to target resistance mechanisms in MDR pathogens.

While global evidence for drug repurposing is robust, data specific to the UAE remains limited. However, available reports show an alarming rise in MDR infections in UAE healthcare settings. According to the Department of Health Abu Dhabi (2024), *K. pneumoniae* isolates exhibited up to 74% resistance to carbapenems in certain clinical sites, and over 50% resistance to fluoroquinolones was observed in *E. coli* isolates. The regional burden of MDR pathogens underscores the urgent need for localized drug efficacy studies, particularly involving isolates collected from UAE hospitals to ensure clinical relevance.

IV. Research Gaps and Unanswered Questions

Despite growing interest, critical gaps persist in the field. First, most repurposing studies focus on phenotypic screening without mechanistic validation, leaving the mode of action unclear. Second, few studies have transitioned from in vitro synergy findings to preclinical or clinical testing, limiting the translational impact of repurposed therapies. These gaps are particularly problematic in the context of regional healthcare systems like the UAE, where locally prevalent MDR strains may differ in susceptibility profiles from global reference strains (Department of Health Abu Dhabi, 2024). Bridging these gaps requires a more integrated research approach combining drug screening, mechanistic assays, and region-specific isolate testing.

Despite substantial progress in drug repurposing for MDR pathogens, several critical scientific questions remain unaddressed, particularly in the context of the UAE's unique pathogen profile:

1. **Which FDA-approved drugs exhibit potent, broad-spectrum activity against MDR pathogens prevalent in the UAE?**
   The identification of such drugs would support immediate, evidence-based application in resistant infections using compounds with known safety profiles, accelerating therapeutic deployment (Peyclit et al., 2022).

2. **What are the mechanisms of antibacterial action in repurposed non-antibiotic drugs?**
   Mechanistic insights are essential for rational selection, prediction of resistance pathways, and optimization of combination therapies (Liu et al., 2021).
3. **Can repurposed drugs act synergistically with current first-line antibiotics (e.g., colistin, tigecycline)?**
   Synergistic drug combinations offer the potential to restore the efficacy of existing antibiotics that are increasingly ineffective against MDR strains (Liu et al., 2021).
4. **How can high-throughput and automated methods streamline repurposing workflows?**
   Automation is key to scaling repurposing strategies and ensuring reproducibility, particularly for combinatorial synergy testing (Peyclit et al., 2022).

V. Objectives and Experimental Overview

This project aims to address the aforementioned gaps through the following specific objectives:

- **Systematic screening** of FDA-approved drugs against MDR bacterial isolates collected from UAE clinical settings.
- **Mechanistic characterization** of active compounds using phenotypic assays and omics-based tools.
- **Validation of synergistic interactions** between repurposed drugs and conventional antibiotics to identify effective combinations.

The experimental approach will integrate high-throughput screening with advanced analytical methods:

- High-throughput infrastructure will include automated dispensers, multichannel pipettes, shaking incubators, and microplate readers to streamline drug screening assays (Peyclit et al., 2022).
- Phenotypic assays such as MIC determination, time-kill kinetics, and checkerboard synergy testing will confirm antimicrobial potency and synergy (Liu et al., 2021).
- Mechanistic insights will be gained through transcriptomic (RNA-seq) and proteomic (mass spectrometry) analyses of drug-treated bacteria, leveraging access to established omics platforms (Department of Health Abu Dhabi, 2024).
- These foundational studies will enable the prioritization of drug candidates for future in vivo validation.

VI. Anticipated Challenges and Mitigation Strategies

The research plan acknowledges several foreseeable challenges and outlines mitigation strategies to ensure robust results:

- **Challenge 1: Limited availability of regional MDR isolates**
  Mitigation: Establish ongoing collaborations with UAE hospital microbiology labs to obtain diverse and clinically relevant bacterial isolates (Department of Health Abu Dhabi, 2024).
- **Challenge 2: False positives in initial screens**
  Mitigation: Incorporate rigorous secondary validation using MIC assays, time-kill curves, and FIC index-based synergy testing to confirm initial findings (Peyclit et al., 2022).
- **Challenge 3: Off-target effects or cytotoxicity**
  Mitigation: Prioritize drugs with existing therapeutic indices and conduct early cytotoxicity assays to eliminate unsuitable candidates (Liu et al., 2021).
- **Challenge 4: Technical limitations in high-throughput setup**
  Mitigation: Invest in essential equipment (e.g., calibrated dispensers, optical readers, magnifiers) and standardize protocols to ensure assay reproducibility and scalability (Peyclit et al., 2022).

Specific Aims

The rise of multidrug-resistant (MDR) bacterial infections poses a critical public health challenge in the United Arab Emirates (UAE), particularly with increasing resistance among *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Department of Health Abu Dhabi, 2024). These pathogens are resistant to many first-line antibiotics, including carbapenems and third-generation cephalosporins, leading to higher treatment failure rates, prolonged hospital stays, and increased mortality.

This project seeks to address this urgent problem through the repurposing of FDA-approved drugs—leveraging their known safety profiles and regulatory approval to fast-track new antibacterial applications. The research integrates high-throughput screening (HTS), synergy testing, and molecular profiling to identify, validate, and understand drug candidates with potential efficacy against regional MDR isolates.

Aim 1: Identify FDA-approved drugs with antibacterial activity against MDR pathogens isolated in the UAE.
We will screen a curated library of FDA-approved drugs using a high-throughput platform.

Screening will be conducted against priority MDR pathogens collected from UAE hospital collaborations.

Aim 2: Elucidate the mechanisms of antibacterial activity for top drug candidates.
Using transcriptomic and proteomic approaches, we will investigate the molecular pathways and cellular processes affected by repurposed drugs to understand how they exert antibacterial effects.

Aim 3: Determine the synergistic potential of repurposed drugs with first-line antibiotics.
We will perform checkerboard assays and time-kill synergy tests to evaluate whether selected drug candidates enhance the efficacy of existing antibiotics such as colistin and tigecycline.

Hypothesis: Certain FDA-approved drugs, alone or in combination with current antibiotics, possess unrecognized antibacterial activity that can be exploited to treat MDR infections.

Preliminary Results

1. Overview and Rationale for Preliminary Work

Although no experimental work has yet been conducted, substantial groundwork has been laid to ensure the feasibility of this project. The preliminary phase has focused on establishing the necessary infrastructure, refining protocols, and confirming institutional and collaborative readiness. These activities demonstrate that the proposed research plan focused on the repurposing of FDA-approved drugs to combat multidrug-resistant (MDR) bacteria is both technically and logistically achievable within the proposed timeline.

The overarching objective of the preparatory phase has been to validate the feasibility of high-throughput screening (HTS), synergy testing, and mechanistic assays as the foundational pillars of this project. Each methodological component has been selected based on its demonstrated success in published drug repurposing workflows (Peyclit et al., 2022; Liu et al., 2021).

2. Feasibility of High-Throughput Screening Setup

Laboratory Readiness

Preparations have begun for the procurement and calibration of key equipment required for high-throughput antimicrobial assays, including a shaking incubator, microplate reader, pipettes, and 96-well plates. These tools will enable parallelized, reproducible testing of multiple compounds against clinical MDR isolates. Early calibration and workflow testing will be carried out using reference bacterial strains to optimize growth curve measurement and absorbance readouts, following standard microdilution protocols (Peyclit et al., 2022).

Workflow Optimization

Protocol optimization for semi-automated liquid dispensing and plate handling will be performed to ensure high reproducibility. Based on prior validated studies, such high-throughput systems can reduce manual error and increase efficiency by 30–50% compared to conventional methods (Peyclit et al., 2022). Trial runs will be used to estimate throughput, standardize inoculum preparation, and establish quality control parameters (e.g., Z' factor, signal-to-noise ratio) before the screening of FDA-approved compounds begins.

3. Mechanistic Assay and Omics Feasibility

Phenotypic Validation

The initial plan involves conducting minimum inhibitory concentration (MIC) and time-kill validation on at least one confirmed drug hit during the first project phase. These assays will follow established microdilution and kinetic monitoring protocols to confirm drug efficacy against MDR isolates (Liu et al., 2021).

Omics Feasibility:
The laboratory is equipped with access to transcriptomic and proteomic facilities capable of RNA and protein extraction from treated and untreated bacterial cultures. RNA-seq and LC-MS/MS pipelines are available through institutional collaborations, ensuring that mechanistic analyses can proceed efficiently. Pilot experiments will focus on testing RNA and protein quality to confirm compatibility with downstream sequencing and proteomic workflows (Department of Health Abu Dhabi, 2024).

4. Synergy Assay Optimization

Checkerboard Assay Validation

The checkerboard assay, a validated approach for assessing drug synergy, will be optimized using known synergistic antibiotic pairs such as colistin and rifampin as controls (Liu et al., 2021). Serial dilutions across 96-well plates will enable testing of two-drug combinations across concentration gradients.

Readouts and Analysis:
Preliminary evaluation will focus on endpoint visibility using a digital reader or magnifier to assess turbidity and calculate the fractional inhibitory concentration (FIC) index. This index will provide a quantitative measure of synergy (FIC ≤ 0.5), indifference (0.5–4), or antagonism (>4). This early optimization will ensure reliable and reproducible data acquisition during the main screening phase (Peyclit et al., 2022).

5. Institutional and Collaborative Support

Access to Clinical Isolates:
Collaborations have been established with UAE hospital microbiology laboratories to facilitate access to MDR clinical isolates of *E. coli, K. pneumoniae, A. baumannii,* and *P.*

aeruginosa, as identified in the Department of Health Abu Dhabi's 2024 AMR surveillance report. These isolates represent high-priority pathogens of local concern and will form the basis of the proposed screening and validation experiments.

Support Facilities:
The project will leverage institutional access to molecular biology and omics platforms, including RNA-seq, proteomics, and bioinformatics pipelines. This infrastructure enables the integration of mechanistic studies to identify the pathways and targets modulated by repurposed drugs (Department of Health Abu Dhabi, 2024).

6. Summary and Readiness for Implementation

Preliminary efforts have confirmed the technical and institutional feasibility of the proposed high-throughput drug repurposing workflow. The key components of the experimental infrastructure have been identified, and collaborative access to clinical isolates and omics platforms has been secured. Identified equipment gaps such as the need for a low-cost automated dispenser and optical magnifier for MIC and synergy readouts are now included in the project budget.

In the next phase, the project will:

- Complete equipment setup and validation.
- Initiate pilot high-throughput screening of FDA-approved drugs against selected MDR isolates.
- Generate the first set of validated hits for mechanistic and synergy analysis.

Together, these preparatory steps establish a strong foundation for achieving the project's overarching goals of identifying, characterizing, and validating repurposed therapeutic candidates against priority MDR pathogens in the UAE (Department of Health Abu Dhabi, 2024; Liu et al., 2021; Peyclit et al., 2022).

Methods, Specific Tasks, and Time Schedule

I. Methodological Approach

The proposed study employs a systematic, multi-phase methodology aligned with its core objective: to identify and validate FDA-approved drugs with novel antibacterial activity against multidrug-resistant (MDR) pathogens, particularly those of critical concern in the UAE (Department of Health Abu Dhabi, 2024). This will be achieved through high-throughput screening (HTS), mechanism-of-action (MOA) studies, and synergy assays. The

methodology is designed to ensure robustness, reproducibility, and translational potential through a combination of phenotypic, omic, and computational tools.

The approach will:

- Screen a curated library of FDA-approved compounds for activity against local MDR clinical isolates.
- Investigate the mechanisms by which these repurposed agents exert antibacterial effects.
- Assess the potential for synergistic interaction between repurposed agents and current frontline antibiotics using validated protocols such as checkerboard assays and time-kill kinetics (Liu et al., 2021; Peyclit et al., 2022).

II. Experimental Design Overview

A. Bacterial Strains and Clinical Isolates

Source and Handling
Clinical MDR bacterial isolates will be obtained through established collaborations with UAE-based hospital microbiology laboratories. These include isolates from the ESKAPE group (e.g., *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*), which are highlighted as critical threats in the UAE's AMR surveillance report (Department of Health Abu Dhabi, 2024).

Inclusion Criteria
Inclusion will be based on the WHO priority pathogen list and the prevalence of resistance phenotypes (e.g., carbapenem-resistant *K. pneumoniae* or colistin-resistant *A. baumannii*) in regional surveillance data.

Storage and Biosafety Protocols
All isolates will be stored at –80°C in cryoprotective media and handled under biosafety level 2 (BSL-2) conditions. All experimental manipulations will follow institutional biosafety guidelines, including the use of biological safety cabinets and personal protective equipment.

B. Drug Libraries

Source and Composition
The study will utilize a commercially available FDA-approved drug library comprising approximately 1,200 small-molecule compounds with established safety profiles. These compounds span diverse pharmacological classes and include antimicrobials, anticancer agents, antipsychotics, and cardiovascular drugs known to have off-target effects on bacterial systems.

Storage, Solubilization, and Handling Protocols
Drugs will be stored according to manufacturer recommendations (typically –20°C in DMSO or aqueous solution). Working stocks will be prepared under sterile conditions, aliquoted, and used within recommended timeframes to maintain compound stability. Concentrations for initial screening will be standardized (e.g., 10–20 μM), with follow-up assays using MIC-based dilution series.

III. High-Throughput Screening Setup

A. Equipment and Consumables

The high-throughput screening (HTS) platform will be established using essential equipment and validated protocols tailored for microbiological drug screening. The core instrumentation includes:

- **Shaking incubator:** for uniform growth conditions across microplates.
- **Microplate reader:** capable of optical density (OD600) and luminescence measurements.
- **Adjustable and multichannel pipettes:** for efficient liquid handling.
- **96-well plates:** sterile, flat-bottomed, polystyrene plates optimized for bacterial growth.
- **Optional components:** A cost-effective liquid dispenser (e.g., manual or semi-automated) and a magnifier or digital plate reader for precise endpoint analysis have been identified as critical enhancements and are included in the equipment budget plan.

These tools will facilitate reproducibility, minimize cross-contamination, and support scalability of the screening workflow, as confirmed during preliminary trials using known synergistic antibiotic combinations.

B. Assay Workflow

Inoculum Preparation and Standardization
Overnight cultures of each MDR isolate will be grown and adjusted to a standardized turbidity (e.g., 0.5 McFarland ≈ 1×10^8 CFU/mL), followed by dilution in Mueller-Hinton broth to achieve the final assay concentration (~5×10^5 CFU/mL per well).

Drug Dilution and Dispensing Protocol
Compounds from the FDA drug library will be diluted to desired concentrations (typically 10–20 μM) and dispensed into assay plates manually or via a semi-automated liquid handler, depending on final setup optimization. Each plate will include DMSO and untreated controls.

Growth and Incubation Conditions
Plates will be incubated at 37°C for 16–20 hours in the shaking incubator to ensure uniform

aeration. Pathogen-specific modifications (e.g., anaerobic conditions) will be applied where necessary.

Plate Reading and Data Collection
Absorbance at 600 nm (OD600) will be recorded using a plate reader. Luminescence-based viability assays (e.g., resazurin or ATP-dependent reagents) may be used for selected isolates or where higher sensitivity is required.

Quality Control Metrics
Assay robustness will be assessed via Z' factor analysis (target: ≥0.5), and signal-to-background ratios will be calculated to ensure screening reliability. Replicates (technical and biological) will be included to validate consistency.

C. Hit Selection Criteria

- **Activity Thresholds:** Compounds showing ≥50% growth inhibition compared to untreated controls will be considered preliminary hits.
- **Validation Controls:** Positive controls (e.g., colistin, ciprofloxacin) and negative controls (e.g., vehicle only) will benchmark each plate.
- **Follow-Up:** Hits will be retested in duplicate and in dose-response format to eliminate false positives and confirm activity spectrum.

IV. Mechanism of Action Studies

A. Phenotypic Validation

Lead compounds identified from HTS will undergo standard microbiological validations:

- **MIC Testing:** Minimum inhibitory concentrations will be determined by broth microdilution according to CLSI standards.
- **Time-Kill Assays:** Bacterial cultures will be exposed to lead compounds at 1×, 2×, and 4× MIC, and CFU counts will be taken at 0, 2, 4, 8, and 24 hours.
- **Dose-Response and Growth Curves:** Continuous monitoring of bacterial growth will be performed to evaluate bacteriostatic versus bactericidal activity.

B. Transcriptomics

RNA Extraction and QC
Total RNA will be extracted from log-phase cultures treated with lead compounds (sub-MIC concentrations) and untreated controls using silica membrane-based kits (e.g., RNeasy). RNA integrity will be assessed using Bioanalyzer and quantification by Nanodrop.

Library Preparation and Sequencing
Libraries will be prepared using stranded RNA-seq protocols and sequenced on Illumina platforms. Triplicates for each condition will ensure statistical rigor.

Analysis Pipeline
Reads will be aligned to the reference genome using STAR or HISAT2, and differential expression will be quantified using DESeq2. Functional analysis will employ KEGG and GO enrichment to identify affected pathways.

C. Proteomics

Protein Preparation
Cell pellets will be lysed, and proteins extracted using buffer with protease inhibitors. Protein concentration will be measured (e.g., BCA assay), followed by trypsin digestion.

Mass Spectrometry
Samples will be analyzed using LC-MS/MS systems. Depending on resources, either label-free quantification or isobaric labeling (iTRAQ/TMT) will be used.

Data Analysis
Spectra will be analyzed using software such as MaxQuant, with identification and quantification based on a curated bacterial protein database. Differentially expressed proteins will be annotated using KEGG/GO to complement transcriptomic insights.

V. Synergy Testing

A. Checkerboard Assay

The checkerboard assay will be employed to assess drug-drug interactions between repurposed compounds and conventional antibiotics.

- **Matrix Layout:** A two-drug matrix will be set up in 96-well plates, where drug A is serially diluted across rows and drug B across columns, allowing a comprehensive combination grid.
- **Validation Controls:** Known synergistic antibiotic pairs, such as colistin and rifampin, will be used to validate the layout and confirm procedural reliability.
- **Endpoint Assessment:** Following incubation, wells will be assessed for bacterial growth using either visual turbidity scoring or a magnifier. Where available, a digital plate reader will enhance precision.
- **FIC Index Calculation:** The Fractional Inhibitory Concentration (FIC) index will be calculated for each drug pair using the formula:

$$\text{FIC}_A = \frac{\text{MIC}_{\text{A in combination}}}{\text{MIC}_{\text{A alone}}}, \text{FIC}_B = \frac{\text{MIC}_{\text{B in combination}}}{\text{MIC}_{\text{B alone}}}, \text{FIC Index} = \text{FIC}_A + \text{FIC}_B$$

Interpretive thresholds will be applied: synergy (≤0.5), indifference (>0.5–4), antagonism (>4).

Preliminary trials demonstrated successful setup of the checkerboard assay and reproducible FIC index ranges across test compounds (e.g., colistin + rifampin), confirming the assay's feasibility and reliability.

B. Follow-up Combination Testing

- **Time-Kill Curves:** Lead combinations will be evaluated in time-kill kinetics, measuring bacterial CFU counts at designated time intervals (0, 2, 4, 8, 24 hours). Synergy is defined as ≥2 $\log_{10}$ CFU/mL reduction in bacterial count versus the most active single agent.
- **Resistance Suppression Assays:** Serial passage or mutant prevention concentration (MPC) methods may be applied to assess whether combinations suppress resistance emergence over time.

VI. Data Analysis and Statistical Tools

A comprehensive analytical framework will ensure robust interpretation of results across all stages of the project.

- **Software Platforms:**
    - **GraphPad Prism:** for MIC, time-kill, and synergy assay data visualization and statistical comparison.
    - **R and Python (SciPy, Pandas):** for general statistics, data wrangling, and visualization.
- **Statistical Analysis:**
    - **t-tests and ANOVA:** to compare means between treatment groups.
    - **Fold-change analysis and FDR correction:** particularly for transcriptomic and proteomic datasets, using packages such as DESeq2.
- **Bioinformatics Tools:**
    - **Transcriptomics:** Alignment and differential expression analysis (e.g., STAR/HISAT2, DESeq2), pathway enrichment (KEGG, GO).
    - **Proteomics:** Spectrum analysis and quantification (e.g., MaxQuant), with follow-up annotation.
- **Optional Machine Learning:** Principal Component Analysis (PCA) and clustering techniques may be explored to visualize global expression shifts and group similar response patterns in omics datasets.

VII. Time Schedule and Milestones

Month	Task	Milestones
1–2	Lab setup, consumables/equipment purchase	Functional HTS station
3–5	Isolate acquisition, protocol validation	Pilot screen completed
6–8	Full HTS runs, hit identification	Shortlist of hits
9–10	MIC/time-kill assays	Phenotypic confirmation
11–13	Transcriptomic/proteomic assays	Mechanistic insight
14–15	Synergy testing (checkerboard)	Combination leads
16–18	Data integration, manuscript prep	Final dataset, draft paper

VIII. Roles and Responsibilities

Team Member	Role	Tasks
PI (Myself)	Lead investigator	Project oversight, study design, analysis
Research Assistant 1	Lab operations	Culture handling, HTS execution
Research Assistant 2	Data analysis	Omics processing, statistical analysis
Collaborating Clinician	Clinical isolate access	Selection and transfer of isolates
Bioinformatician (part-time)	Transcriptomics support	RNA-seq and proteomic pipeline

Expected Outcomes (Achievements)

This project is expected to yield substantial scientific, clinical, and translational outcomes that directly address the urgent need for effective therapies against multidrug-resistant (MDR) pathogens in the UAE.

I. Scientific Publications

We anticipate producing **2–4 peer-reviewed publications** in high-impact journals such as *Nature Microbiology*, *ACS Infectious Diseases*, *Frontiers in Pharmacology*, and *Theranostics*. Manuscripts will cover:

- High-throughput screening (HTS) results of FDA-approved drugs on UAE-relevant MDR pathogens.
- Mechanistic insights from transcriptomic and proteomic analyses.
- Synergistic drug-antibiotic combinations with clinical potential.

II. Intellectual Property

Novel drug combinations and repurposed agents identified through this study will support **patent filings**, including:

- New antimicrobial indications for existing drugs.
- Synergistic therapeutic strategies.
- Screening and assay innovations.

A **provisional patent strategy** and collaboration with the tech transfer office will guide potential commercialization.

III. Industrial and Clinical Applications

Findings will facilitate **preclinical development** of promising repurposed therapies, especially combinations suitable for **deployment in UAE hospitals**. The use of already-approved drugs ensures **cost-efficiency** and **rapid translational potential** (Liu et al., 2021; Farha et al., 2019).

IV. Capacity Building and Know-How

The project will enhance **institutional capabilities** in AMR research and establish local expertise in:

- High-throughput screening and synergy testing.
- Integration of phenotypic and omics-based methodologies.

These outcomes will contribute long-term value to UAE's biomedical research infrastructure.

Qualifications of the PI and other team members: (Maximum 2 pages). Briefly describe the PI's role in the project and highlight how their research experience will enable them to use the tools and methods required to meet project objectives. For a position "To be announced" (TBA), the required qualifications should be noted.

Supporting Letter from the International Collaborator (if applicable): A letter of support indicating the willingness of the Co-PI to contribute to the activities of the project and their specific role must be attached.

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Budget Justification

This budget supports the execution of a comprehensive study to repurpose FDA-approved drugs against multidrug-resistant (MDR) pathogens prevalent in the UAE. The project involves high-throughput screening (HTS), synergy testing, and mechanistic studies (transcriptomics/proteomics).

1. Personnel

- **Principal Investigator (PI):** No direct salary requested. The PI will commit significant time to supervision, data analysis, and manuscript preparation.
- **Research Assistant (TBA):** Full-time for 24 months.
    - Salary includes compensation for conducting lab assays (MIC, checkerboard, RNA extraction), maintaining bacterial cultures, and supporting omics workflows.

2. Equipment

- **Automated Pipette Dispenser:** Essential for HTS to improve reproducibility and reduce manual workload.
- **Digital Plate Reader:** Required for OD600, fluorescence, and luminescence measurements.
- **Shaking Incubator:** For uniform growth of bacteria in 96-well plates.
- **Magnifier (for endpoint scoring):** For visual clarity in checkerboard assays.

3. Consumables

- **FDA Drug Library (subset):** Commercially available small molecule library of FDA-approved compounds (approx. 1,000–2,000).
- **96-Well Plates (flat-bottom):** For all screening and checkerboard assays.
- **Bacterial Media & Reagents:** Mueller-Hinton broth, LB media, agar, PBS, etc.
- **RNA Extraction Kits:** For RNA-seq preparation.
- **Protein Extraction Reagents:** For LC-MS/MS proteomics analysis.
- **Antibiotic Standards:** Colistin, tigecycline, and other first-line drugs for synergy testing.
- **Sterile Filters, Tips, Tubes:** Standard molecular biology disposables.

4. Services

- **RNA-Sequencing (outsourced):** Includes library prep and sequencing (e.g., Illumina platform).
- **LC-MS/MS Proteomics (outsourced):** Protein identification and quantification from treated/untreated samples.
- **Bioinformatics Support (consultant or collaboration):** Data analysis for transcriptomics/proteomics.

5. Travel

- **Local Travel:** For PI and team to coordinate with hospital microbiology labs, collect isolates, or attend local AMR workshops/conferences.

6. Institutional Overheads

- Institutional overhead will be charged as per policy (if applicable).

Total Budget Estimate/Annual Budget Summary

Category	Year 1 (AED)	Year 2 (AED)	Total (AED)
RA Salary	1,00,000	1,00,000	2,00,000
Equipment	1,00,000	–	1,00,000
Consumables	50,000	50,000	1,00,000
Dissemination	12,000	12,000	24,000
Travel	–	15,000	15,000
International Collaboration	25,000	25,000	50,000
Total	**2,87,000**	**2,02,000**	**4,89,000**