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Novel Nanoparticle therapeutics as alternatives to antibiotics to control *Escherichia coli* bacterial infection in ruminants in Ethiopia:

A Strategic Alternative to Antibiotics

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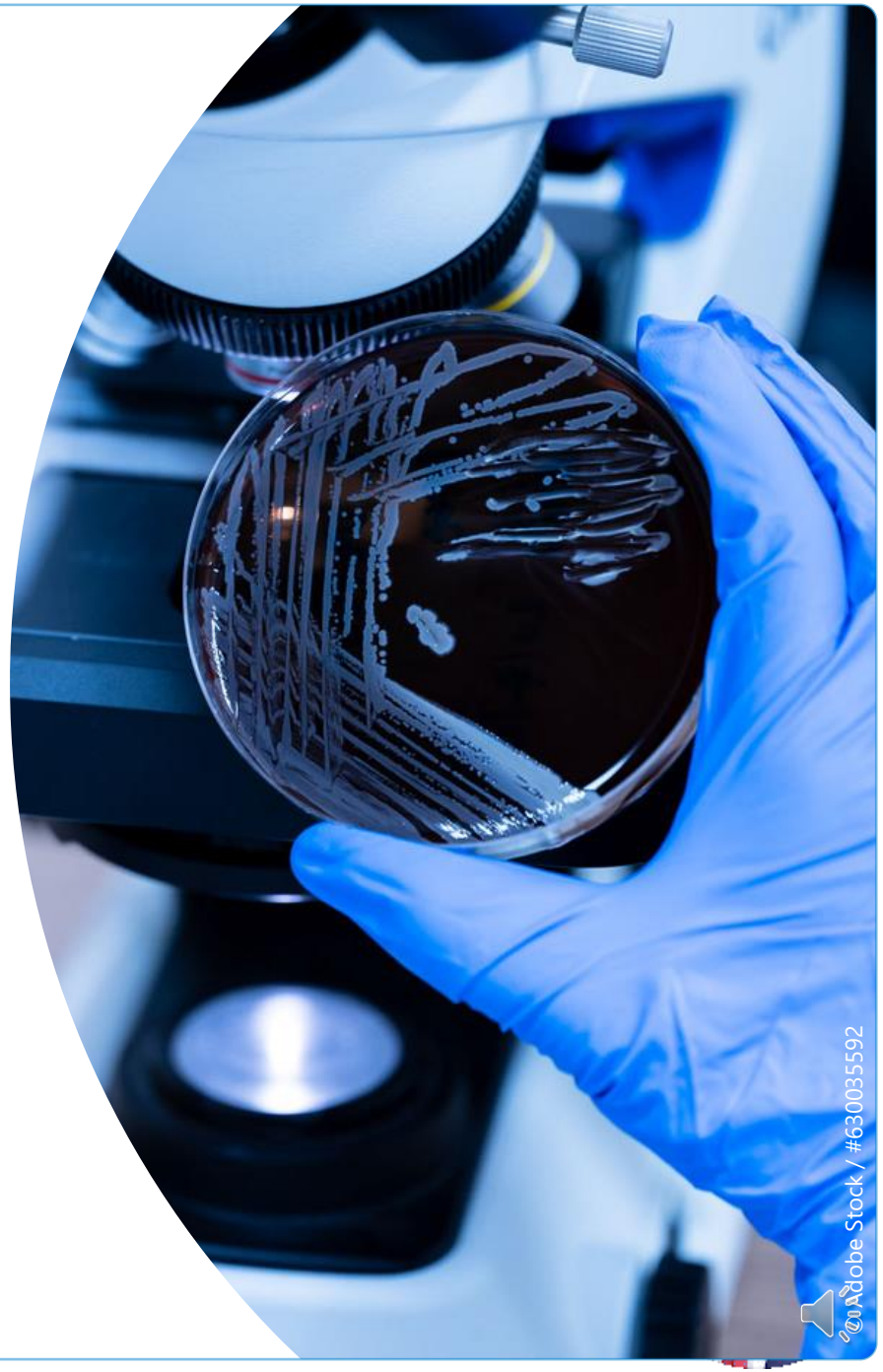
1. Introduction

- AMR:
  - Global concern:
    WHO designates AMR as one of the top 10 global health threats
  - Ethiopia's context: High burden of infectious diseases, limited surveillance, and antibiotic misuse



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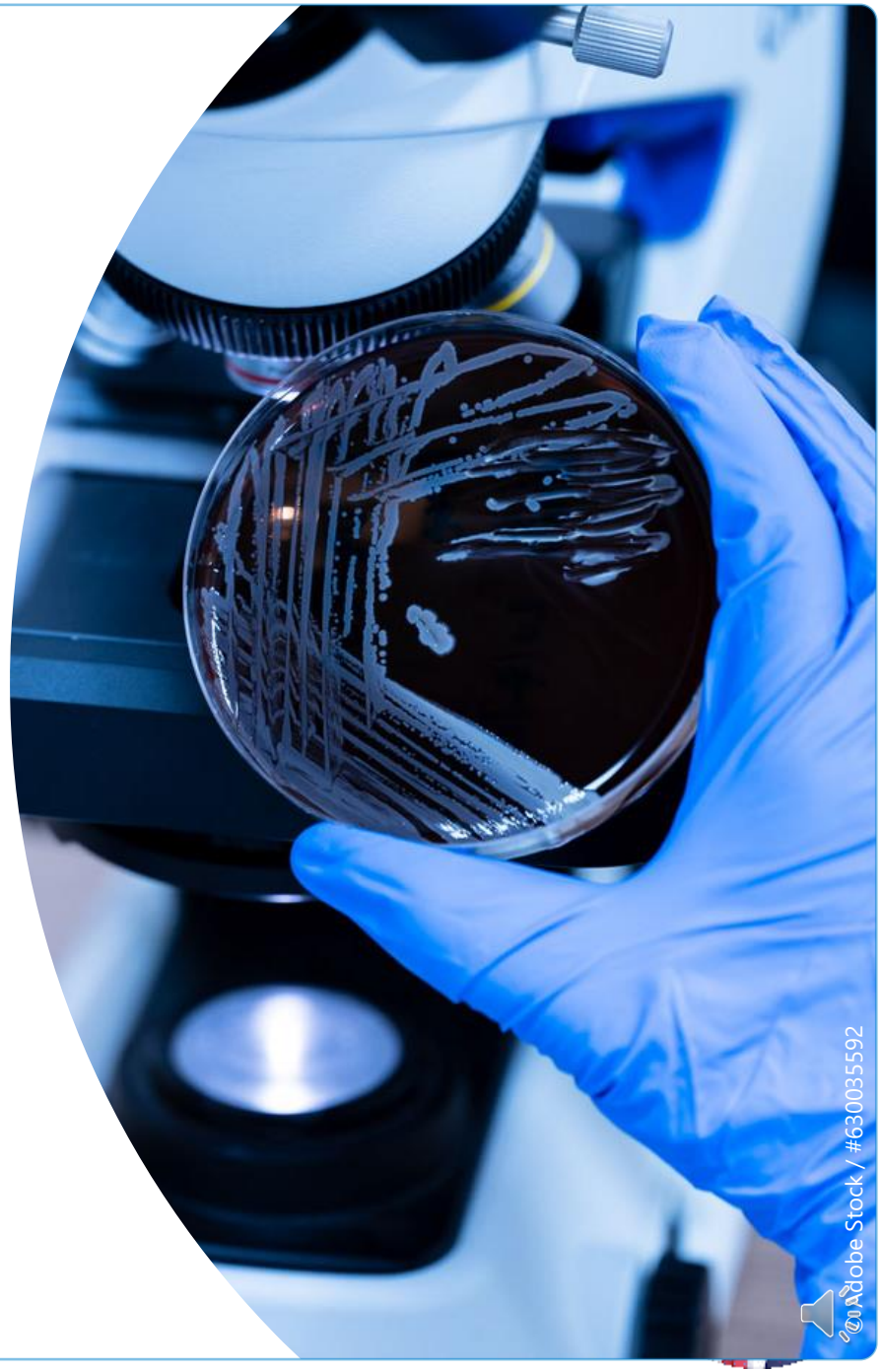
AMR Landscape in Ethiopia

- Rising resistance in livestock and human pathogens (e.g., *E. coli*, *Salmonella*, *Staphylococcus*)
- Drivers of AMR:
  - Overuse/misuse of antibiotics in veterinary and human medicine
  - Lack of regulatory enforcement
  - Informal drug markets and self-medication
  - Surveillance and Data Gaps (Limited national AMR surveillance systems, Fragmented reporting across sectors, Need for One Health integration)
- Consequences:
  - Treatment failures
  - Economic losses in agriculture
  - Threat to food safety and public health



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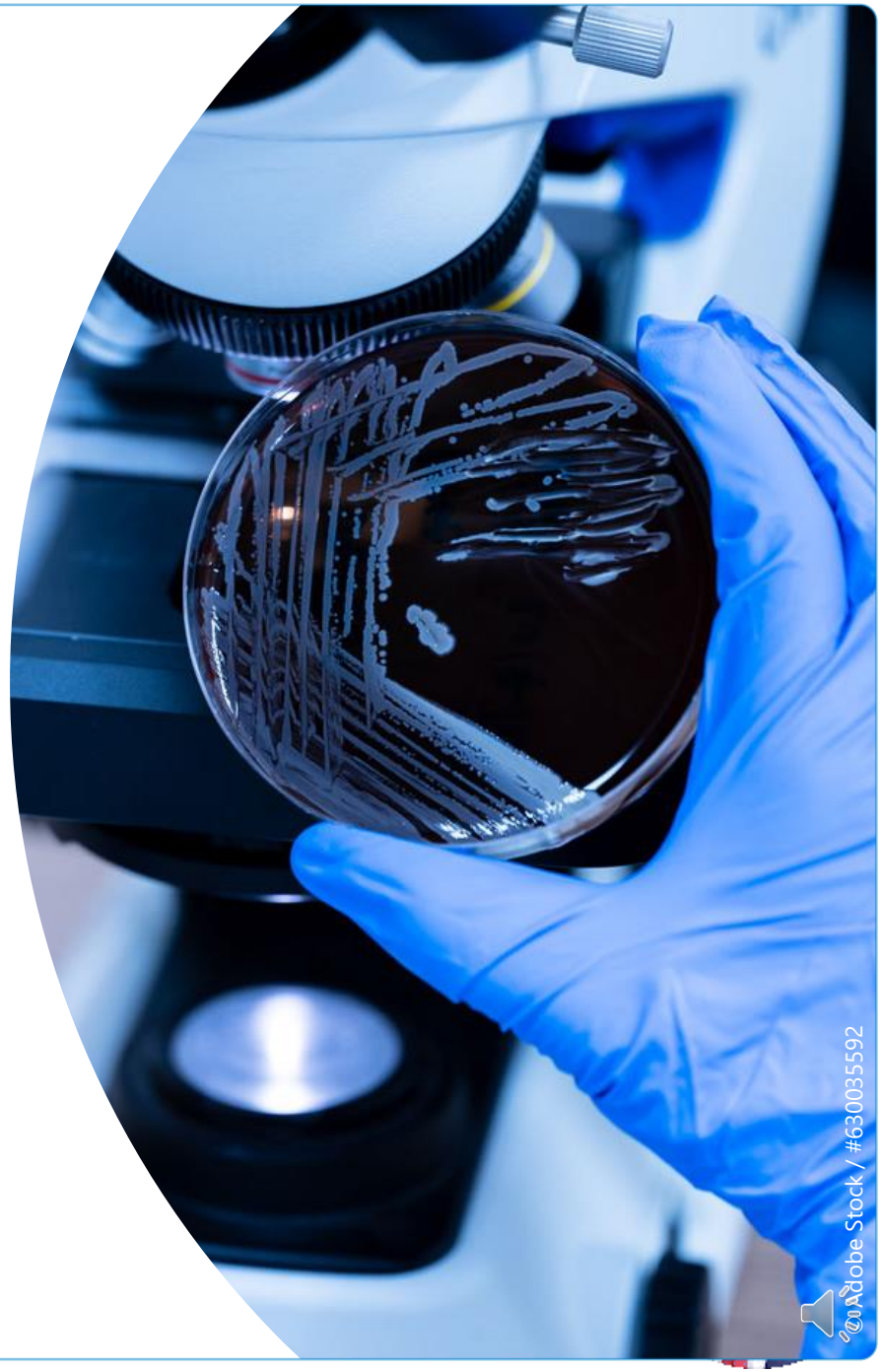
AMR Mitigation Strategies

1. **Vaccines**
2. **Rational Use of Antibiotics**
3. **Antimicrobial Alternatives** (Probiotics, prebiotics, and phytogenics in livestock feed, Bacteriophage, Immunomodulators)
4. **Public Awareness**



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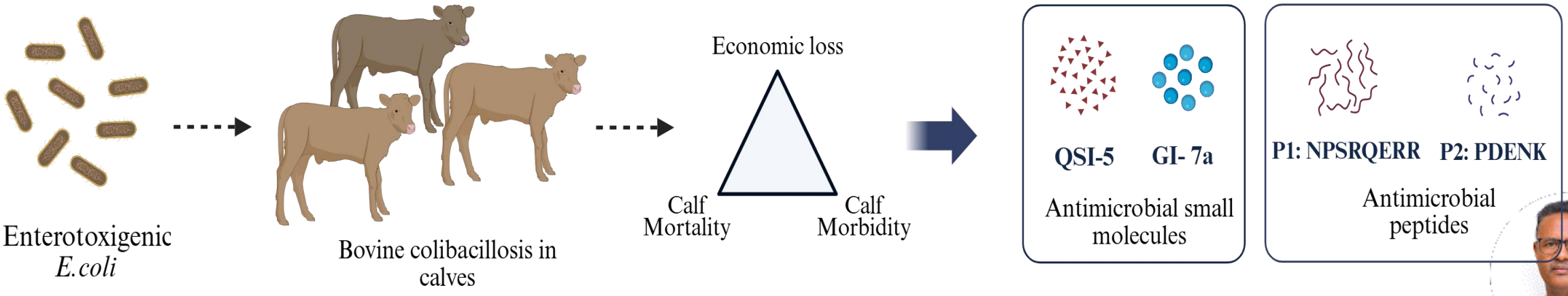
2. Project Background

- **Major Constraints and threats of AMR in Ethiopian Dairy Sector:**
  - Ethiopia has Africa's largest cattle population; dairy is rapidly growing.
  - Calf mortality (14.8%) and mastitis are leading production challenges
  - **There is high ETEC Burden in young calves and AMR Threat**
  - About 32% of diarrheagenic E. coli in calves are ETEC.
  - E. coli acts as a reservoir for AMR genes
  - 90% of isolates show multidrug resistance to ≥3 antibiotic classes

- **Limitations of Current Treatments**
  - High resistance rates to common antibiotics used:
    - tetracycline (63.8%),
    - ampicillin (55.8%),
    - sulfamethoxazole + trimethoprim (75%).
  - No effective vaccines exist due to strain diversity
- **Antimicrobial small molecules (QSI-5 & GI-7) and antimicrobial peptides (P1 & P2) could be alternative solutions in treat antibiotic resistant bacteria**



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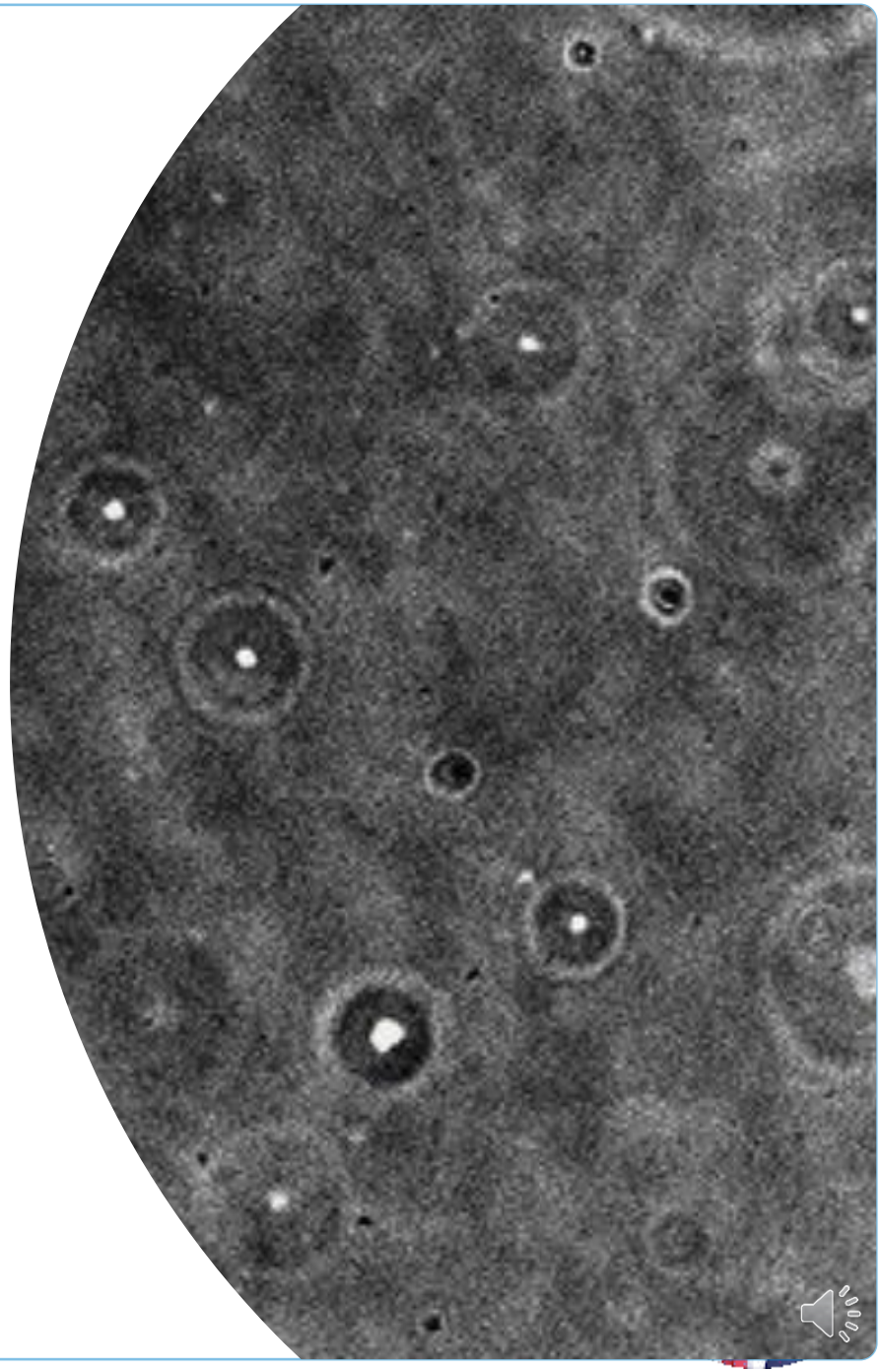
WHY ANTIMICROBIAL SMALL MOLECULES AND PEPTIDES?

- QSI-5 disrupts bacterial communication via quorum sensing inhibition
- GI-7a compromises bacterial membrane integrity
- Peptides P1 & P2 target membrane proteins and gene expression



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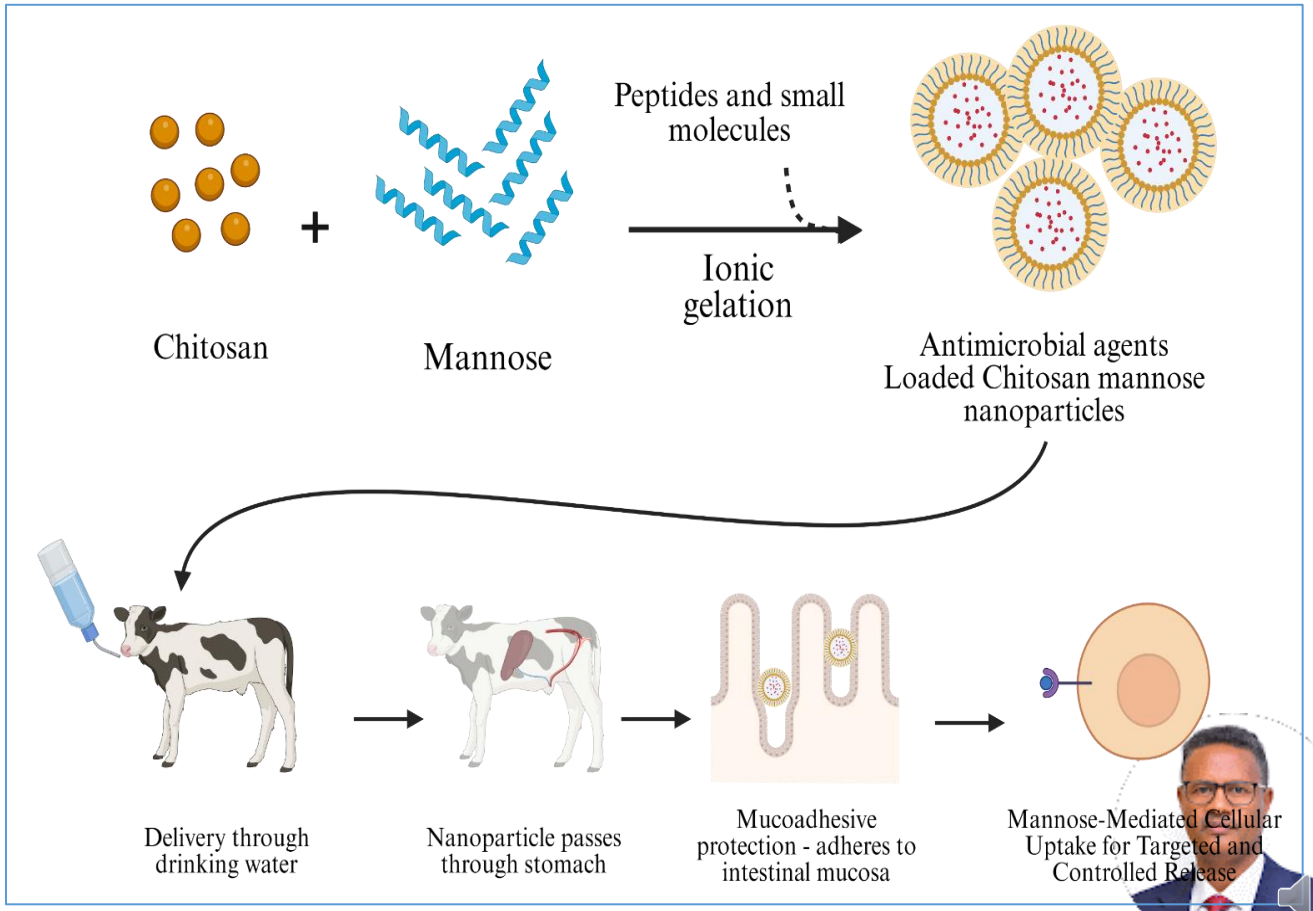
CHITOSAN - MANNOSE NPs: EMERGING AS AN EFFECTIVE ORAL DELIVERY PLATFORM

- Biocompatible, biodegradable, and mucoadhesive polymer NPs, **ideal for oral delivery**, are efficiently taken up by **intestinal M cells** and **dendritic cells**.
- Mannose-functionalized chitosan NPs **enhance targeted uptake** and are **promising carriers** for small molecule inhibitors and antimicrobial peptides (AMPs) to treat calf diarrhea.



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3. Project Objectives

Objective 1

Synthesis and Characterization

(University of Illinois, Urbana Champaign, UIUC)

Synthesis & development of NPs

- Analysis part (NMR, IR, MS, Size, zeta potential, UV, XRD, SEM)
- Drug stability

Objective 2

Safety & efficacy evaluation (Animal Experiment)

(National Veterinary Institute, NVI)

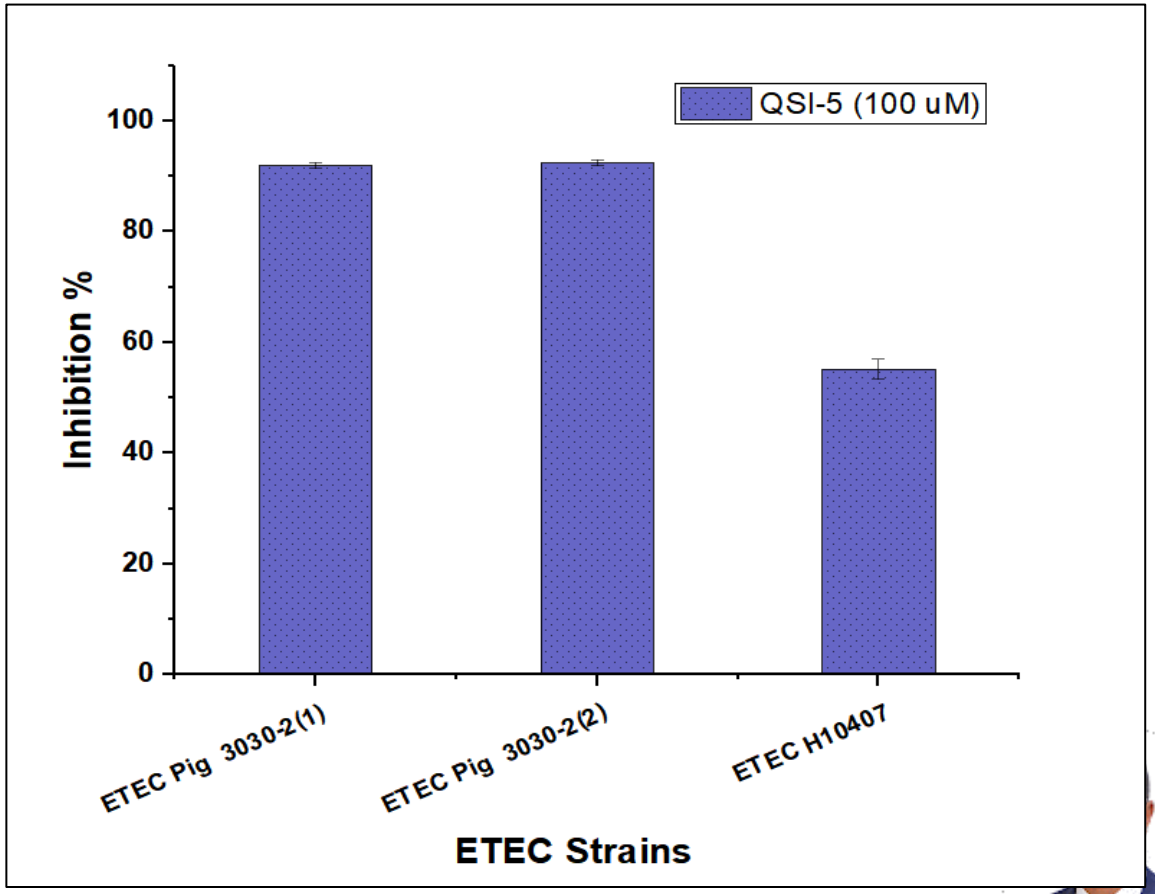
- ETEC Challenge dose optimization in calves
- Drug dose optimization in calves
- POC experiment in calves to evaluate the Efficacy, safety, and applicability of lead-microbial (Drug loaded NPs)
- PK and PD studies

4. Results

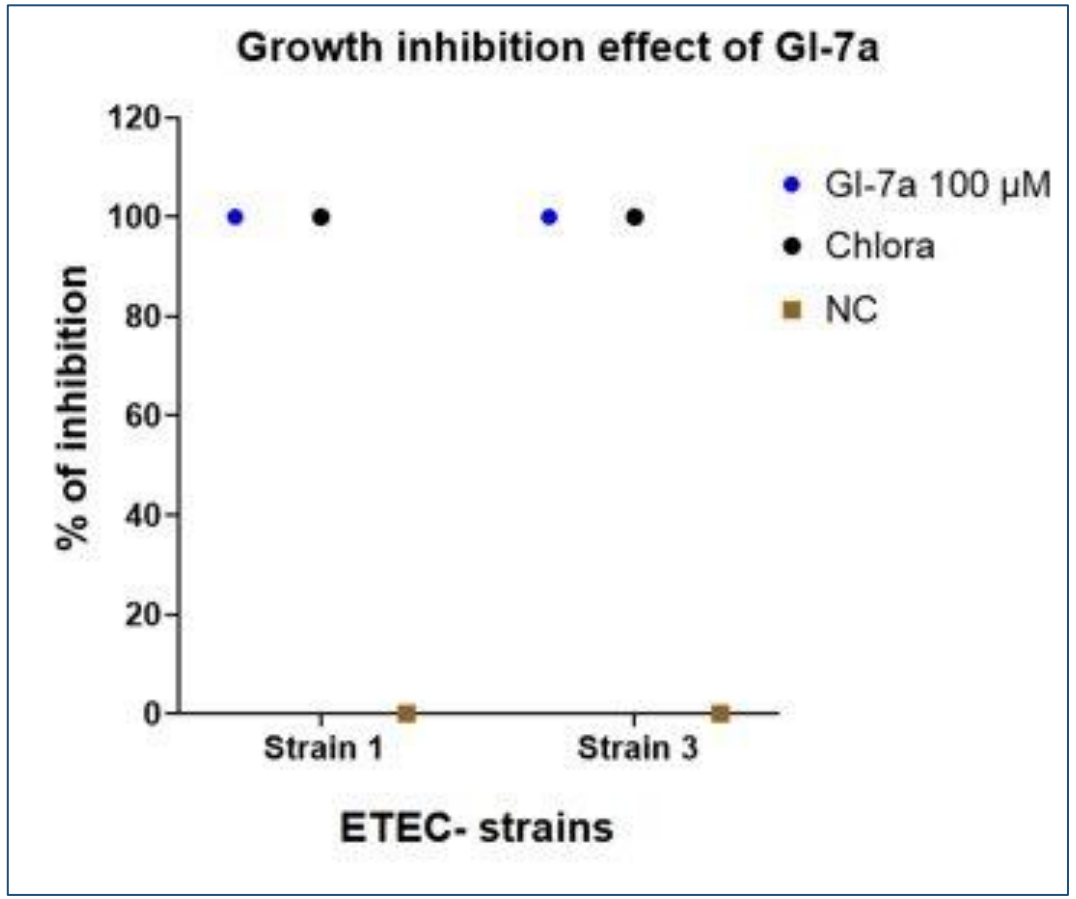
IN VITRO ANALYSIS OF SMALL MOLECULES AND PEPTIDES

QSI-5 Suppresses AI-2 signaling in pig and human ETEC

- QS-5 demonstrated **significant inhibition of AI-2 up to 92%** in both Pig ETEC strains [ETEC Pig 3030-2, ETEC Pig 3030-2
- A **moderate inhibition of 52%** was observed in the ETEC Human-10407 strain.



EVALUATION OF GI-7a FOR GROWTH INHIBITION IN PIG AND HUMAN ETEC ISOLATES



- The graph shows the growth inhibition of ETEC strains **treated with GI-7a (100 µM),** compared to chloramphenicol (20 µg/ml) as a positive control and 1% DMSO as a negative control.

- Both GI-7a and chloramphenicol achieved nearly 100% **inhibition in pig (strain 1) and human (strain 3) isolates**, while the negative control showed no activity.

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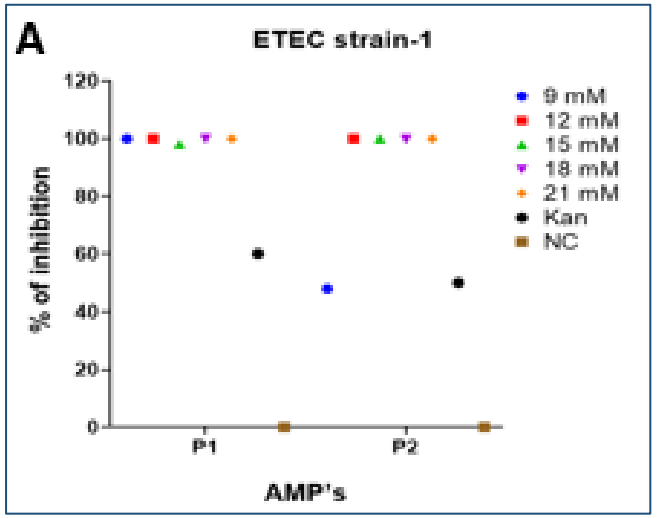
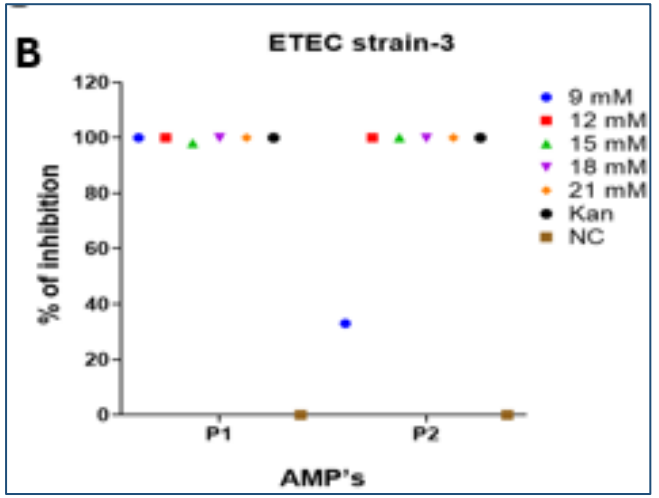
ASSESSMENT OF GROWTH INHIBITION BY P1 AND P2 PEPTIDES ON ETEC

- Peptides P1 and P2 were tested at concentrations of **9 mM, 12 mM, 15 mM, 18 mM, and 21 mM** for the treatment. Bacteria grown in **1% DMSO (Negative control; NC), kanamycin-50 µg/mL** (Kan) were used as controls.





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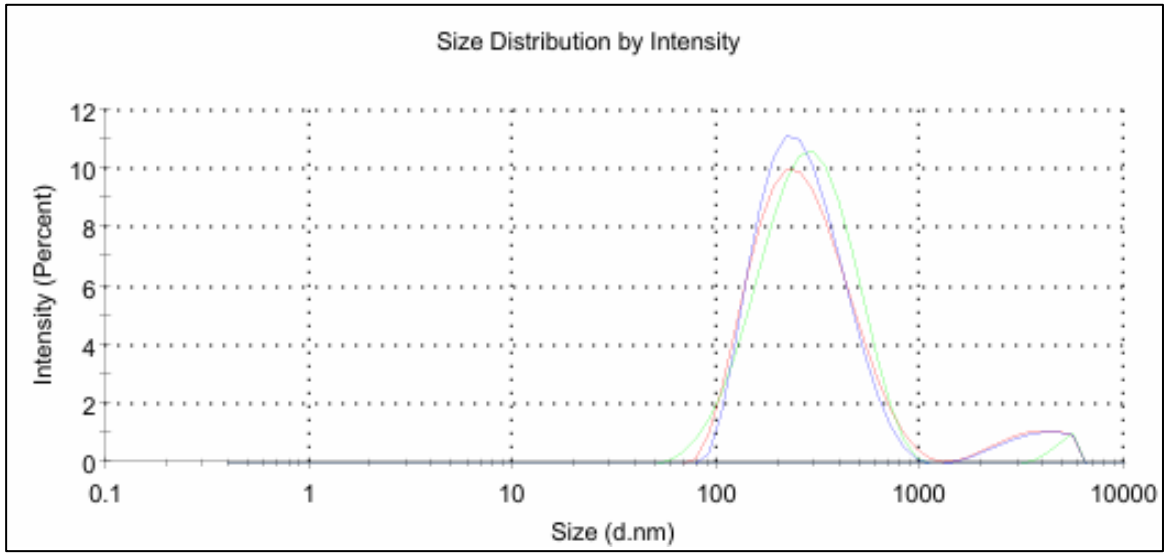
CHARACTERISTICS OF CHITOSAN MANNOSE NANOPARTICLE



Nanoparticle Size Distribution Graph

Nanoparticle	Size (nm)	Polydispersity Index	Zeta Potential
QS-5	285.9	0.3	+30
P1	198.6	0.259	+33.8
P2	271	0.358	+37

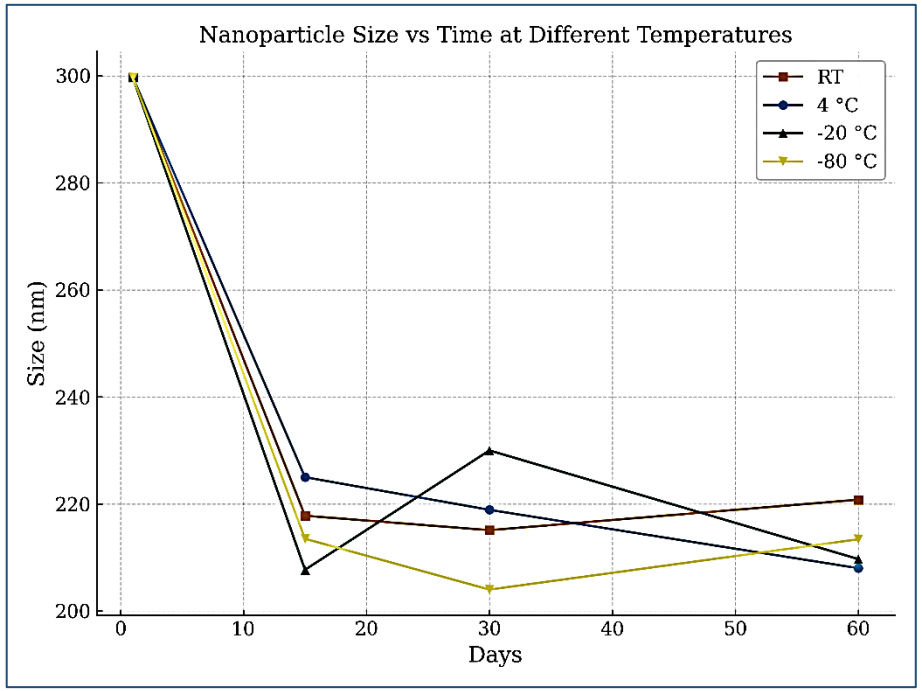
LYOPHILIZATION AND CRYOPROTECTANT ANALYSIS

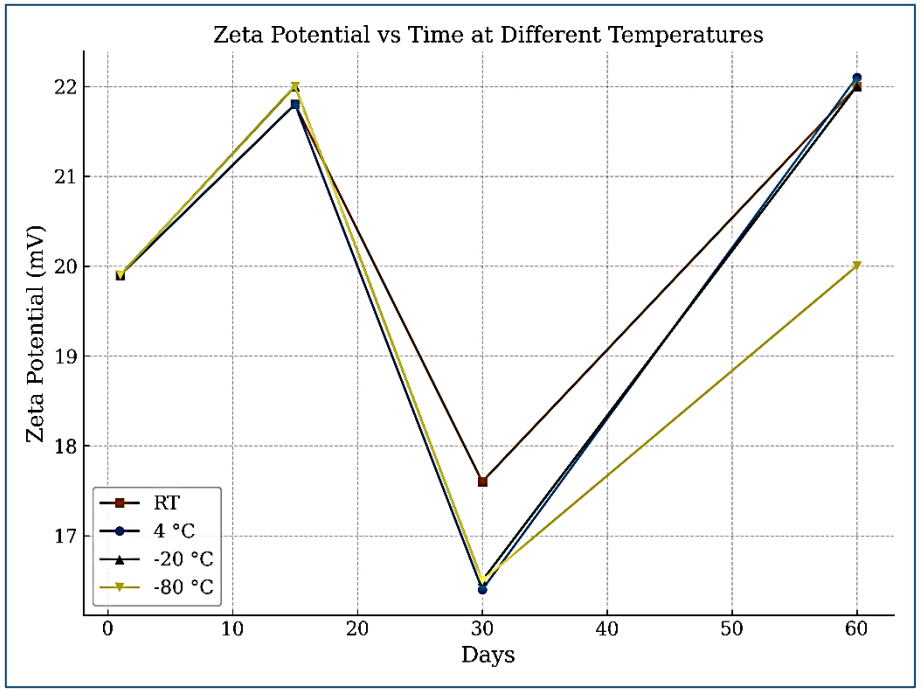
- Nanoparticles in liquid form can aggregate or degrade over time.
- **Lyophilization (freeze-drying)** removes water under low pressure after freezing at –50 °C.
- Cryoprotectants like **sucrose** are added to **prevent structural damage** during freezing and drying.
- Cryoprotectants enhance the stability of nanoparticles during lyophilization.

Nanoparticle sample	Size (nm)	Zeta potential	Inference
Before lyophilization	308.3	+ 24.2	Stable with accurate size
1% sucrose	298.4	+ 19.3	No changes and Ideal
5% sucrose	299.7	+ 19.9	No changes
10% sucrose	283.8	+ 16.5	No changes
Without cryoprotectant	150,000	+ 17.1	Size increased

TEMPERATURE STABILITY ASSESSMENT OF NANO PARTICLES

- QSI-5 nanoparticles (lyophilized with sucrose) were stored at RT, 4 °C, −20 °C, and −80 °C for 60 days and were assessed for stability.
- Particle size remained stable (**204–300 nm**). Zeta potential showed minimal change **(+16.4 to +19.9 mV**).





No substantial impact of storage temperature on nanoparticle stability over 60 days

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Safety and efficacy evaluation of Nano-particle therapeutics in Animal experiments at NVI, Ethiopia

- **Preparation of challenge ETEC strains**
  - ETEC strains isolated from cases of calf diarrhoea
  - Identification and characterization of ETEC strains done (phenotypic and molecular)
  - Nalidixic resistant challenge ETEC strain generated
- **Challenge dose optimization experiment is underway**

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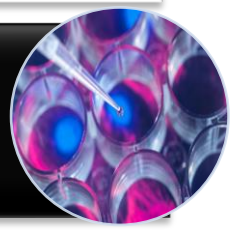
6. Future plans

- Encapsulation efficiency
- Loading capacity
- Temperature & pH stability
- In vitro release study and kinetics

INVITRO STUDIES

- Nanoparticle required for dose optimization studies in animal models will be prepared and shipped to Ethiopia

BULK NANOPARTICLE PRODUCTION



- The produced QS-5, GI-7a, P-1 and P-2 nanoparticles will studied in Calf models at NVI, Ethiopia
  - Optimum dose determination
  - POC experiment to evaluate the safety & efficacy of drug loaded NPs

CALF MODEL TRIALS

7. Strategic Impact of the project

- **Supports Ethiopia's One Health goals**
- **Strengthens dairy sector resilience**
- **Provides scalable platform for broader bacterial disease control in LMICs**

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