The Synergistic Potential of Silver Nanoparticles and Cefotaxime In Extended Spectrum Beta Lactamase Producing *Escherichia coli* isolates in Tertiary Care Hospital

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Presenter
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INTRODUCTION

- **Extended-Spectrum Beta-Lactamases (ESBLs)** are enzymes synthesized by bacteria, like *Escherichia coli*, that impart resistance to many beta-lactam antibiotics, making infections harder to treat and increasing healthcare burdens.
- In 2021, about 4.71 million deaths were associated with **Antimicrobial Resistance** (AMR) and 1.14 million deaths were directly attributable to it.
- It is predicted to increase to 1.91 million attributable deaths and 8.22 million associated deaths in 2050

Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050, *Naghavi, Mohsen et al. The Lancet, Volume 404, Issue 10459, 1199 - 1226*

RATIONALE

- **Silver nanoparticles (AgNPs)** have numerous properties including antimicrobial properties.

- This study aims to explore synergistic potential of AgNPs and antibiotic (Cefotaxime) in Extended Spectrum Beta Lactamase (ESBL) producing *E. coli* from tertiary care hospital.

OBJECTIVES

1. To characterize in-house synthesized AgNPs by Particle size assay and X-ray diffraction

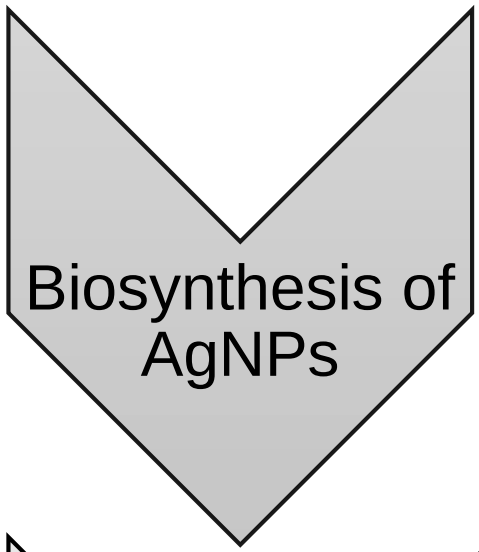
2. To evaluate the antimicrobial activity/ property of synthesized AgNPs and cefotaxime in the ESBL producing *E. coli* isolates by determining Minimum Inhibitory Concentration (MIC) of individual agent

3. To investigate the synergistic effect of AgNPs and antibiotic (cefotaxime) in the ESBL producing *E. coli* isolates from MIC of their combination

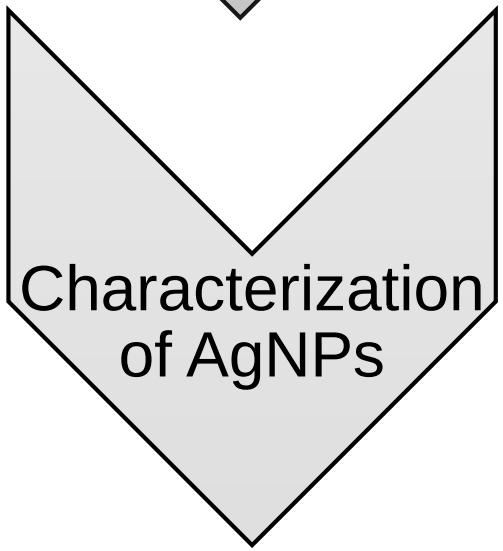
METHODOLOGY

- **Study Design**: Experimental, laboratory-based study.
- **Study site**: Department of Microbiology, Dhulikhel Hospital
- **Study population**: 32 phenotypically confirmed ESBL producing *E.coli* isolates from clinical specimens.
- **Data collection tools**:
  - Antibiotic Sensitivity tests: i.e. Kirby Bauer Method, Micro broth dilution method
  - Green synthesis of AgNPs
  - Characterization tools for silver nanoparticles: i.e. Particle size analyzer and X-ray diffraction

FLOW CHART OF PROCEDURE



- Culture *Pseudomonas aeruginosa* in Trypticase Soya Broth at 37^{o}C for 48 hours
- Prepare a culture filtrate
- Prepare 1-2 mM of silver nitrate ($AgNO_3$)
- Mix the culture filtrate and $AgNO_3$ solution in equal amount and incubate in 37^{o}C for 48 hours
- Purify the product and dry in Hot air Oven at 45^{o}C for 24 hours.



- Particle size analyzers
- X-ray Diffraction

FLOW CHART OF PROCEDURE (CONT.)

Screening of ESBL *E. coli* – with one of the two antibiotics Ceftazidime (30µg)/ Cefotaxime(30µg) as recommended by Clinical & Laboratory Standards Institute (CLSI)

Phenotypic confirmation of ESBL *E.coli* by combined disk test of Ceftazidime (30µg) and ceftazidime (30µg) + clavulanic acid (30/10 µg)

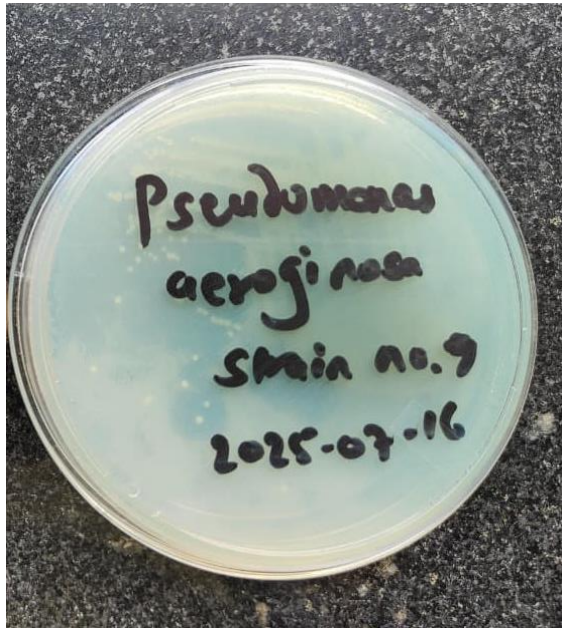
Determination of MIC by broth microdilution Method for AgNPs and Cefotaxime separately

Synergy testing by checkerboard assay to determine Fractional Inhibitory Concentration Index (FICI)

Data recording and Analysis in Excel sheet, SPSS (Wilcoxon Signed-Ranks Test)

RESULTS

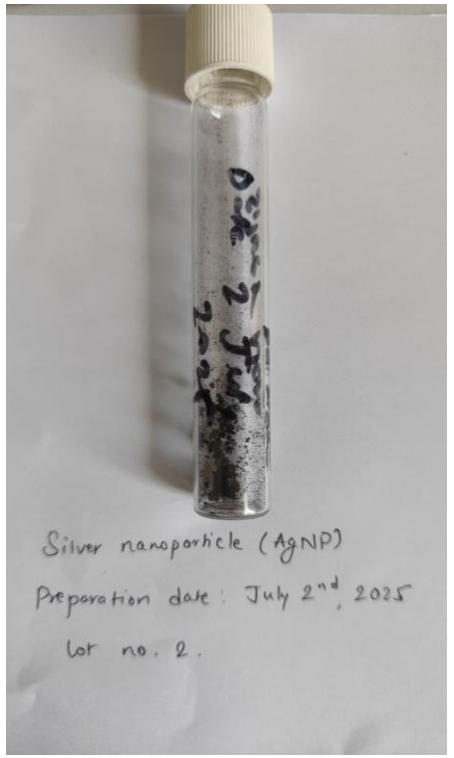
Biosynthesis of AgNPs



Pseudomonas aeruginosa

Control

AgNPs

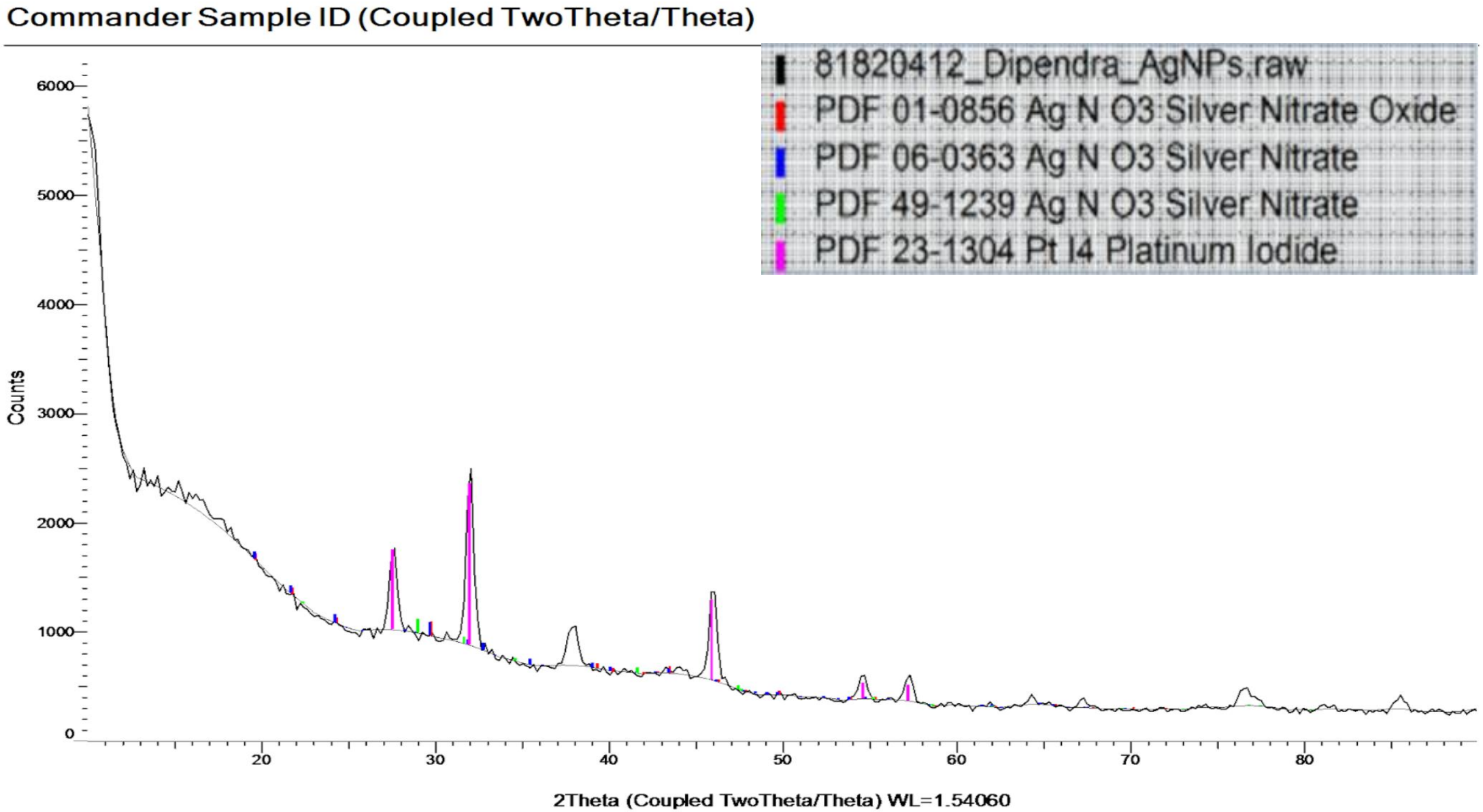


AgNPs powder

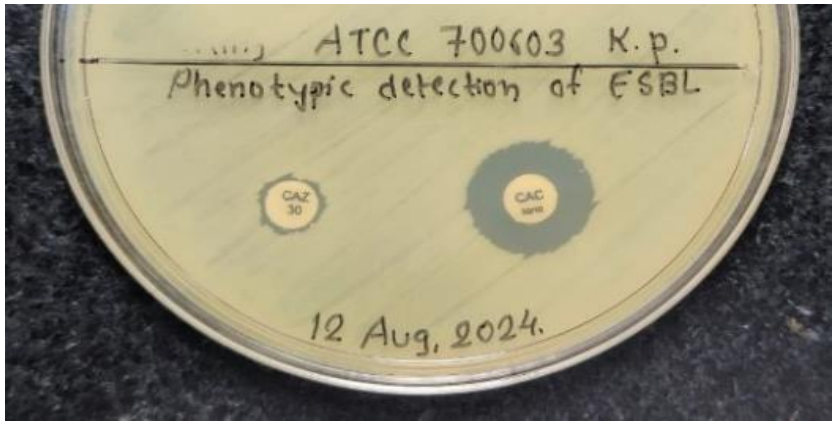
Particle size analyzer

S.N.	Characteristics	Value
1	Scattering Angle	173
2	Temperature of the Holder	24.8 ^{o}C
3	Dispersion Medium Viscosity	0.899 mPa
4	Transmission Intensity before Measurement	795
5	Representation of Result	Scattering Light Intensity
6	Count Rate	2615 kCPS
7	**Peak size (Mean)**	**100.3 nm**
8	Standard deviation	31.6 nm
9	**Total mode**	**87.8 nm**

X-ray Diffraction

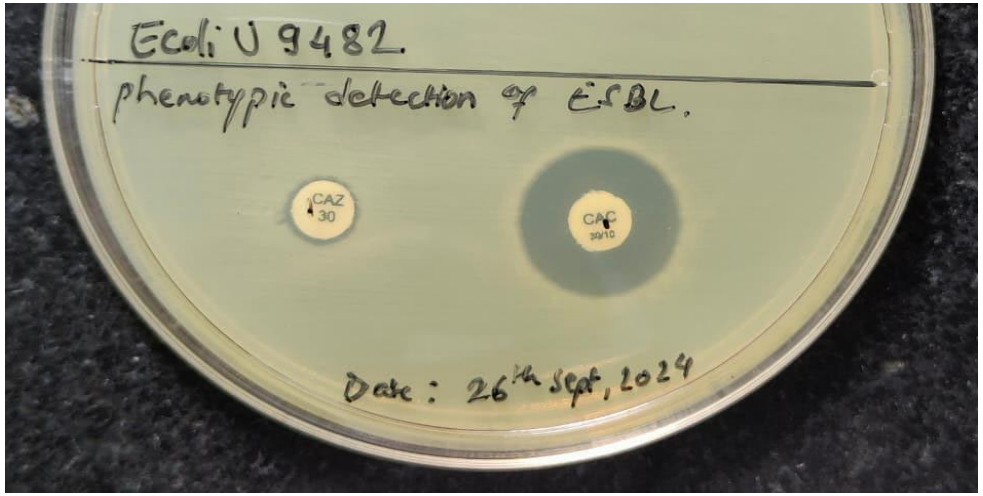


Phenotypic confirmation of ESBL *E. coli* using ceftazidime (CAZ) and ceftazidime clavulanate (CAC)



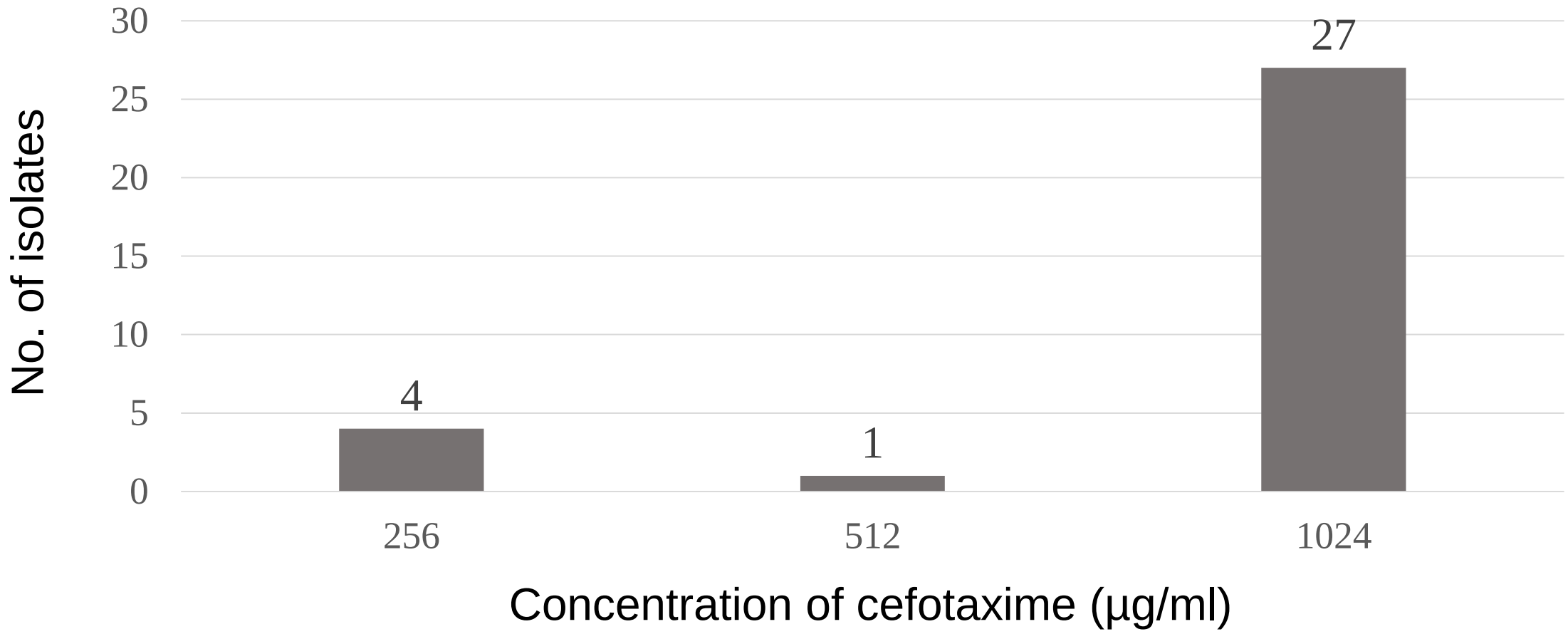
Klebsiella quasipneumoniae ATCC 700603

E. coli ATCC 25922

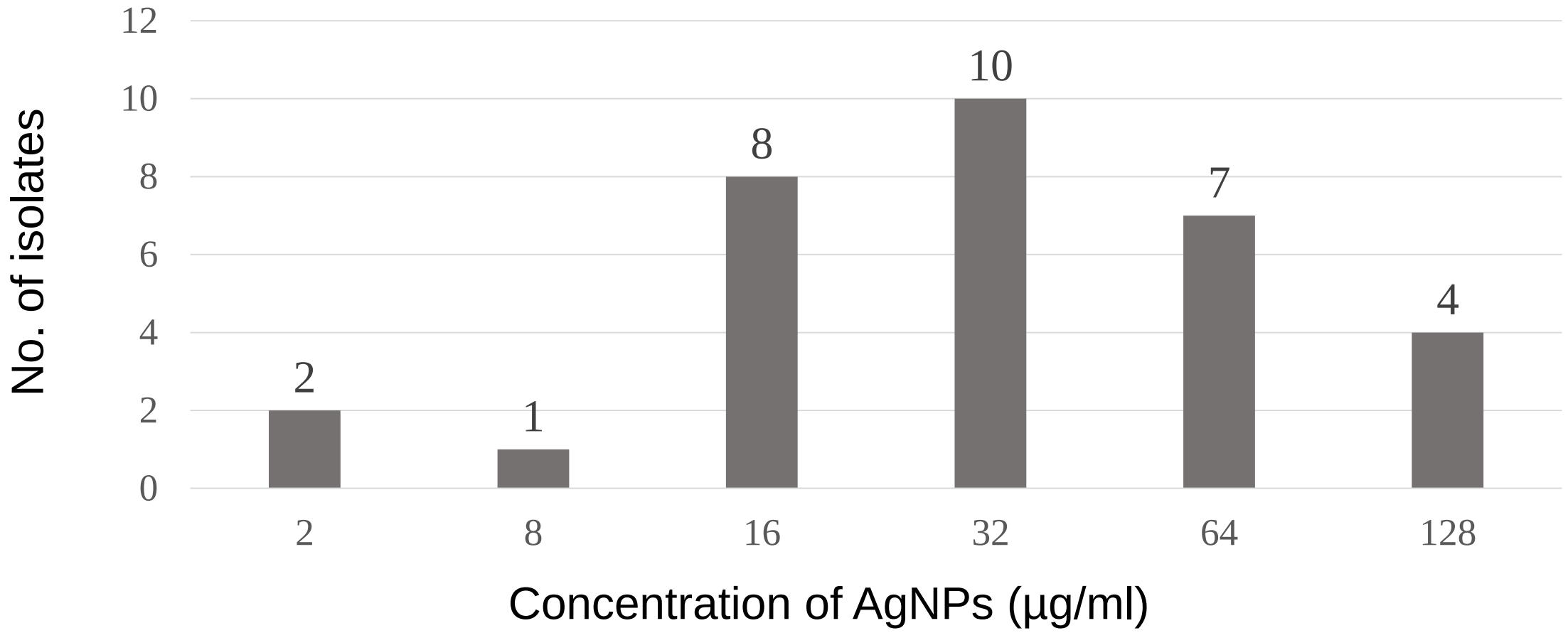


E. coli of Sample

Minimum Inhibitory Concentration of Cefotaxime



Minimum Inhibitory Concentration of Silver Nanoparticles (AgNPs)



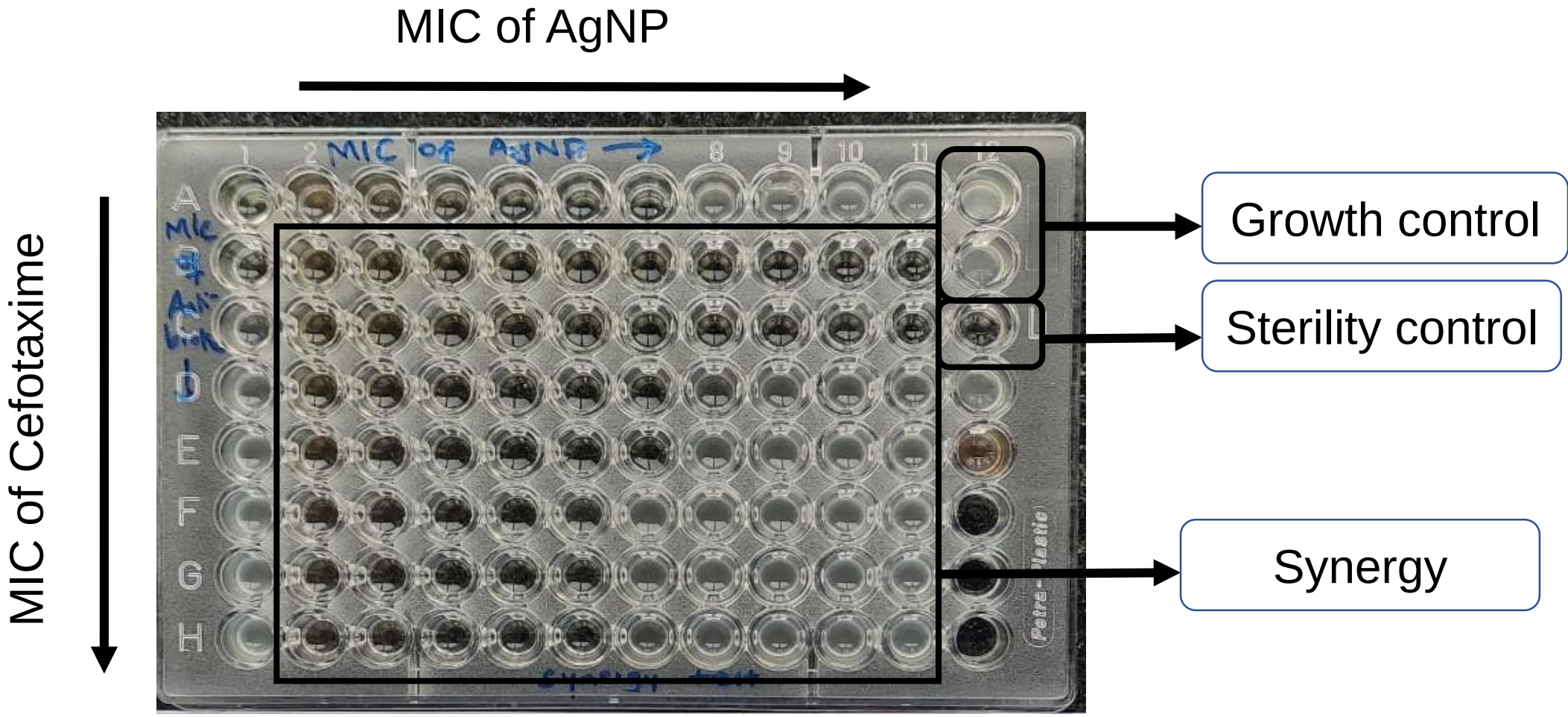
MIC of Cefotaxime in combination

Concentration (µg/ml)	Frequency	Percentage	p-value
2	12	37.5%	
4	17	53.1%	
8	2	6.3%	<0.001
16	1	3.1%	
Total	32	100%	

MIC of AgNPs in combination

Concentration (µg/ml)	Frequency	Percentage	p-value
0.125	2	6.3%	<0.001
0.250	3	9.4%	
0.500	1	3.1%	
2.000	1	3.1%	
4.000	10	31.3%	
8.000	9	28.1%	
16.000	5	15.6%	
32.000	1	3.1%	
Total	32	100%	

Synergistic effect of Cefotaxime and silver nanoparticles



Synergistic effect of AgNP and Cefotaxime in ESBL *E. coli*

Synergistic effect of Cefotaxime and silver nanoparticles

FICI	Frequency	Percentage %	Interpretation
<0.5	23	71.90%	Synergistic effect
0.5 to 1	9	28.10%	Partial synergistic effect

FICI ≤ 0.5 → Synergistic effect

0.5 < FICI < 1 → Partial synergy

FICI = 1 → Additive effect

2 ≤ FICI < 4 → Indifference

FICI > 4 → Antagonism

CONCLUSION

- The combination of AgNPs and cefotaxime demonstrated a strong synergistic antibacterial effect against ESBL producing *E. coli*.
- This suggests that AgNPs can act as potential antibiotic adjuvants, restoring or enhancing the efficacy of existing antibiotics.
- Such combinations may serve as a promising strategy to combat multidrug-resistant infections in other bacteria as well.
- Furthermore, the studies involving in vivo modalities and clinical trials are necessary to assess safety, pharmacodynamics and therapeutical potential of AgNPs and its combination with antibiotics.

Dipendra Bajracharya, a final-year postgraduate student of Medical Microbiology at Kathmandu University School of Medical Sciences has interests in infectious diseases and antimicrobial resistance.

Contribution to research: “First case report of *Schistosoma japonicum* in Nepal” “Fungal Infection among Diabetic and Non-diabetic Individuals in Nepal”

“Multidrug-Resistant Bacteria from Raw Meat of Buffalo and Chicken, Nepal”.