

Expression of *bla*_{OXA-58} is enhanced against sub-inhibitory concentration of carbapenems

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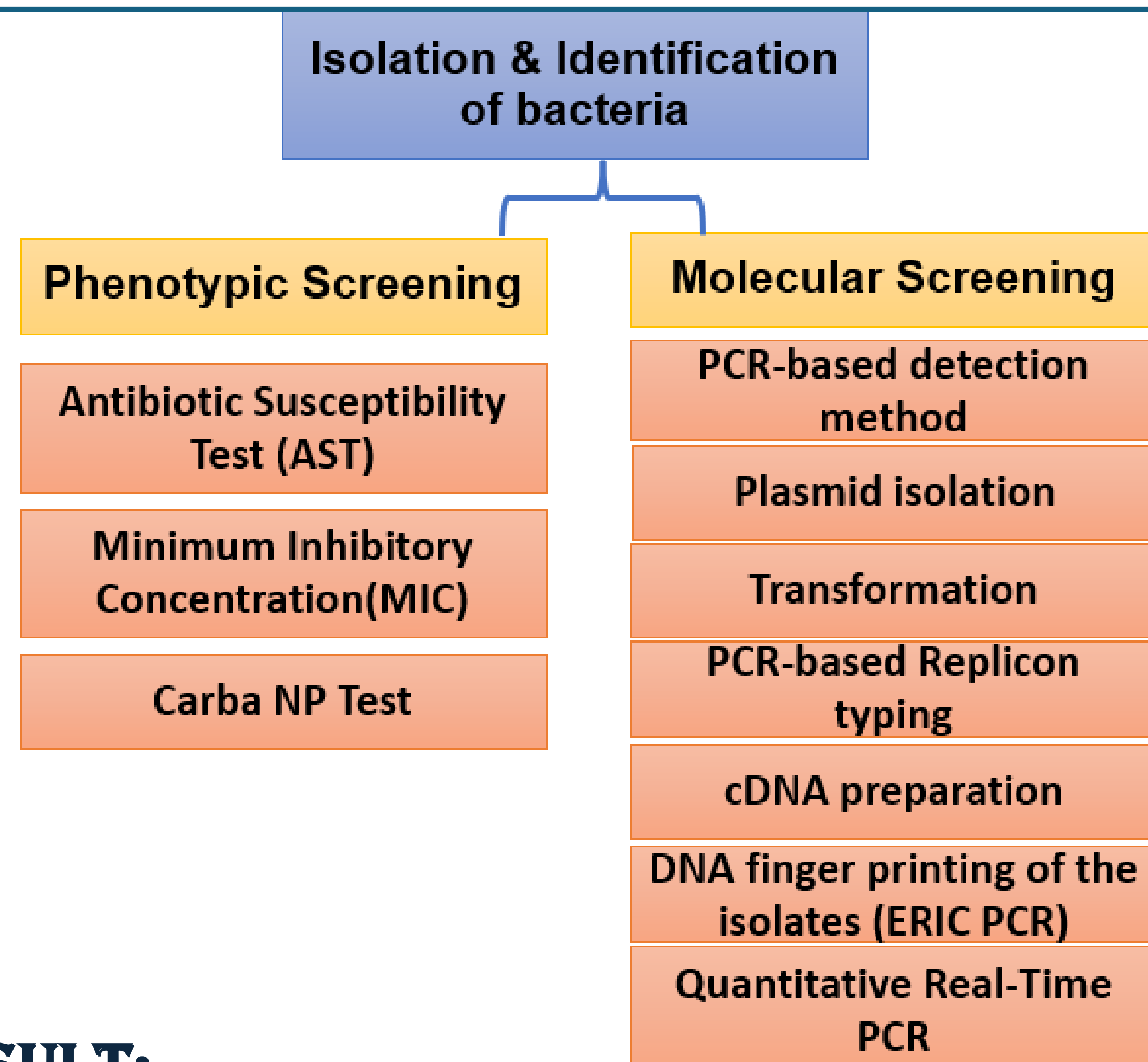
1 INTRODUCTION :

- Carbapenem resistance is primarily attributed to the production of carbapenemases. On the growing clinical significance of class D carbapenemases, the present study investigates the transcriptional response of *bla*_{OXA-58} gene in vitro when exposed to a single dosage of sub-inhibitory concentration of carbapenems
- The first member of the OXA-58 group of enzymes was identified in France in 2003. It was found in a multidrug-resistant *A. baumannii* clinical isolate that also demonstrated carbapenem resistance

2 OBJECTIVES :

- To characterize carbapenem hydrolysing class D beta-lactamase in *Escherichia coli* (*E.coli*)
- To analyse transcriptional response of *bla*_{OXA-58} under sub-inhibitory concentration

3 METHODOLOGY :



4 RESULT:

- Among 264 isolates, 46 isolates exhibited resistance towards carbapenems and are detected in Rapidec® Carba NP test

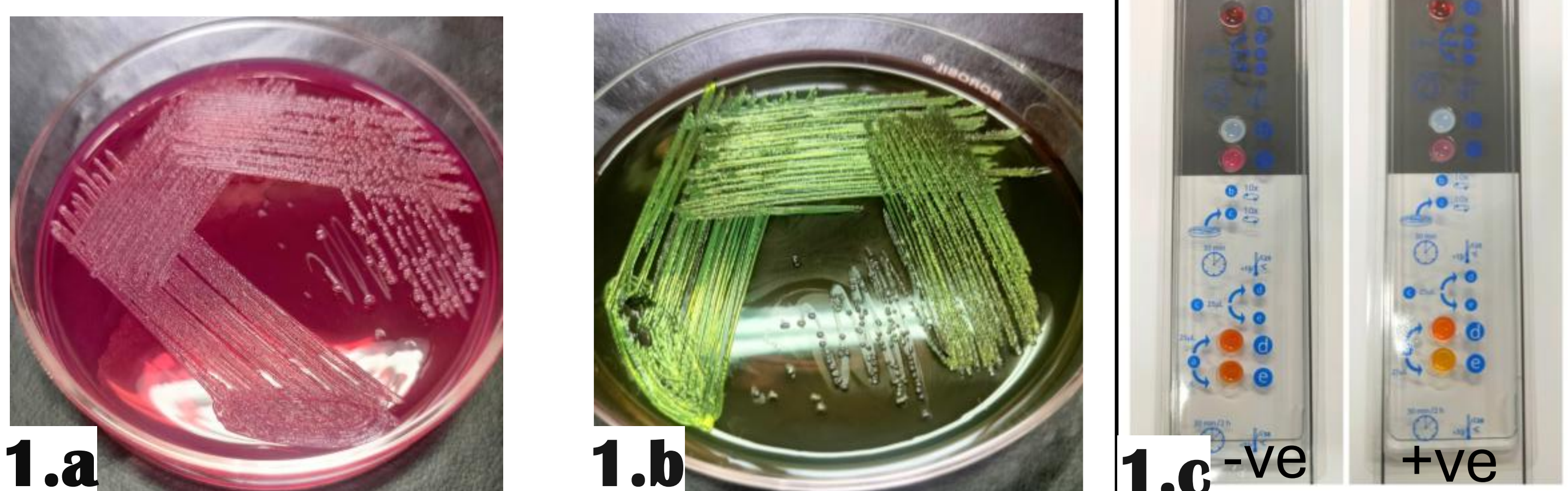


Fig1: a) Growth of *E. coli* on MacConkey agar, b) Growth of *E. coli* on EMB agar, c) Screening of carbapenemase producing *Escherichia coli* using Rapidec® Carba NP test.

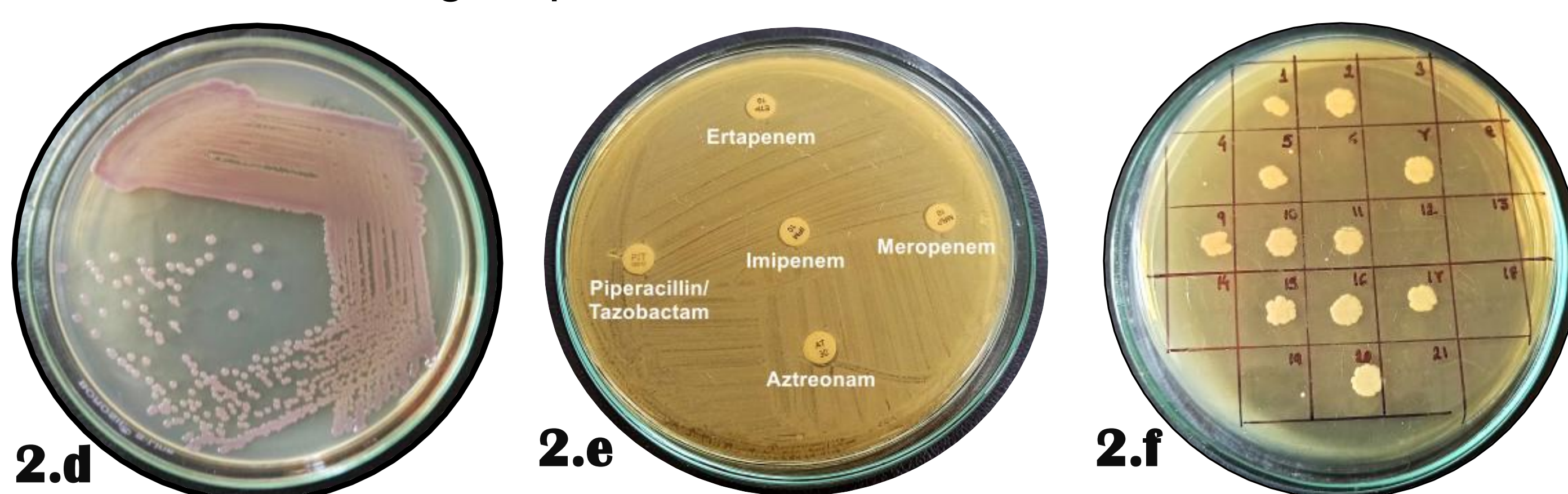


Fig2: d) Screening of carbapenemase producing *E. coli* using HiCrome™ KPC Agar e) Antibiotic susceptibility testing of isolates f) Minimum Inhibitory Concentration determination of isolates.

- Four *bla*_{OXA-58} isolates were identified, and exhibited resistance towards the antibiotics used in antibiotic susceptibility testing and showed high MIC values against carbapenems (≥ 64 μ g/ml)

PCR RESULT:

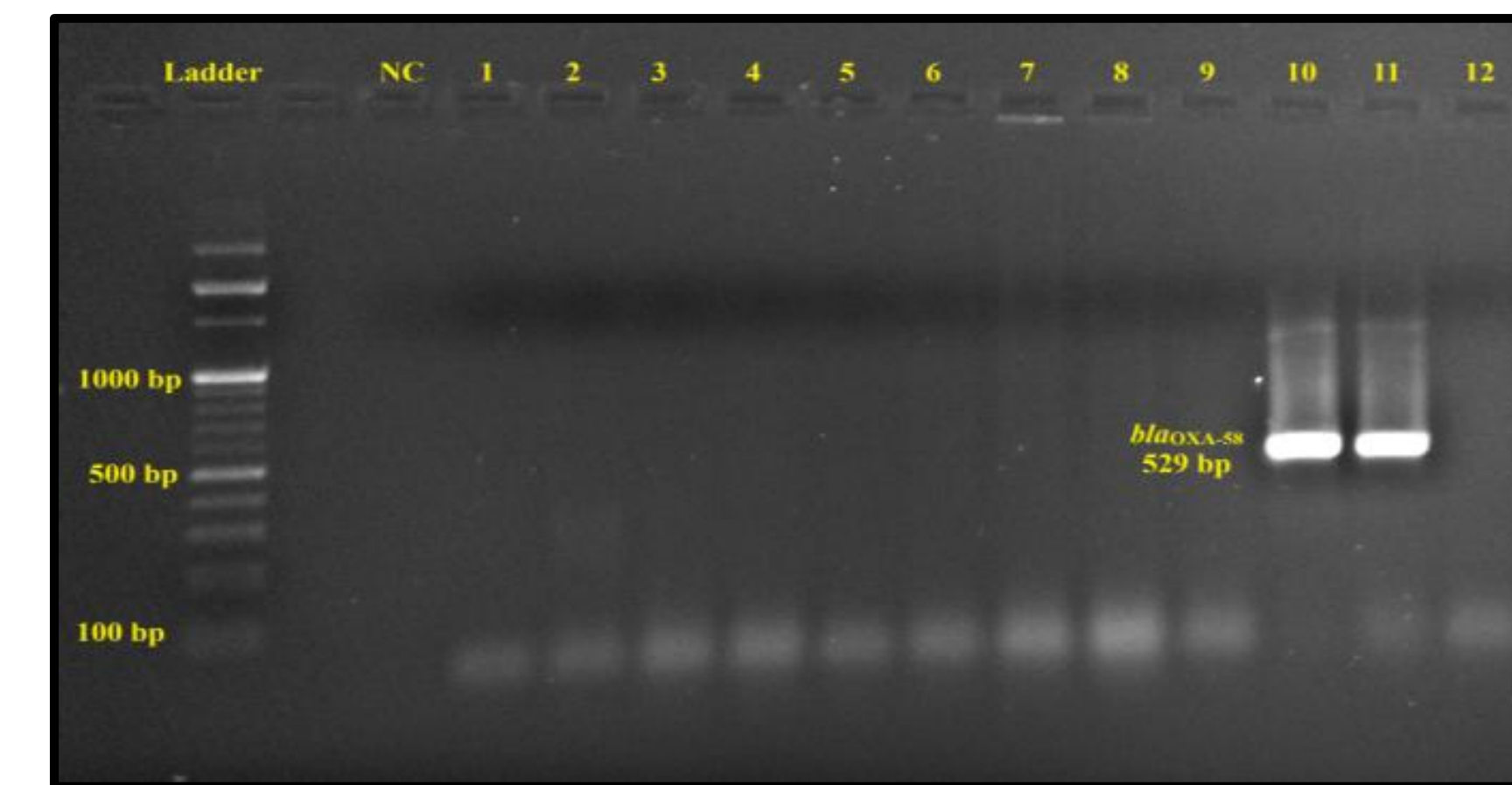


Fig 3 : Gel image showing amplification of *bla*_{OXA-58} (529 bp). Ladder: 100 bp, NC: Negative Control, and Lane 1-12: test isolates .

HORIZONTAL GENE TRANSFERABILITY & PCR BASED REPLICON TYPING:



Fig 4: Transformants plate carrying OXA-58 on LB agar

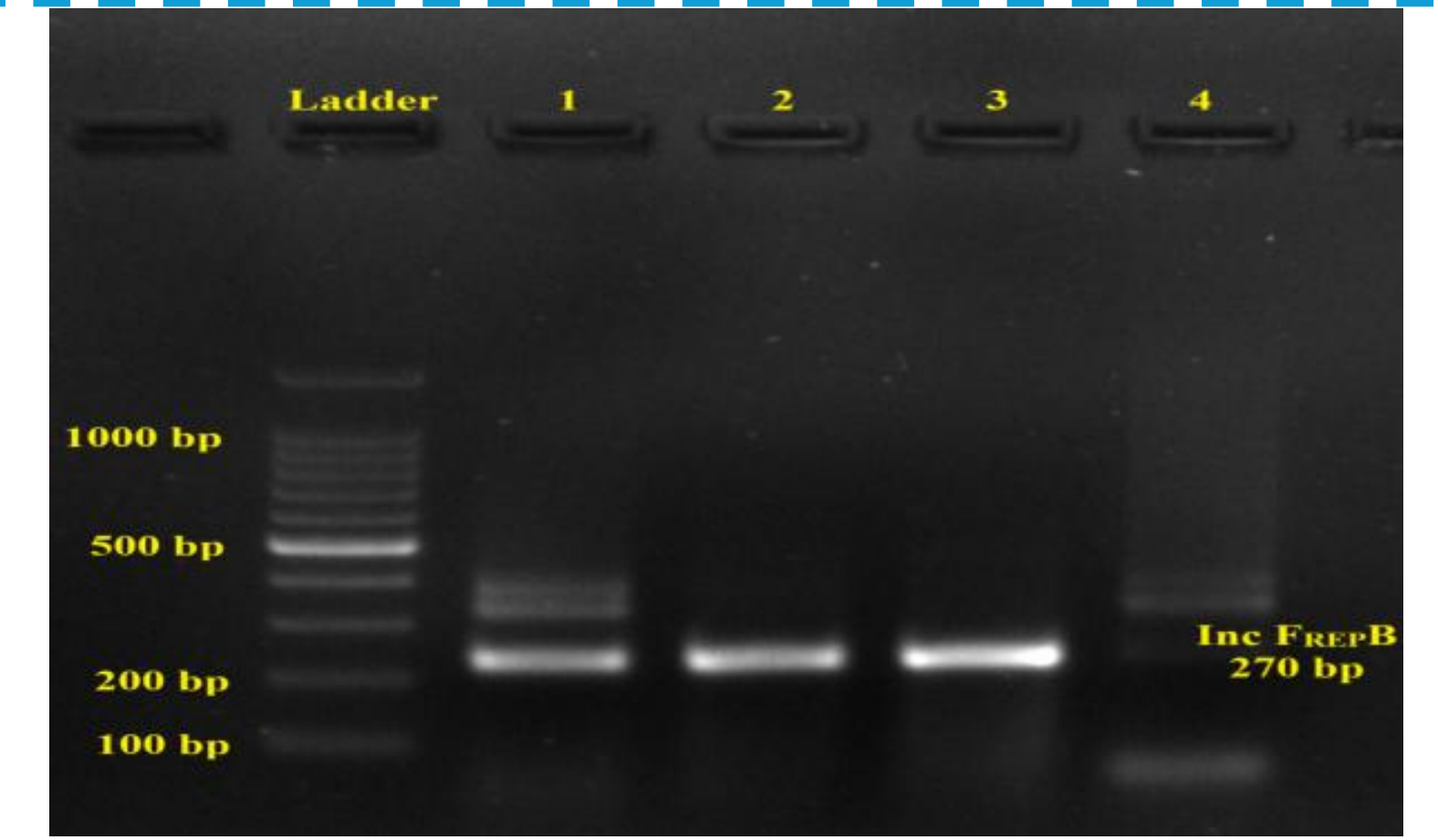


Fig 5 : Gel image showing amplification of replicon typing Inc F_{REP}B (270 bp). Ladder: 100 bp, NC: Negative Control, Lane 1-4: test isolates

DNA FINGERPRINTING OF THE ISOLATES

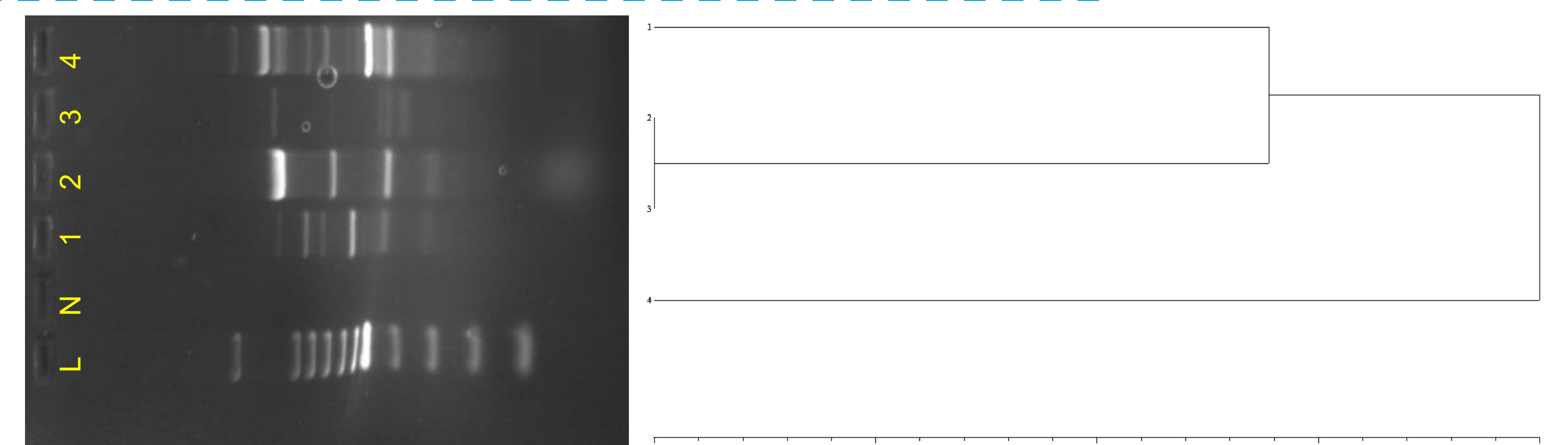


Fig 6: Dendrogram and gel image showing clonal diversity of *E. coli* isolates by ERIC PCR.

TRANSCRIPTIONAL EXPRESSION:

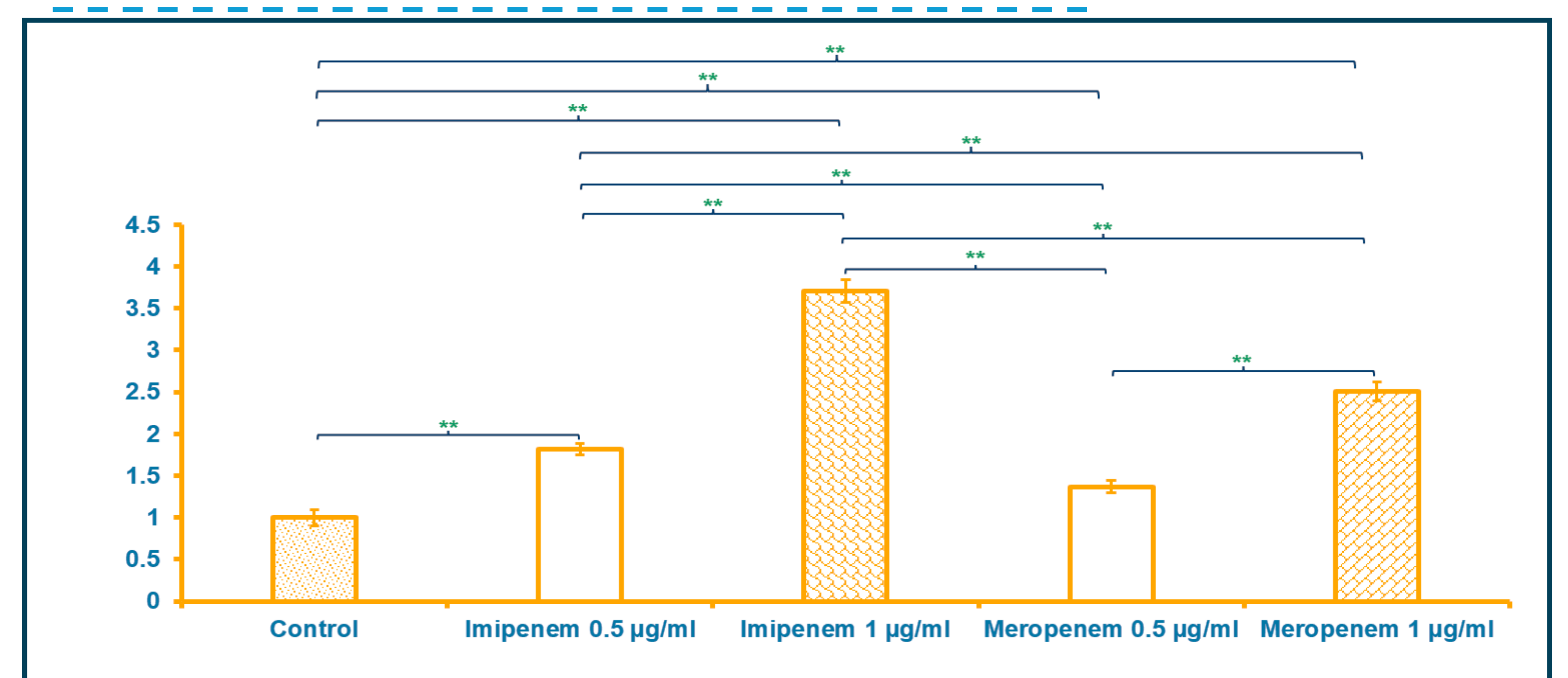


Fig 7: Transcriptional expression of *bla*_{OXA-58} gene in *Escherichia coli* isolates in control condition (without carbapenem exposure) and under sub-inhibitory concentrations of carbapenems. The fold change in relative quantification (RQ) values of the expression of *bla*_{OXA-58} gene is plotted on the Y-axis. The error bars represent Standard Deviation (SD) and asterisks indicates the level of significance (* : $p \leq 0.05$, ** : $p \leq 0.01$ and *** : $p \leq 0.001$).

- The transcriptional responses of *bla*_{OXA-58} gene were dose-dependent and with the increase in concentration of carbapenems, the gene expression levels also increased
- The expression is nearly doubled (1.8-fold) at 0.5 μ g/ml imipenem and increased to more than three-and-a-half-fold at 1 μ g/ml exposure.
- Under meropenem pressure, the gene expression nearly increased to one-and-a-half-fold (1.4-fold) at 0.5 μ g/ml concentration and increased by two-and-a-half-fold at 1 μ g/ml concentration

5 CONCLUSION:

- Four isolates were identified harbouring *bla*_{OXA-58} gene within transferable IncF_{IB} and IncF_{REP}B plasmids.
- The transcriptional response of *bla*_{OXA-58} in with or without subinhibitory concentration of carbapenems, and that revealed that the expression of *bla*_{OXA-58} gene was enhanced under imipenem & meropenem exposure.

References:

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