

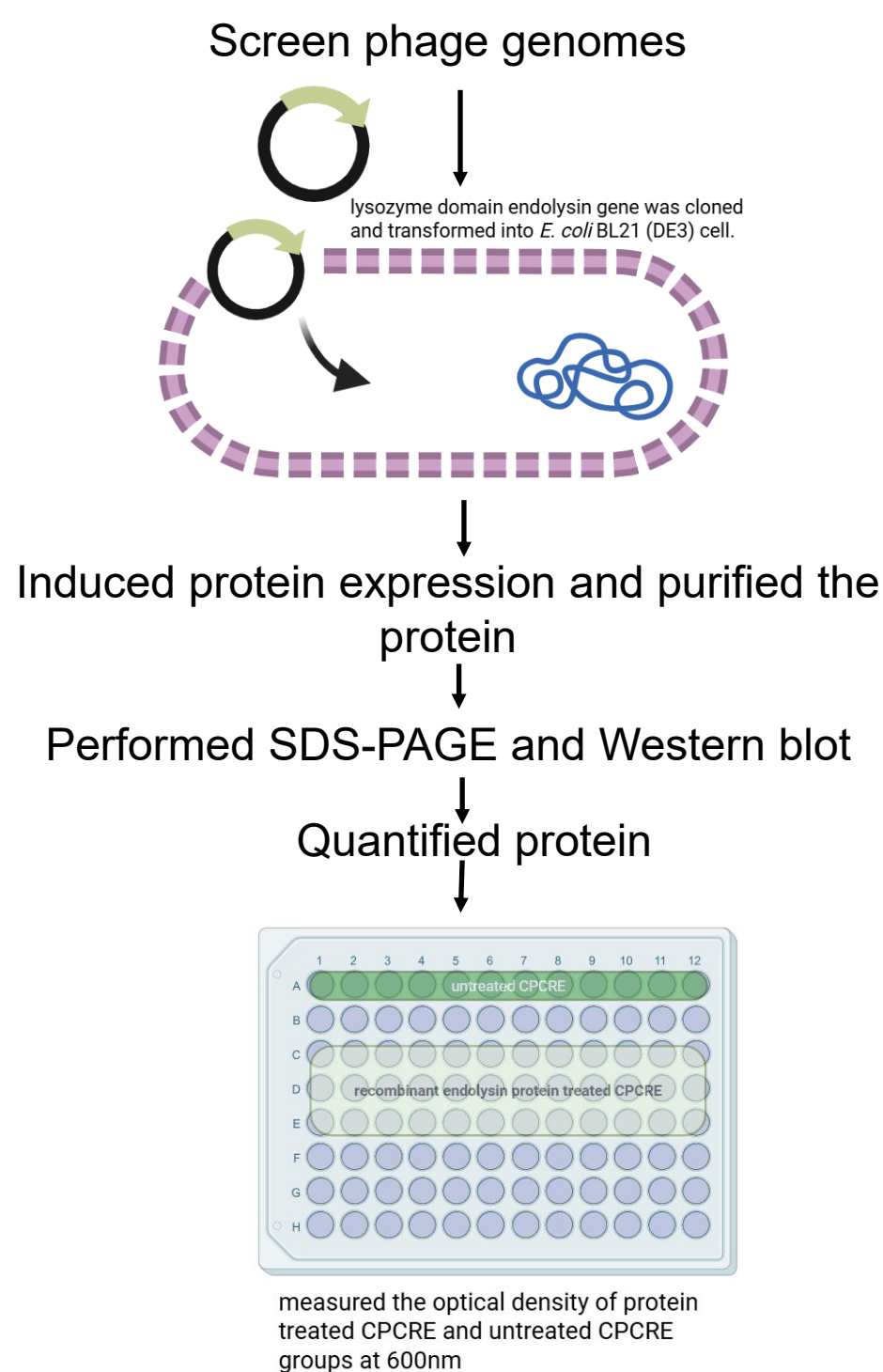
# Assessment of antimicrobial activity of recombinant endolysin against carbapenemase-producing carbapenem-resistant *Enterobacterales*

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## BACKGROUND

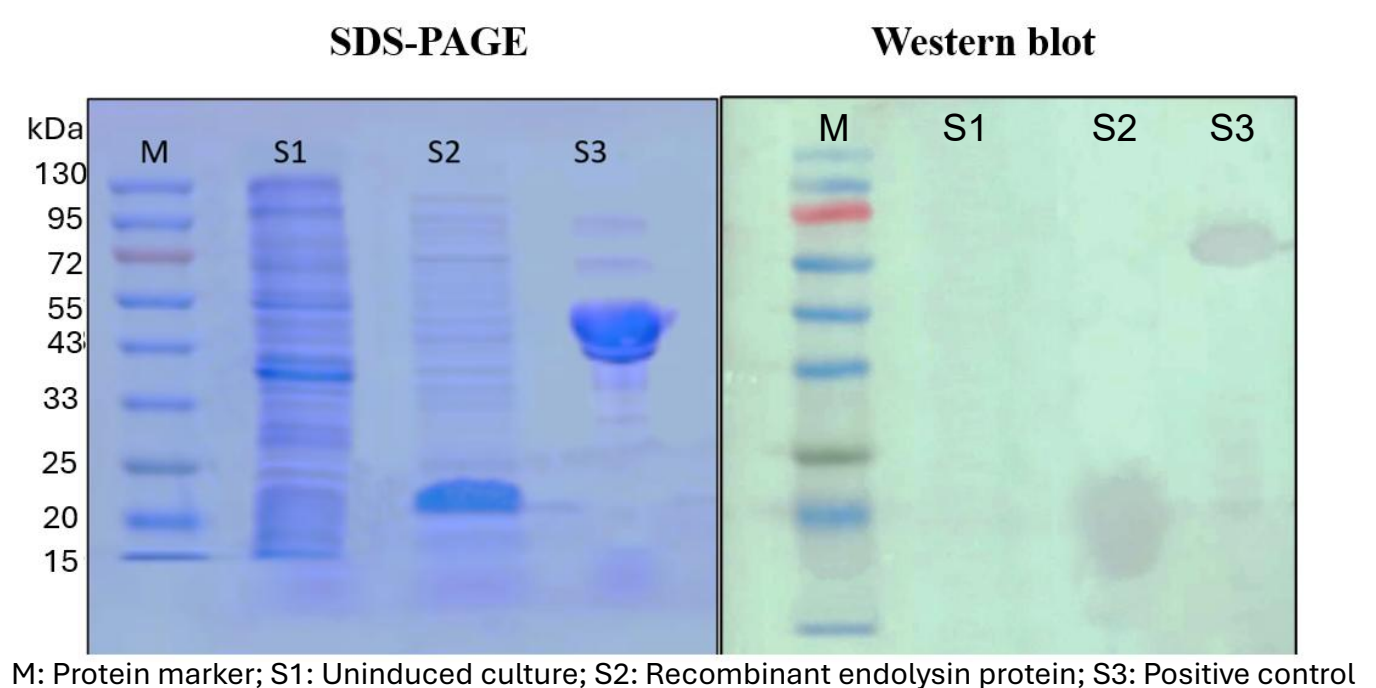
Carbapenem-resistant *Enterobacterales* (CRE) represent a critical threat in modern healthcare. These Gram-negative bacteria have developed resistance to carbapenems, a class of  $\beta$ -lactam antibiotics often regarded as the last line of defence against multidrug-resistant bacterial infections. The emergence of CRE highlights the dire need for novel substitutes to antibiotics. In contrast, phage-derived endolysins, enzymes capable of lysing bacterial peptidoglycan layer, are being extensively investigated as potential agents to combat antimicrobial resistance. Thus, the aim of this study is to develop an alternative antimicrobial agent targeting CRE.

## METHODOLOGY



## RESULTS

- Enterobacter* E-2 phage has a lysozyme domain with highly positive charged amino acids at C-terminal.



Sample	Absorbance at 600nm				
	Sample only	100 $\mu$ g/mL	250 $\mu$ g/mL	500 $\mu$ g/mL	
<b>Concentration</b>					
<i>K. pneumoniae</i> 1706	0.355	0.048	0.045	0.040	quality control
<i>E. coli</i> 25922	0.393	0.051	0.043	0.039	
<i>E. cloacae</i> 1143	0.596	0.065	0.050	0.042	
<i>K. pneumoniae</i> (blaNDM-1)	0.444	0.288	0.088	0.042	Growth inhibited: OD600 of treated bacteria is lesser than sample only.
<i>K. pneumoniae</i> (blaNDM-1+KPC)	0.329	0.238	0.102	0.041	
<i>K. pneumoniae</i> (blaKPC)	0.311	0.102	0.042	0.041	
<i>K. pneumoniae</i> (blaNDM-1+OXA-48)	0.409	0.268	0.099	0.042	Growth undetectable: OD600 of treated bacteria is equivalent to the MHB broth.
<i>K. pneumoniae</i> (blaOXA-48)	0.429	0.087	0.041	0.040	
<i>E. coli</i> (blaNDM-1+KPC)	0.200	0.041	0.040	0.040	
<i>E. cloacae</i> (blaNDM-1)	0.297	0.125	0.041	0.041	
<i>E. kobei</i> (blaNDM-1+KPC)	0.498	0.179	0.041	0.041	
MHB broth	0.042	-	-	-	
Tris-HCl buffer	0.040	-	-	-	

## DISCUSSION

- ✓ The findings align with the concept that endolysins function as peptidoglycan-degrading enzymes, whereby increased enzyme concentrations accelerate and intensify cell wall breakdown, ultimately resulting in osmotic lysis<sup>1</sup>.
- ✓ Stronger antibacterial activity was observed on blaNDM-1 + blaKPC-producing *E. coli*, due to the differences in peptidoglycan and outer membrane composition<sup>2</sup>.
- ✓ Minor susceptibility variations observed among *K. pneumoniae* isolates indicate that capsule thickness and lipopolysaccharide structure may influence the protein's activity<sup>3</sup>.

## CONCLUSION

The recombinant endolysin protein exhibits broad-spectrum antibacterial activity against multiple CPCRE species. Hence, it may be a potential alternative therapy to treat CRE infections.

## ACKNOWLEDGMENT

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