



Meta-analysis on New Delhi metallo-β-lactamase 1 (NDM-1) from India, Pakistan, South Africa and UK

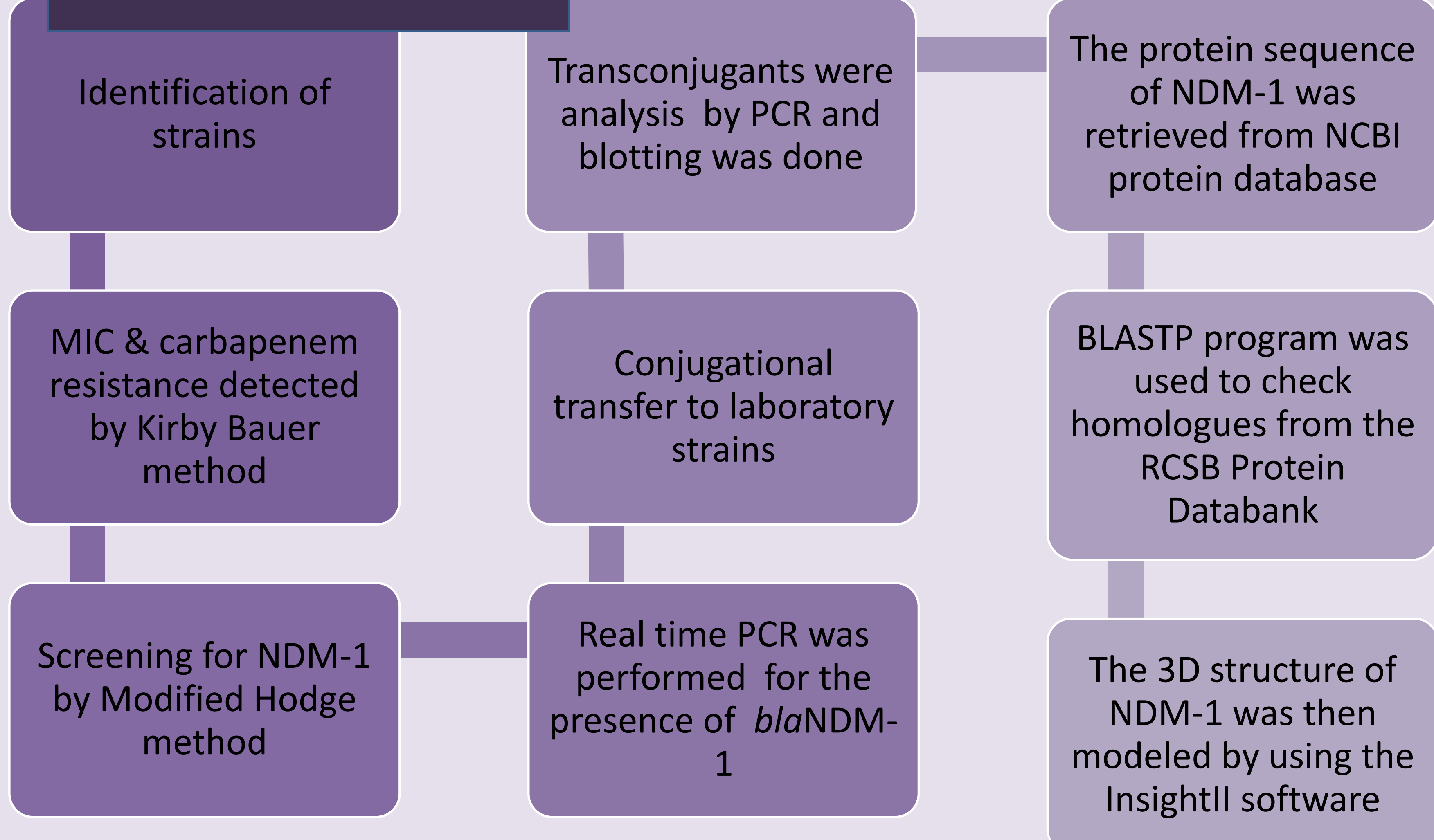
Undergraduates of Department of Microbiology & Molecular Genetics, University of the Punjab
Nimrah Farooq, Farzeen Zehra, Ayesha Ali, Saba Tariq, Madiha Habib, Faseeha Khan & Maham Khalid



INTRODUCTION

- Mobile genes on the plasmids, air travel and migration between countries and continents are the cause of increasing resistance in Gram-negative bacteria which results in bacterial plasmids and clones transportation.
- Carbapenems have long been a reliable last line of defence in the treatment of infections caused by Gram negative pathogens
- However, the emergence of resistance in Enterobacteriaceae mediated via carbapenemase is a major public health concern .
- The New Delhi Metallo-β-lactamase (NDM-1) was first reported in 2009 in a Swedish patient and in South Africa reported in August 2011 both cases have the history of travelling to subcontinent.
- 3D structure analysis and NDM-1/antibiotics complex. It revealed that the hydrolytic mechanisms are highly conserved

METHOD



RESULTS

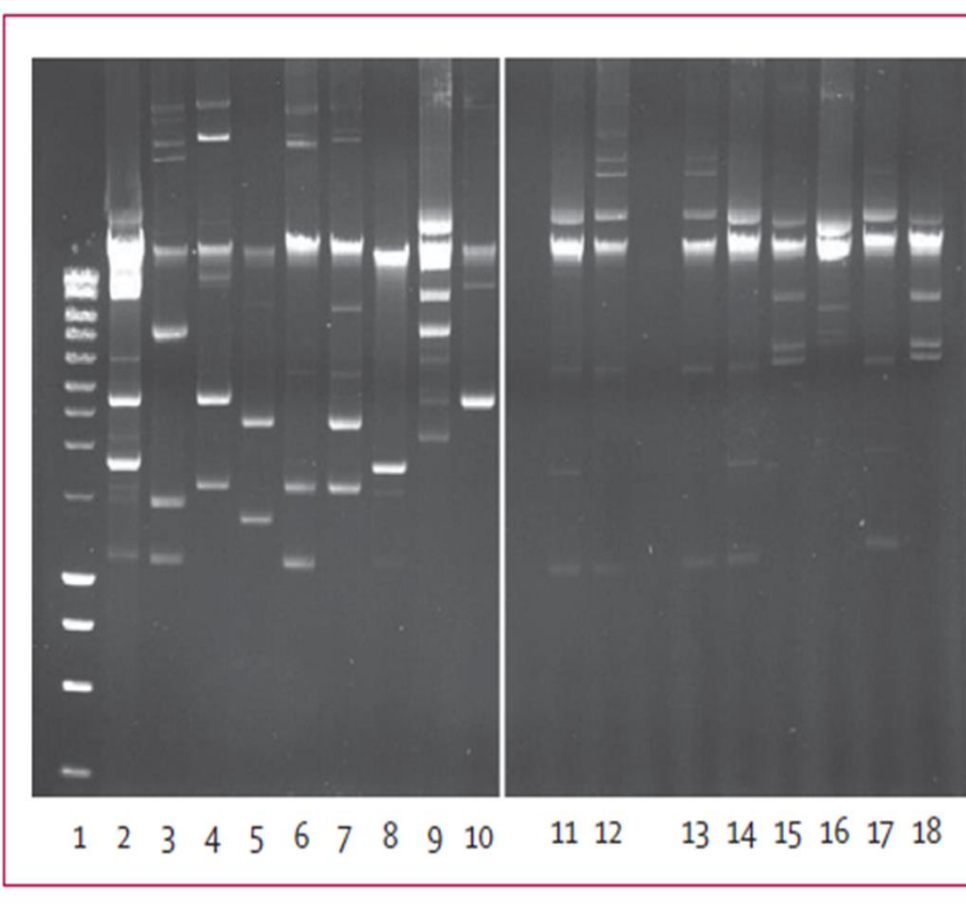
Table1: Antibiotic susceptibilities for NDM-1-positive Enterobacteriaceae isolated in the UK and north (Chennai) and south India (Haryana)

Antibiotic	UK (n=2)		Chennai (n=4)		Haryana (n=2)	
	MIC ₅₀ (mg/L)	Proportion susceptible	MIC ₅₀ (mg/L)	Proportion susceptible	MIC ₅₀ (mg/L)	Proportion susceptible
Imipenem	32-32	0%	64-128	0%	32-128	0%
Meropenem	32-32	0%	32-32	0%	32-32	0%
Piperacillin-tazobactam	>64-64	0%	>64-64	0%	>64-64	0%
Ceftazidime	>256-256	0%	>256-256	0%	>256-256	0%
Cefepime	>64-64	0%	>64-64	0%	>64-64	0%
Aztreonam	>64-64	0%	>64-64	0%	>64-64	0%
Ciprofloxacin	<8-8	0%	<8-8	0%	<8-8	0%
Gentamicin	>32-32	0%	>32-32	0%	>32-32	0%
Tobramycin	>32-32	0%	>32-32	0%	>32-32	0%
Amikacin	>64-64	0%	>64-64	0%	>64-64	0%
Mirofloxacin	32-32	0%	32-32	0%	32-32	0%
Tigecycline	3.4	100%	4.4	100%	1.2	100%
Colistin	62.5	100%	1.2	100%	1.2	100%

Table: 2 Bacterial strain MIC result isolated in South Africa

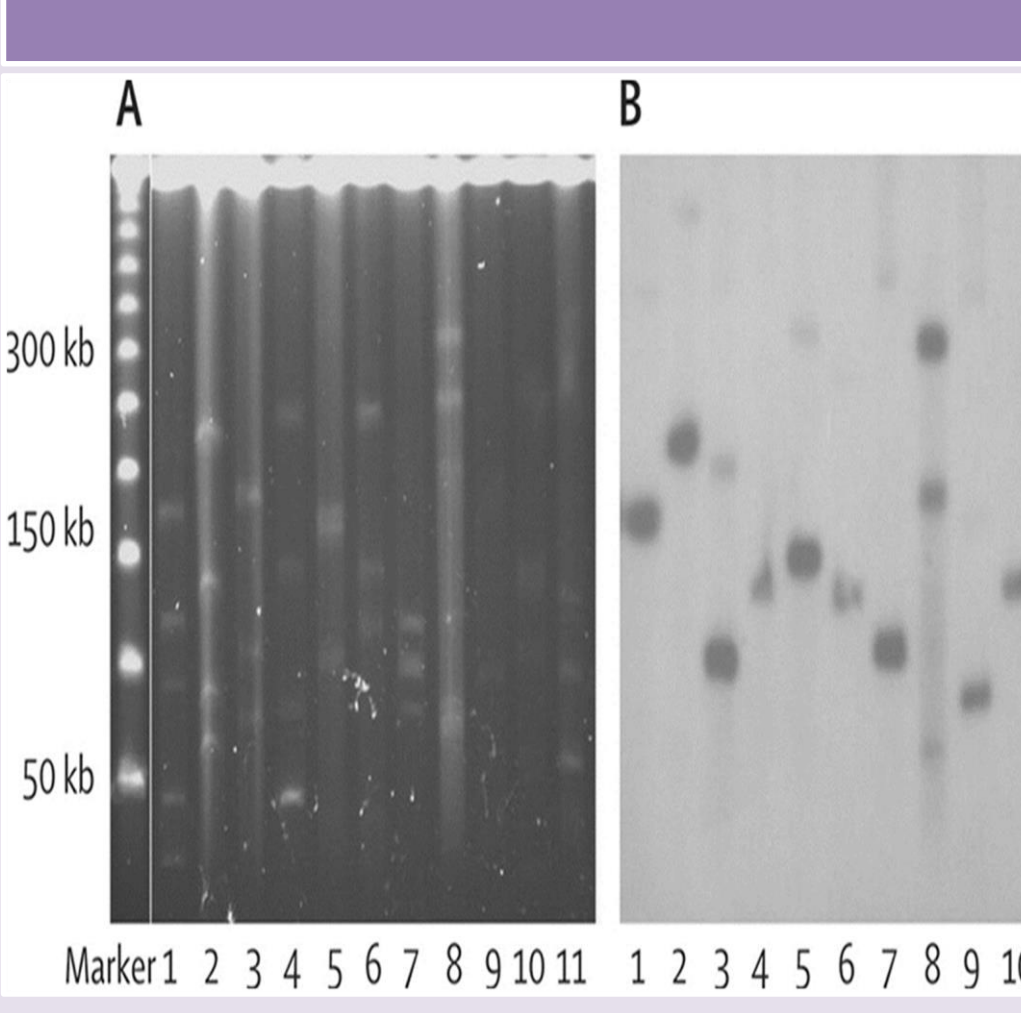
Antimicrobial agents	Vitek 2		Interpretation		E-test		Interpretation	
	MIC (μg/ml)	S/R/B	MIC (μg/ml)	S/R/B	MIC (μg/ml)	S/R/B	MIC (μg/ml)	S/R/B
Ampicillin	>32	R	NT	NT	NT	NT	NT	NT
Amoxicillin-clavulanic acid	>32	R	NT	NT	NT	NT	NT	NT
Piperacillin-tazobactam	>128	R	NT	NT	NT	NT	NT	NT
Cefepime	>64	R	NT	NT	NT	NT	NT	NT
Ceftazidime	>64	R	NT	NT	NT	NT	NT	NT
Cefepime	>64	R	NT	NT	NT	NT	NT	NT
Ceftriaxone	>64	R	NT	NT	NT	NT	NT	NT
Colistin	>64	R	NT	NT	NT	NT	NT	NT
Daptomycin	NT	NT	16	R	NT	NT	NT	NT
Ertapenem	>8	R	NT	NT	NT	NT	NT	NT
Imipenem	>16	R	12	R	NT	NT	NT	NT
Meropenem	>16	R	24	R	NT	NT	NT	NT
Gentamicin	>16	R	NT	NT	NT	NT	NT	NT
Amikacin	>32	R	NT	NT	NT	NT	NT	NT
Tigecycline	0.5	R	0.5	S	NT	NT	NT	NT
Ciprofloxacin	>4	R	NT	NT	NT	NT	NT	NT
Chloramphenicol	>320	R	NT	NT	NT	NT	NT	NT
Fusidic acid	NT	NT	NT	NT	NT	NT	NT	NT
Colistin	62	S	NT	NT	NT	NT	NT	NT

Figure 1: The difference in plasmid numbers from a selection of Indian isolates



Tracks 1–10 show the number of plasmids in isolates from Chennai and tracks 11–18 from Haryana. In Chennai there was greater variation

Figure 2: Hybridization results of UK isolates with blaNDM-1



(a). Pulsed-field gel of S1-treated plasmid DNA. (b). Autoradiogram of gel A probed with a blaNDM-1 probe.

Figure 3: Distribution of NDM-1-producing Enterobacteriaceae strains in Asia, and the UK

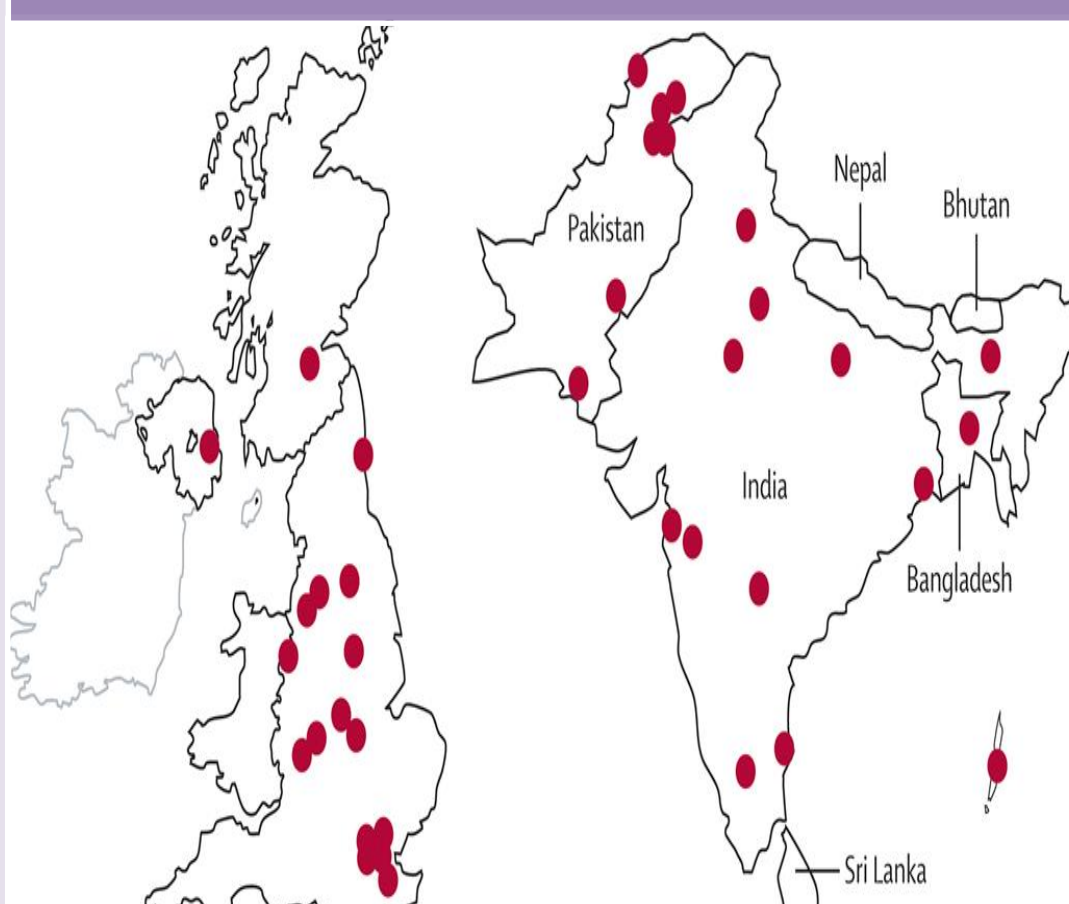
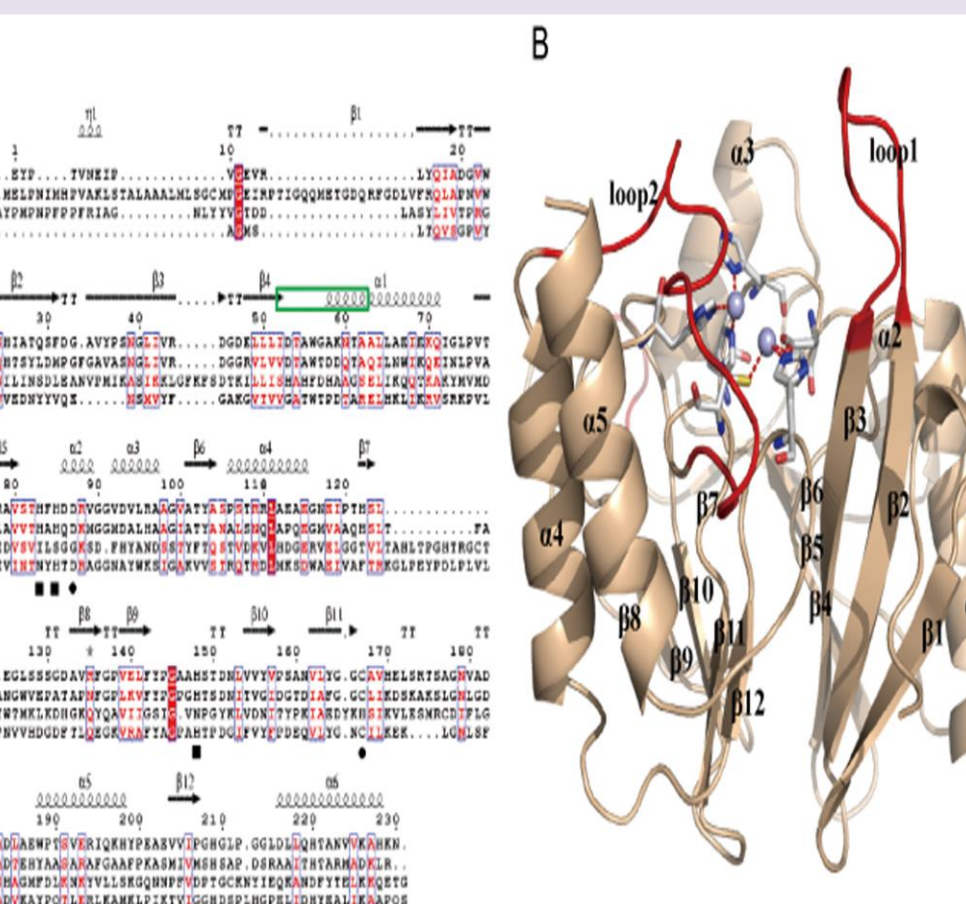
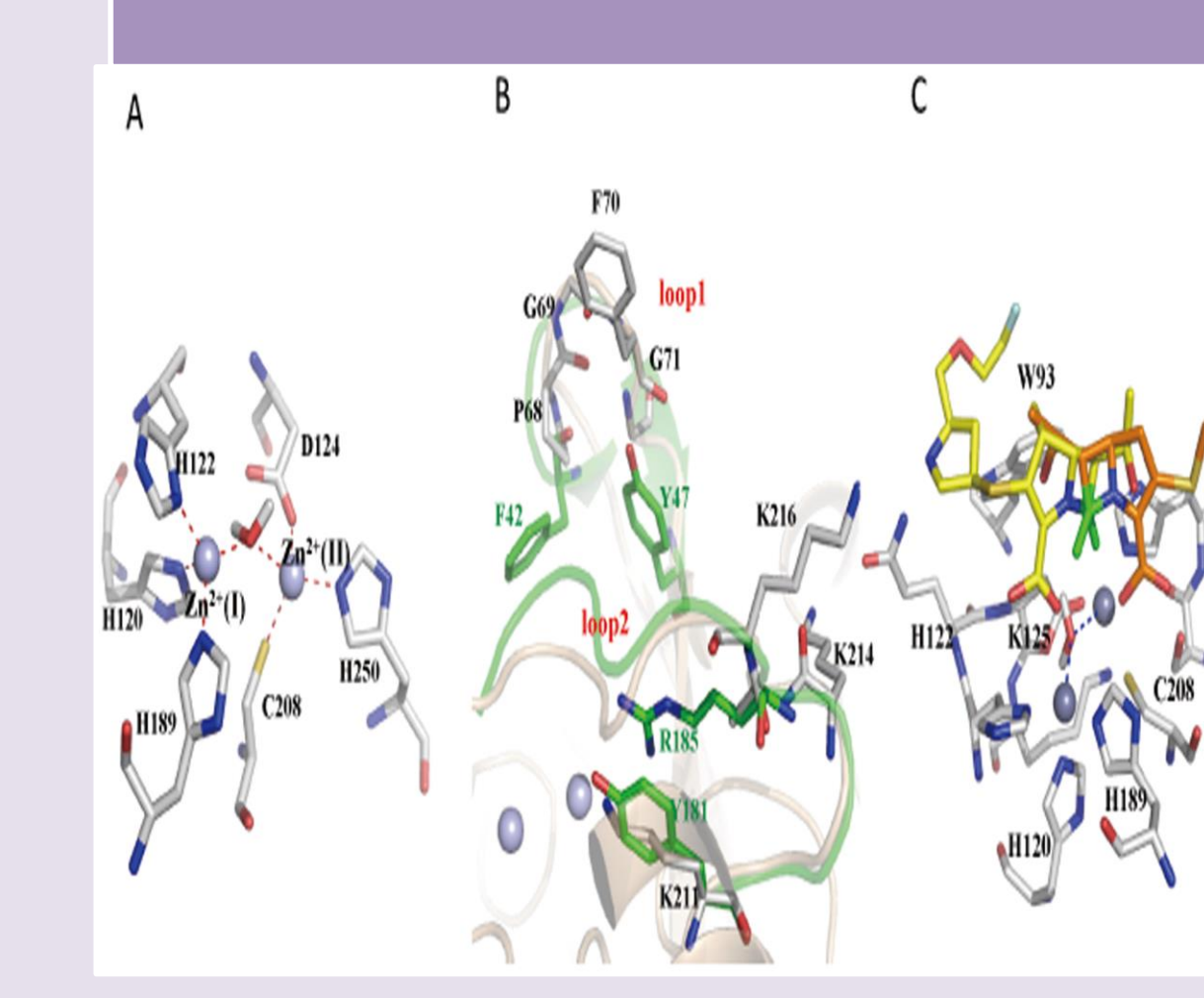


Figure 5: NDM-1 sequence alignment with its homologue proteins.



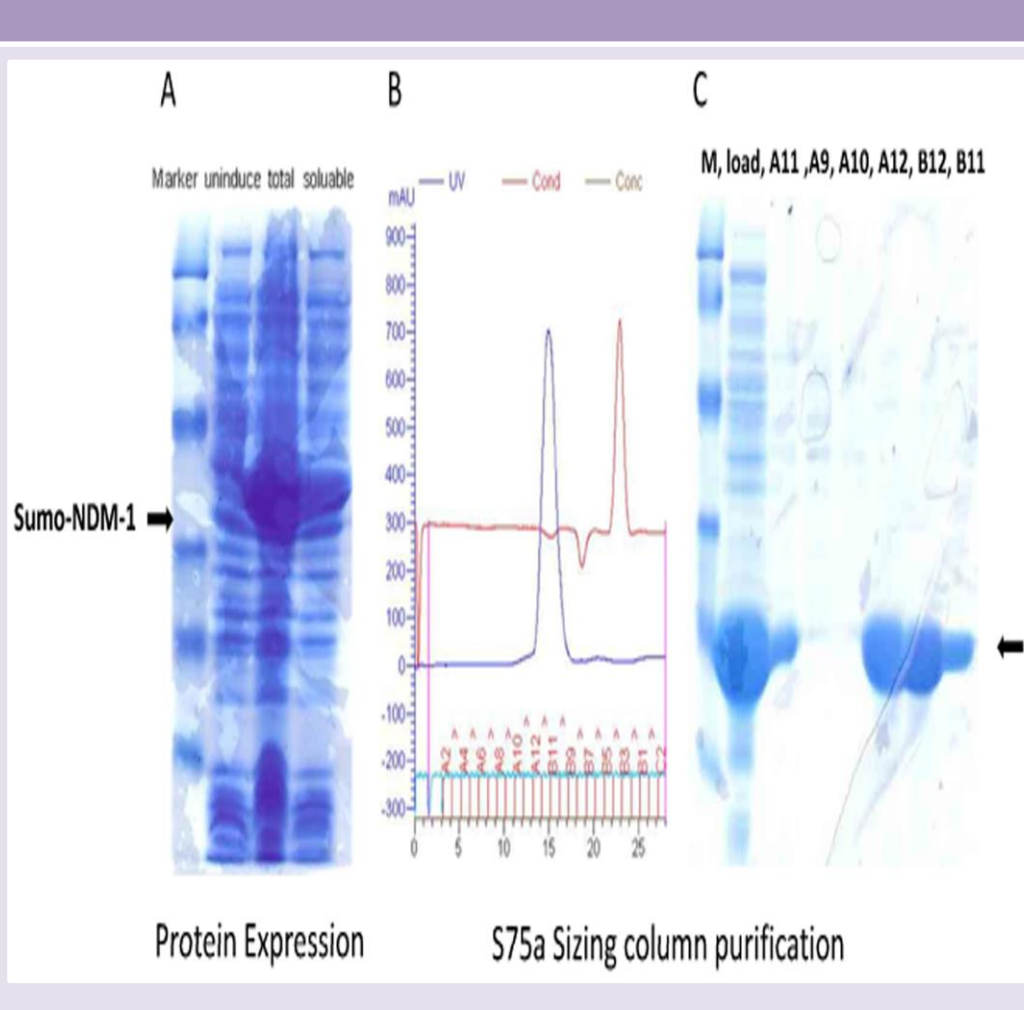
A. Sequence alignment of NDM-1 with homologue. B. Cartoon representation of the overall structure of NDM-1

Molecular models of NDM-1 and its complex with antibiotics.



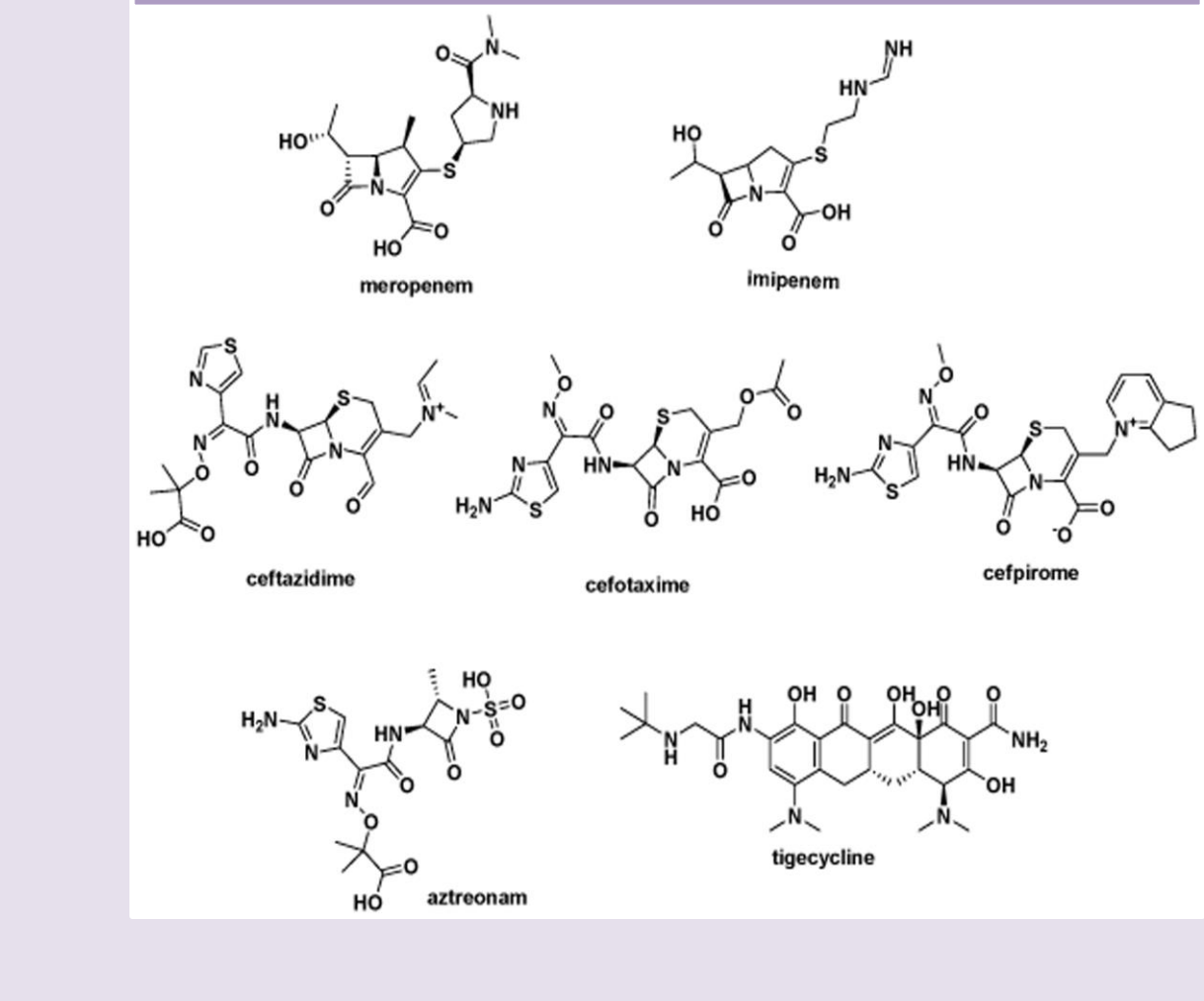
A. The active site of NDM-1 with two zinc ions and the coordinating residues. B. The comparison of the two loops in NDM-1 and VIM-2C. C. The binding modes of antibiotics imipenem and carbapenem in the active site of NDM-1.

NDM-1 protein expression and purification

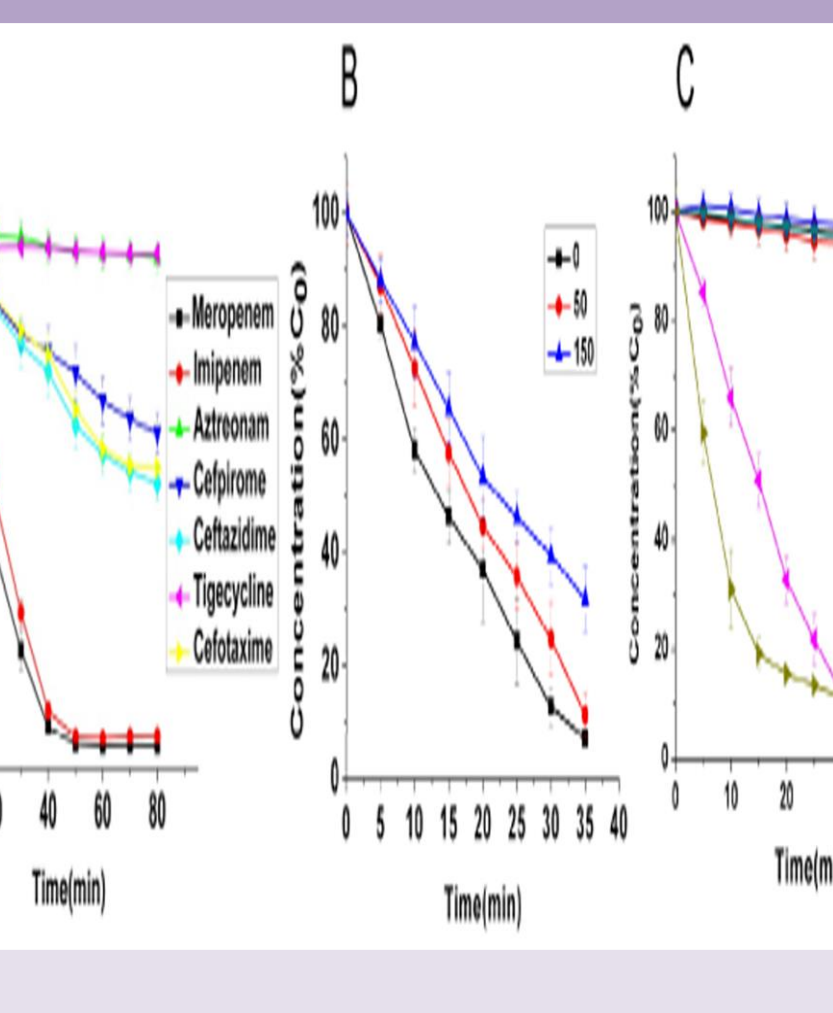


A. 6×His and sumo (small ubiquitin-related modifier) tagged NDM-1 was overexpressed in E.coli BL21(DE3) strain B. The cleaved NDM-1 protein was purified by S75S gel filtration chromatography. C. SDS-PAGE gel shows the purity of NDM-1 more than 95%.

The chemical structures of the seven antibiotics tested in the assay of NDM-1 catalytic activity.



Hydrolysis activity of NDM-1 by enzymatic assays.



A. Seven antibiotics were hydrolyzed by NDM-1 protein. B. Tigecycline inhibited the hydrolysis activity of NDM-1 to meropenem weakly. C. Compare with the wild type enzyme (pink line),

CONCLUSION

To our knowledge, this is the first documented case of an NDM-1 Enterobacteriaceae in INDIA and the first case in SA where a direct epidemiological link to the Indian subcontinent has been established. Since the detection of this isolate, other cases in several hospitals have been identified. Ongoing vigilance and strict infection control measures need to be maintained.

ACKNOWLEDGMENT

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