NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

Laboratory testing for carbapenems resistant Enterobacteriacae (CRE)

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9th March 2013

Layout

- Introduction to carbapenemases producing Enterobacteriaceae
- Referral to Antimicrobial Resistance Reference Laboratory (AMRRL)
- Discussion on diagnostics and screening methods

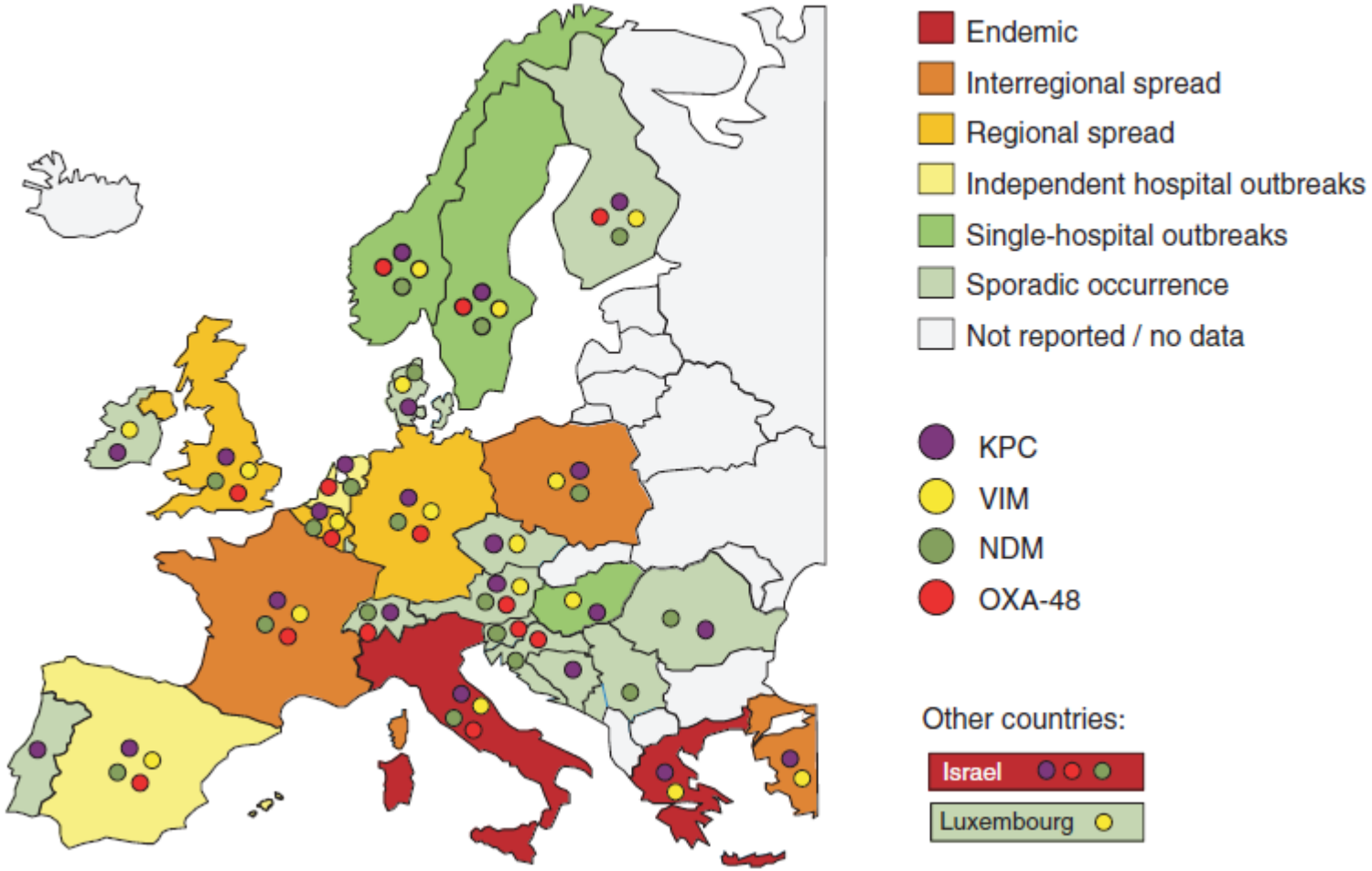
CREs

- Carbapenemases in Enterobacteriaceae are mostly plasmid encoded, which largely explains their common association with other resistance markers and their multidrug resistance patterns.
- The type of CREs depend on the country, and might be associated with historical/cultural relationships and exchange of populations with other countries of high prevalence. Cross border transfer of patients, travel, medical tourism and refugees might also play an important role.

Main Carbapenemases: distribution and molecular epidemiology

Type of carbapenemases	Geographical spread	Molecular epidemiology
NDM	*K. pneumoniae* and *E. coli* in India Imported to UK firstly and other countries via patients with travel (hospitalization) in India. There have been a cases of cross-infections. In South Africa since 2011.	Widespread in Enterobacteriaceae. Diverse strain type in UK. Plasmid spread among strains and species is more important than clonal spread among patients.
VIM	Scattered globally, endemic in south Europe (Greece)	Plasmid spread among strains is more important than clonal spread of producer strains.
IMP	Scattered worldwide; no clear associations	Mostly plasmid spread.
KPC	USA since 1999. Prevalent worldwide.	Plasmid spread from mostly among *K. pneumoniae*, occasionally to other Enterobacteriaceae.
OXA-48	Widespread *K. pneumoniae* in Mideast, Africa, Europe.	Mixture of plasmid and clone spread.
GES	GES enzymes have been identified worldwide, with reports from Greece, France, Portugal, South Africa, French Guiana, Brazil, Argentina, Korea, and Japan	The genes encoding the GES family of enzymes are located in integrons on plasmids and were initially classified as extended-spectrum -lactamases. Their hydrolysis spectrum was expanded in 2001 to include imipenem, with the report of GES-2 in a clinical isolate of *P. aeruginosa* from South Africa.

European situation regarding carbapenemase-producing Enterobacteriaceae

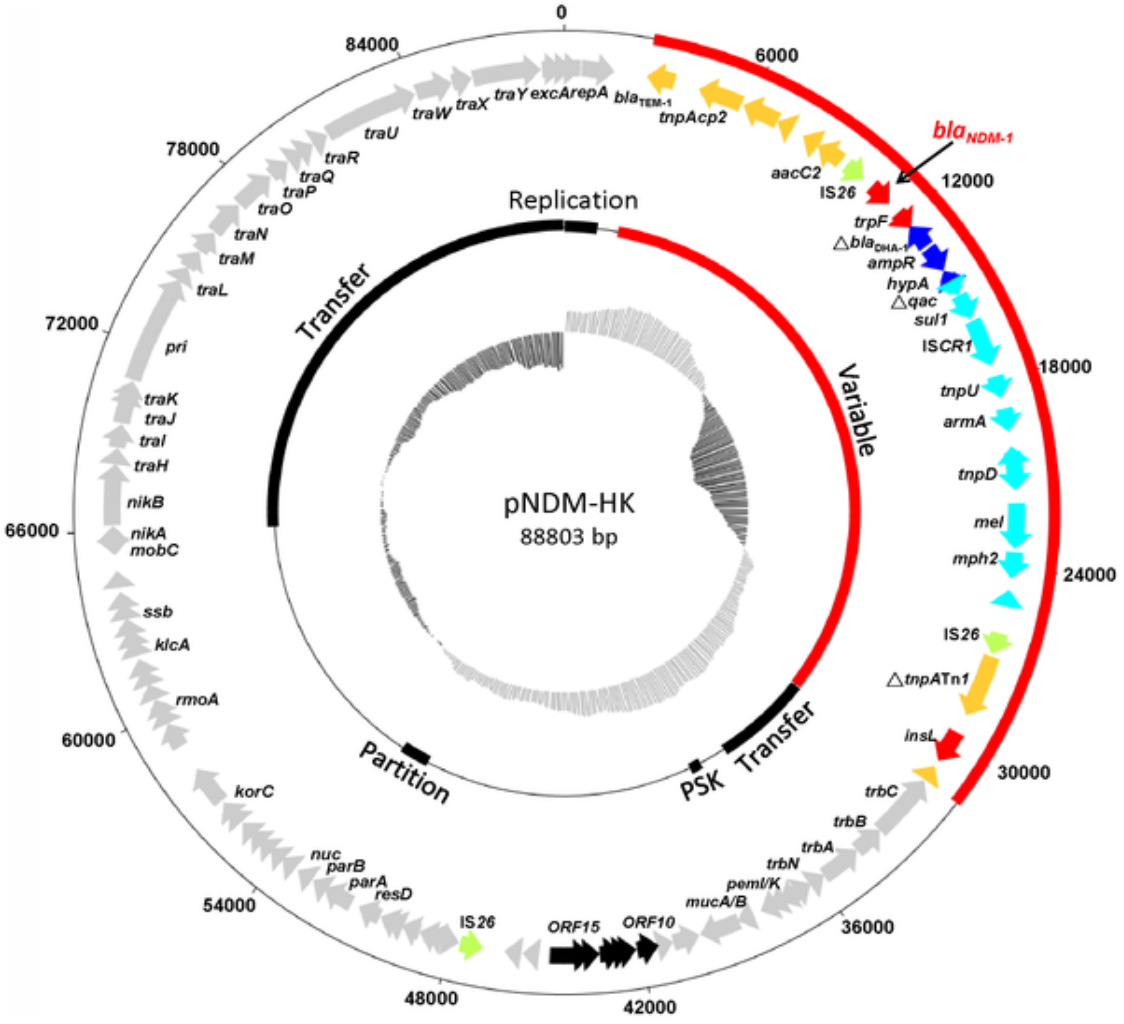


R. Canto´n

NDM-1, new emerging

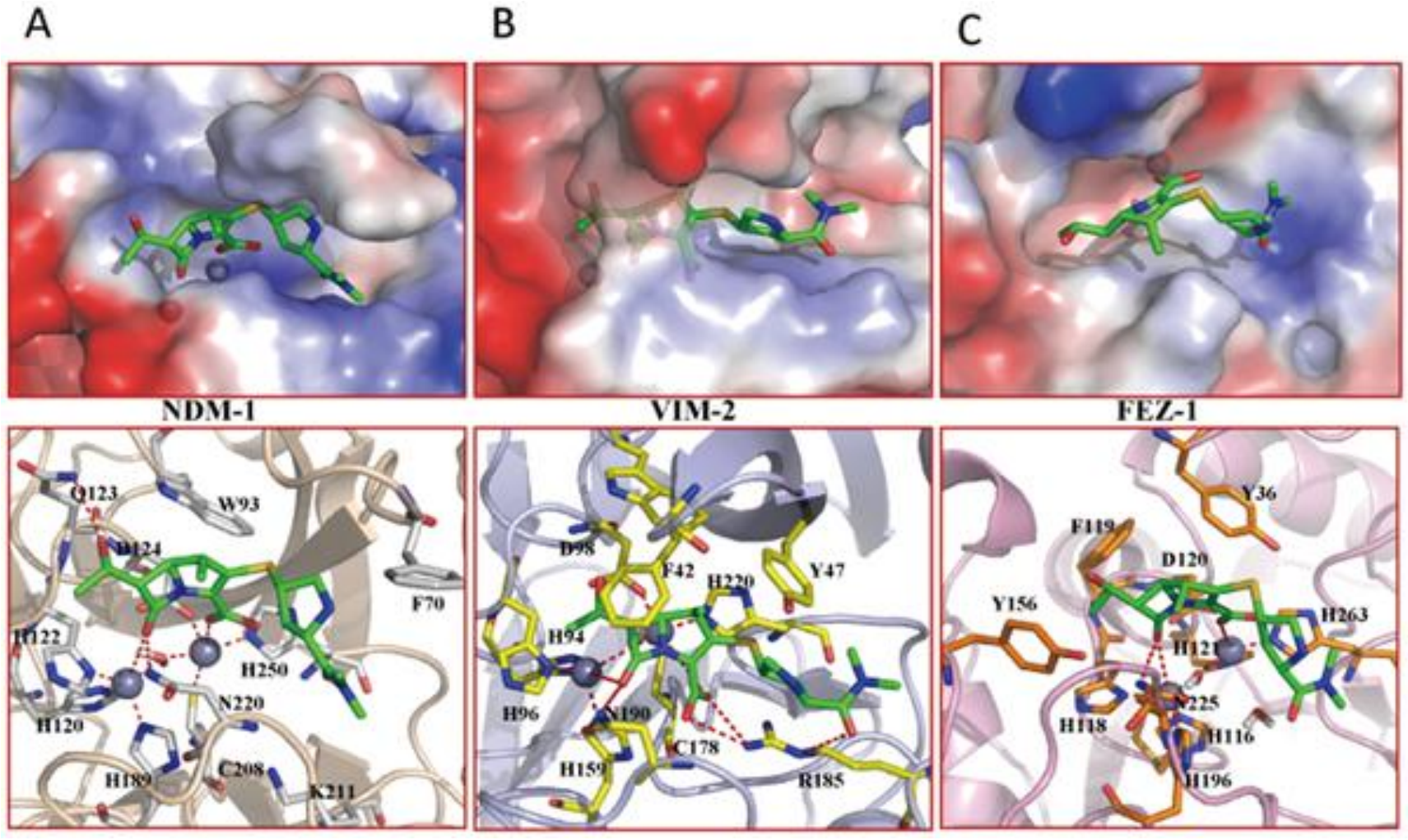
- NDM-1 enzyme is highly potent to degrade carbapenem antibiotics.
- NDM-1 belongs to the Metallo-β-lactamase (MBL, class B) family containing Zn^{2+} and other divalent cations as cofactors.
- It inactivates almost all classes of β-lactams antibiotics including carbapenems by catalyzing the hydrolytic cleavage of the substrate amide bond.
- On the basis of the protein sequence similarities, three different lineages, named as subclass B1, B2 and B3 have been characterized.

Figure 1. An overview of the blaNDM-1 encoding plasmid, pNDM-HK.



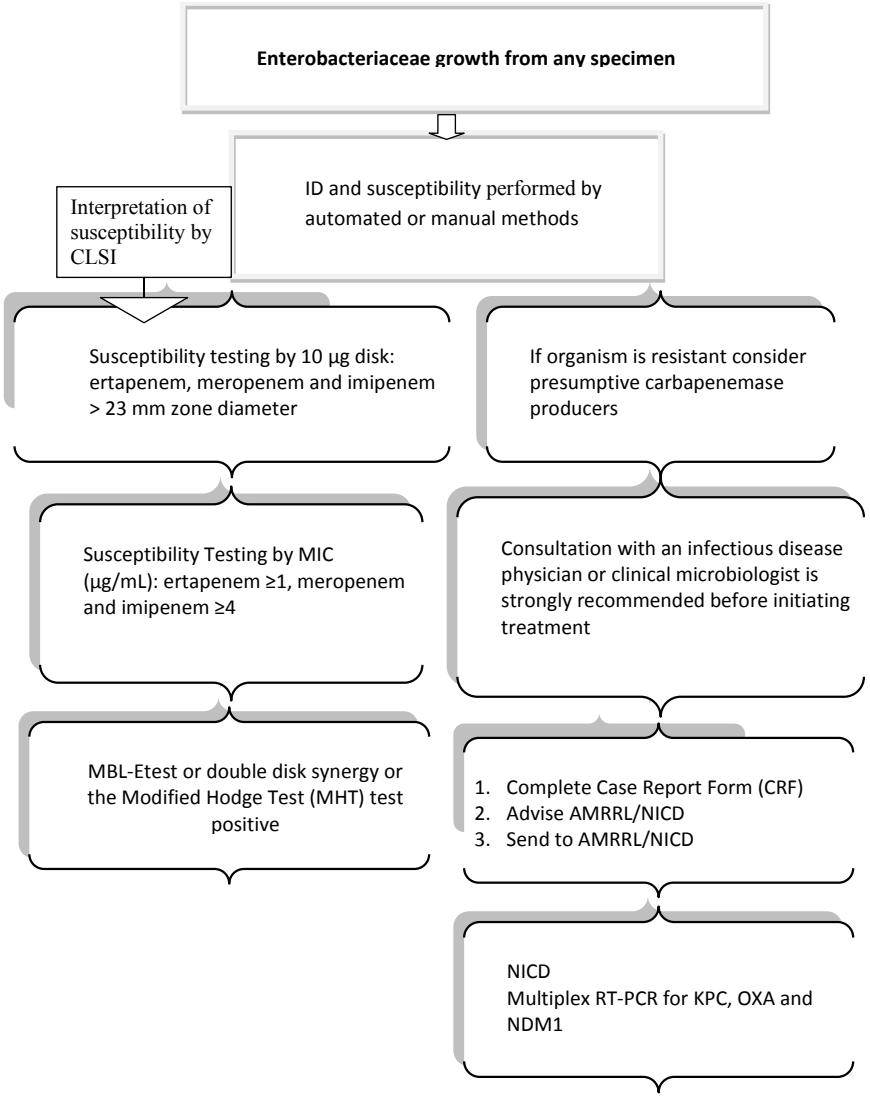
Ho PL, Lo WU, Yeung MK, Lin CH, et al. (2011) Complete Sequencing of pNDM-HK Encoding NDM-1 Carbapenemase from a Multidrug-Resistant Escherichia coli Strain Isolated in Hong Kong. PLoS ONE 6(3): e17989. doi:10.1371/journal.pone.0017989
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0017989

Figure 4. Complex models comparison between NDM-1(A), VIM-2(B) and FEZ-1(C).



Liang Z, Li L, Wang Y, Chen L, et al. (2011) Molecular Basis of NDM-1, a New Antibiotic Resistance Determinant. PLoS ONE 6(8): e23606. doi:10.1371/journal.pone.0023606
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0023606

Carbapenemase producers: AMRRL referrals from public and private laboratories



Case Report Form for CRE referral to NICD

CARBAPENEMASE RESISTANCE SURVEILLANCE: CASE REPORT FORM

1. Please complete a new case investigation form for each isolate that meets inclusion criteria.
2. Inform AMRRU by telephone (011) 555 0342 or by email to ashikas@nicd.ac.za and olgap@nicd.ac.za
3. Send completed form with isolate to AMRRU; see advisory for further details.

1. PATIENT DETAILS

1.1 Surname: _____ Name/s:_____

1.2 Hospital Number:_____ 1.3 Occupation: _____

1.4 Date of birth D D M M Y Y Y Y Age: _____ Both DOB/age unknown: ☐

1.5.Gender: Male ☐ Female ☐

2. DETAILS OF CURRENT CONSULTATION/ADMISSION

2.1 Name of clinician: | Contact number/s of clinician:

2.2 Healthcare facility name: | Province:

2.3 If hospitalised: Adult Ward ☐ Paediatric Ward ☐ ICU ☐ Other ☐ Specify:
Outpatient: ☐

2.4 Date of admission: D D M M Y Y Y Y

2.5 Current working diagnosis:

2.6. Antibiotics prescribed during this admission? Yes ☐ No ☐ Unknown ☐
If yes complete the table below

Antibiotic	Route (po/IV IM)	Date started(enter date prescribed)	Duration (days) of treatment	Antibiotic	Route (po/IV/ IM)	Date started	Duration (days) of treatment
		D D M M Y Y Y Y				D D M M Y Y Y Y	
		D D M M Y Y Y Y				D D M M Y Y Y Y	
		D D M M Y Y Y Y				D D M M Y Y Y Y	

3. PAST MEDICAL AND TRAVEL HISTORY

3.1 Underlying illness : Yes ☐ No ☐ Unknown ☐
If yes, give details:

3.2 Travel outside of South Africa in year prior to this admission/consultation: Yes ☐ No ☐ Unknown ☐
If yes, country: Date of travel and return (dd/mm/yyyy):

3.3 Received medical care in a foreign country during the year prior to this admission/consulation? Yes ☐ No ☐ Unknown ☐
If yes, what type of medical care? surgical operation ☐ ICU admission ☐ admission to general ward ☐
other ☐ (specify)_____ unknown ☐
Country where medical care received:

3.4 Received medical care in South Africa during the year prior to this admission/consulation? Yes ☐ No ☐ Unknown ☐
If yes, what type of medical care? surgical operation ☐ ICU admission ☐ admission to general ward ☐
other ☐ (specify)_____ unknown ☐

Healthcare facility	Reason for admission	Date of admission	Duration of admission

3.5 Antibiotic use in the last 6 months? Yes ☐ No ☐ Unknown ☐ | If yes, list the antibiotic/s:

4. OUTCOME

4.1 Outcome known: Yes ☐ No ☐
If yes: Discharged ☐ Deceased ☐

4.2 CRE categorisation known: Yes ☐ No ☐
If yes: colonisation ☐
invasive infection ☐

Details if available:

4.3 Antibiotic management of CRE known: Yes ☐ No ☐
If yes, was CRE treated with antibiotics: Yes ☐ No ☐
If yes, which antibiotic/s?

5. ADDITIONAL COMMENTS

Genotypic Methods

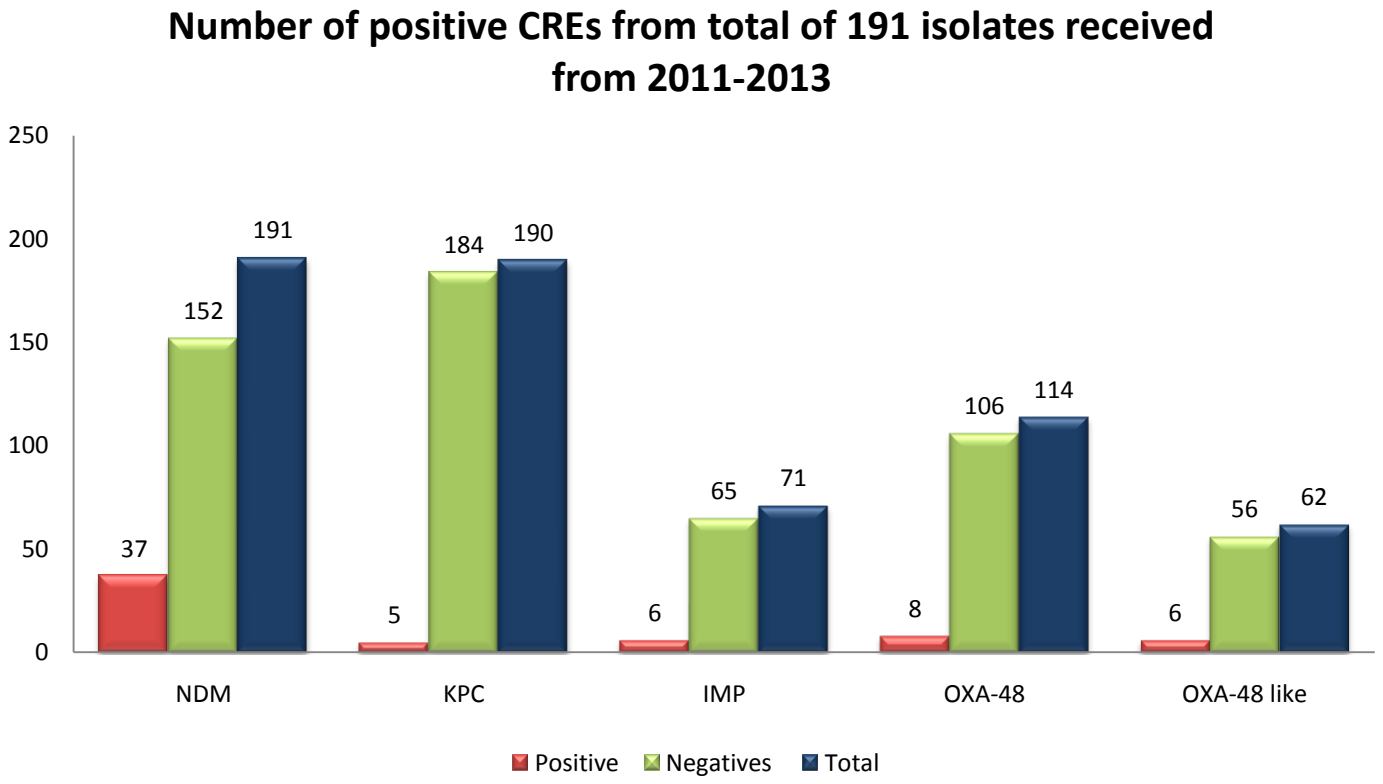
- **DNA Extraction**
  - **Crude**
- **Screening of antimicrobial resistance genes by real time PCR**
  - The LightCycler 480 (Roche Applied Science, Germany) instrument for the real-time polymerase chain reaction (PCR).

Multiplex real-time PCR used by AMRRL

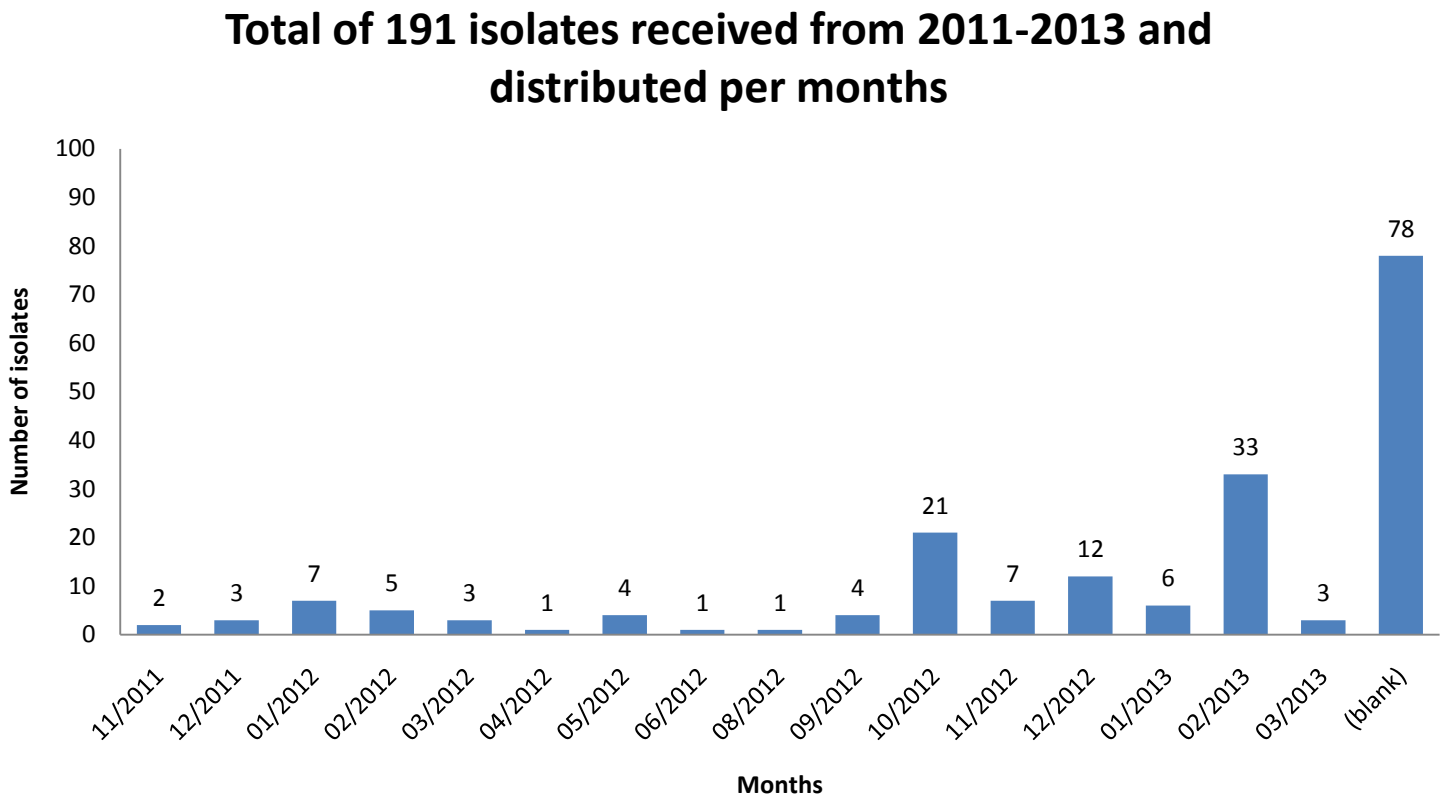
Genes	Primers and probes
KPC	Forward 5'- GGC CGC CGT GCA ATA C -3'
	Reverse 5'- GCC GCC CAA CTC CTT CA -3'
	KPC Probe 5'- YAK- TGA TAA CGC CGC GCG CAA TTT GT -BBQ -3'
NDM	Forward 5'- GAC CGC CCA GAT CCT CAA -3'
	Reverse 5'- CGC GAC CGG CAG GTT -3'
	Probe 5'- FAM- TGG ATC AAG CA+GGA+GAT -BBQ -3'
OXA-48 and its variants	OXA-48 Forward 5'-TTCGGCCACGGAGCAAATCAG-3
	Reverse 5'-GATGTGGGCATATCCATATTCATCGCA-3'
	Forward variant 5' – gCgTggTTAAggATgAACAC-3
	S variant 5'- CATYTCgggCAATgTAgACAg-3'
	Probe '-FAM-CTGGCTGCGCTCCGATACGTGTAACTTATTG-BBQ-3'
	Probe (all) 5'-CY5 - CATTggCTTCggTCAgCATggCT—BBQ-3'
IMP	IMPgenF1 5'- GAATAG(A/G)(A/G)TGGCTTAA(C/T)TCTC -3'
	IMPgenR1 5'- CCAAAC(C/T)ACTA(G/C)GTTATC -3'

Reference:
Multiplex Real-Time PCR Detection of Klebsiella pneumoniae Carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1)

Testing for CREs from public and private referral laboratories



Number of isolates tested at AMRRL

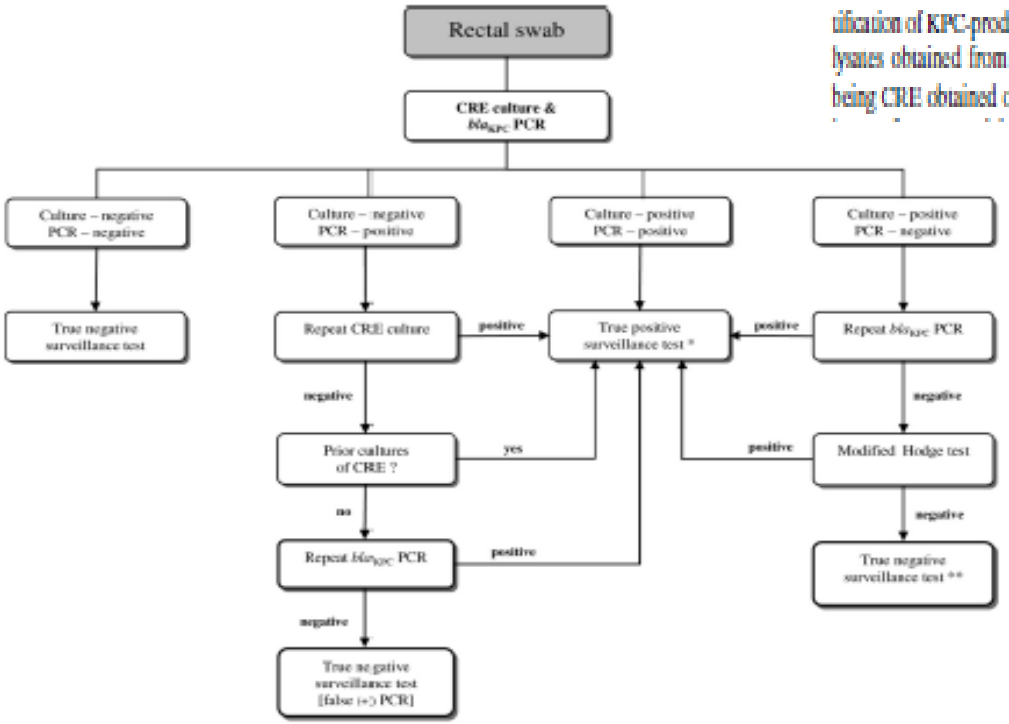


Evaluation of PCR-based testing for surveillance of CRE

Vered Schechner,1 Keren Straus-Robinson,2 David Schwartz,3 Iris Pfeffer,1 Jalal Tarabeia,1
Rina Moskovich,1 Inna Chmelnitsky,2 Mitchell J. Schwaber,1
Yehuda Carmeli,1 and Shiri Navon-Venezia2*
Division of Epidemiology,1 the Laboratory for Molecular Epidemiology and Antibiotic Research,2 and the Clinical Microbiology Laboratory,3 Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2009, p. 3261–3265

- Culture-based and molecular-based processing of 755 rectal swabs for CRE detection

Molecular-based method for KPC-producing CRE detection. Molecular identification of KPC-producing CRE was performed by bla_{KPC} PCRs using bacterial lysates obtained from swab-inoculated broths and from colonies suspected as being CRE obtained on imipenem-containing MacConkey plates. Bacterial cell



* Positive surveillance test for bla_{KPC} or other carbapenemase producing CRE
** Negative surveillance test for carbapenemase producing CRE

FIG. 1. Culture-based and molecular-based processing of 755 rectal swabs for KPC-producing CRE detection.

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2011, p. 718–721 Vol. 49, No. 2
0095-1137/11/$12.00 doi:10.1128/JCM.01773-10

How To Detect NDM-1 Producers▽

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Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics, Hôpital de Bicêtre

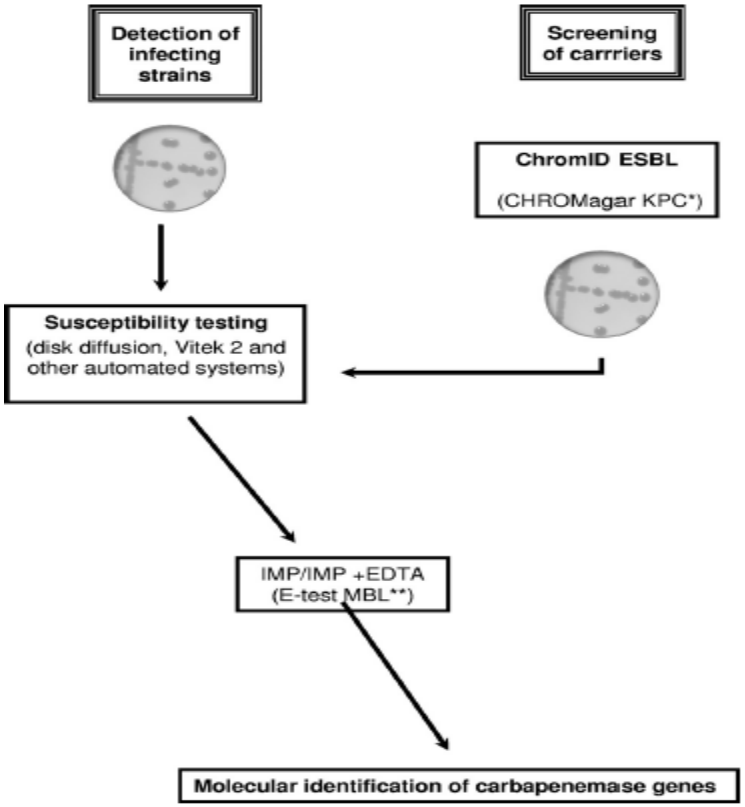

FIG. 1. Strategy for identification of NDM-1 producers as a source of clinical infections and for detecting carriers of NDM-1 producers. *, this culture medium can be used for surveillance of outbreaks of infections with NDM-1 producers after validation of its detection sensitivity for the specific strain responsible for an outbreak. **, Etest MBL is reliable when the MIC of imipenem is not too low.

Stools or rectal swabs (with or without enrichment in the presence of a carbapenem) should be plated on selective media. The problem is that the level of resistance to carbapenems displayed by carbapenemase producers varies significantly, making their detection difficult unless they show high-level carbapenem resistance [38,39].

Screening of colonized patients

- The first marketed screening medium was the CHROM agar KPC medium, which contains a carbapenem (CHROMagar, Paris France)
  - It detects carbapenem-resistant bacteria only if they exhibit high-level resistance to carbapenems. Its main disadvantage is lack of sensitivity; it does not`detect a low level of carbapenem resistance, as observed for several MBL or OXA-48 producers.
- The second screening medium also contains a carbapenem (CRE Brilliance, Thermo Fisher Scientific,UK)
  - It detects KPC and MBL producers well, and most but not all OXA-48 producers.
- Finally, one of the most recently developed screening media (SUPERCARBA) contains cloxacillin, zinc and ertapenem
  - It shows excellent sensitivity and specificity for detection of any kind of carbapenemase producer (not only high-level carbapenem-resistant isolates).
  - Compared with the two other media, it shows improved sensitivity and specificity for detecting all types of carbapenemase producers (including the OXA-48 producers) when present in low amounts in stools. Once carbapenem-resistant isolates are selected on SUPERCARBA medium, we recommend use of the Carba NP test for detecting carbapenemase activity. If needed, molecular identification of the carbapenemase genes may be performed.

Non-molecular tests for carbapenemases production

- Some have good sensitivity and specificity but none 100%,
  - Modified Hodge Test-low sensitivity and specificity and time consuming,
  - MALDI-TOF – detection of CREs,
  - Carba NP test-in vitro hydrolysis of the carbapenems and change the pH value of the indicator.

Conclusions

- Standardized methods for screening and identifications are needed in South Africa.
- Evidence based methods should be introduced.
- Evaluation of new methods or technique should be performed before developing diagnostic algorithms.

Thank you for your attention!