

The Laboratory Approach to KPC Identification



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Carbapenems

- Bactericidal, beta lactam family of antibiotics, target cell wall, disrupts different stages in peptidoglycan synthesis.
- FDA approved for clinical use:
 - imipenem, meropenem, ertapenem, and doripenem.
- Broad-spectrum activity
- Treatment of life-threatening infections:
 - Septicaemia
 - treatment of multi-drug-resistant GNB infections (*Pseudomonas aeruginosa*, *Acinetobacter* spp., ESBL's).

Carbapenem resistance

- impaired permeability due to porin mutation
- efflux pumps
- “carbapenem-hydrolysing enzyme”
 - hydrolyse the carbapenem nucleus and alter porin channels in the bacterial cell wall, reducing permeability of the drug.
 - 3 classes of the Ambler classification system, A, B, and D.

Classification Table

Classification	Enzyme	Most common Bacteria	Inhibitor	Active site
Class A	Chromosomal: IMI, SME, NMC Plasmid: KPC, GES	<i>Enterobacteriaceae</i>	Boronic acid	Serine
Class B	Metallo- β -lactamases: IMP, GIM, VIM, SPM	<i>Ps. aeruginosa</i> , <i>Enterobacteriaceae</i> <i>Acinetobacter</i>	EDTA	Zinc
Class D	OXA β -lactamases	<i>Ps. Aeruginosa</i> , <i>Enterobacteriaceae</i> <i>Acinetobacter</i>	Oxacillin	Serine

Class A

- 3 major families of class A serine carbapenemases:
 - NMC/IMI (not metalloenzyme carbapenemase/imipenem-hydrolysing beta-lactamase)
 - SME (*Serratia marcescens* enzyme),
 - KPC (*K. pneumoniae* carbapenemases)
- Hydrolytic mechanism requires serine at their active site
- Class A chromosomally encoded enzymes ; SME, NMC, IMI.
- Class A Plasmid- encoded enzymes; KPC, Guiana extended spectrum (GES).

Klebsiella Pneumoniae Carbapenemase

- First described in North Carolina in 1999.
- Progressive spread of KPC in US and worldwide.
- KPC confers resistance to all β -lactams including extended-spectrum cephalosporins and carbapenems.
- Gene (bla_{KPC}) that encodes these enzymes are located on plasmids-mobile genetic elements.
 - increases risk transfer.
- Occur in *Enterobacteriaceae*
 - Most commonly in *Klebsiella pneumoniae* or *Escherichia coli*.
 - Also reported in: *K. oxytoca*, *Citrobacter freundii*, *Enterobacter* spp., *Salmonella* spp., *Serratia* spp.
- 2007 data from CDC regarding health care associated infections –
 - indicated that 8% of all *Klebsiella* isolates were carbapenem resistant, compared with fewer than 1% in 2000.

Limerick



Home News

Limerick hospital 'superbug' outbreak

Mid-Western patients hit by multi-drug resistant bug

EITHNE DONNELLAN
Health Correspondent

ATTEMPTS ARE being made to contain an outbreak of a highly drug-resistant and potentially lethal bug at the Mid-Western Regional Hospital in Limerick. It is the first outbreak of KPC, or *Klebsiella pneumoniae carbapenemase*, in an Irish hospital. To date seven patients in the hospital have

been affected, and a further case has been identified at a long-stay facility in the midwest. The cause of the outbreak, which doctors have described as of serious concern, is being investigated. Symptoms include vomiting and diarrhoea. Visiting restrictions have been put in place and a programme of intensive deep cleaning has been

instituted in wards where transmission is suspected of occurring. Dr Fideima Fitzpatrick, clinical lead for healthcare-associated infections with the Health Service Executive and the Royal College of Physicians of Ireland, said the bug was serious because it was multi-drug resistant. It is resistant to a range of antibiotics, including penicillin and carbapenem antibiotics. "It would be sensitive to only one or two antibiotics and, ironically, it is sensitive to some of the older antibiotics we haven't used for years," said Dr Fitzpatrick. The bug was also of concern, she added, because it was in the bowel and was "almost impos-

sible" to get rid of, unlike MRSA which was on the skin and could be treated with washes and creams. She said that in patients with underlying conditions, the bug could be life-threatening. "Up to recently the only cases we noted in Ireland were sporadic and only associated with travel to other countries. That is what is unusual about this outbreak." There have been outbreaks in a number of hospitals abroad, including in the US and Greece. Dr Kevin Kelleher, head of health protection with the HSE, said: "This is the first time that there have been a number of cases

in an Irish hospital and unfortunately, it got to another hospital." He said there had been seven confirmed cases of the bug at the Mid-Western Regional Hospital in Limerick but only one patient had become ill as a result. The other patients were only colonised with the bug. Studies suggest the bug kills about 40 per cent of people who become infected, according to Dr Arjun Srinivasan, a medical epidemiologist with the US Centers for Disease Control and Prevention. "Typically, these are older, frail patients with multiple medical problems and compromised immune systems.

Meanwhile, visitors are asked not to visit the Mid-Western Regional Hospital in Limerick, including its emergency department, unless it is absolutely essential. Visiting is restricted to critically ill patients, with one visitor allowed for each critically ill patient and confined to visiting times (2pm-4pm, 6.30pm-8.30pm). The hospital says the elderly, children, pregnant women or young adults, those with chronic illnesses or vulnerable others are also advised not to visit. Outpatient and daycare services, as well as routine hospital admissions, are not affected.

Regional is hit by killer bug outbreak

■ One patient in intensive care unit as lethal superbug breaks out in hospital

Mike Dwane

SEVEN people have been infected in the Mid-Western Regional Hospital, Dooradoyle, after the HSE confirmed an

bacterium *Klebsiella pneumoniae* that live on the skin and in the mouth and guts of humans and can cause pneumonia and urinary tract infections," the HSE said.

A spokesperson for the hospital advised that "KPC-producing bacteria are a common type of bacterium that has evolved into a major challenge for infection control as

CRE in Ireland

Rapid communications

FIRST IDENTIFICATION OF CLASS A CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* IN THE REPUBLIC OF IRELAND

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February 2009.

Highly resistant to all β – lactams and had reduced susceptibility to three other classes of non- β lactam antibiotics.

RTI-treated with piperacillin-tazobactam.

When isolate identified, patient discharged, clinically well.

- Laboratory MSc Jan 2010
- Jan 2010 the CLSI breakpoint (S if MIC \leq 4 mg/L imipenem or meropenem).
- Difficulty detection from clinical specimens
 - Some strains that harbour blaKPC have carbapenem MIC values susceptible range.
 - Pasteran *et al.* 2009, CLSI 2009, \leq 21mm cut-off used initially.
 - Resistant organisms 2 or more cephalosporins
- March 2010 - CLSI reduced their clinical breakpoints for carbapenems.
- *Enterobacteriaceae*, the meropenem screening breakpoint to detect carbapenemases is set at \geq 0.5mg/L or a zone of \leq 23mm. (Cohen Stuart and Leverstein-Van Hall 2010).

Clinical Breakpoints

TABLE 1

Clinical breakpoints defined by minimum inhibitory concentrations in mg/L for the categories S=susceptible and R=resistant according to recommendations of CLSI and EUCAST

Antibiotic compound	CLSI 2010		EUCAST 2010		
	S ^a	R	S	R	ECOFF for <i>E. coli</i> and <i>K. pneumoniae</i> ^b
Imipenem	≤1 (≤4) ^c	≥4 (≥16)	≤2	>8	≤0.5 for <i>E. coli</i> ≤1 for <i>K. pneumoniae</i>
Meropenem	≤1 (≤4)	≥4 (≥16)	≤2	>8	≤0.125
Ertapenem	≤0.25 (≤2)	≥1 (≥8)	≤0.5	>1	≤0.06
Doripenem	≤1 (ND)	≥4 (ND)	≤1	>4	≤0.12

CLSI: Clinical Laboratory Standards Institute; ECOFF: epidemiological cut-off values; EUCAST: European Committee on Antimicrobial Susceptibility Testing; MIC: minimum inhibitory concentration; ND: no data.

^a I=intermediate is implied by the values between the S-breakpoint and the R-breakpoint.

^b ECOFF for *E. coli* and *K. pneumoniae* define the top end of the wildtype distribution; bacteria with MICs ≥ ECOFF have acquired some mechanism of resistance.

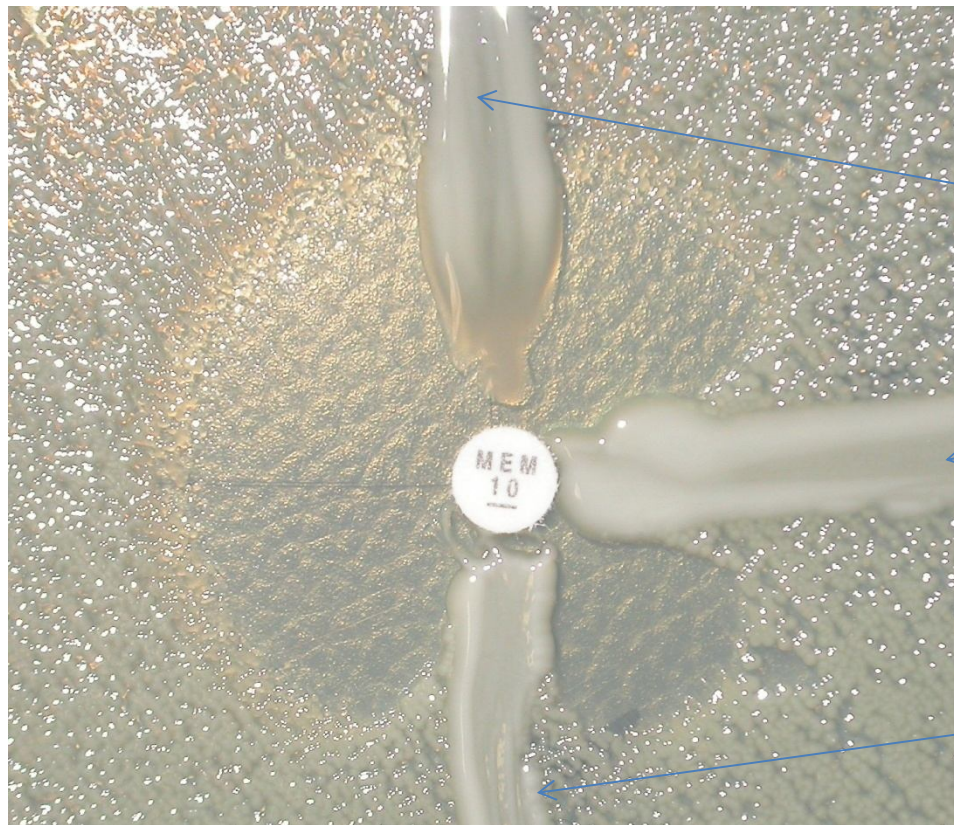
^c Values in parentheses indicate the CLSI breakpoints recommended before June 2010.

Grundmann *et al.* 2010

Laboratory Approach to KPC Identification

1. Disc diffusion with meropenem 10 μ g on Mueller Hinton agar.
2. Phenotypic confirmation
 - Modified Hodge test
 - Microbiological tests with inhibitors
3. Genotypic confirmation
 - Molecular tests

2. Phenotypic confirmation Modified Hodge Plate



Negative
control

Positive test
sample

Positive
control

2. Phenotypic confirmation

Modified Hodge Test

- Modifications of the cloverleaf or Hodge test (Hodge *et al.* 1978).
- Leakage of carbapenemases from the producer into the surrounding agar.
- High sensitivity 95-100% (Cohen Stuart J & Leverstein-Van Hall 2010).
- Different classes of carbapenemases cannot be distinguished.
- False positives lack specificity – AmpC or ESBL producing strains

2. Phenotypic confirmation

Disk diffusion synergy tests

- carbapenem antibiotic synergy with carbapenemase-inhibiting compounds.

- Phenylboronic acid has an affinity for the active site serine residue of class A β -lactamases.
- Prepared locally or can be purchased (ROSCO).
- Test positive for detection of Class A enzyme when the diameter of the growth-inhibitory zone around the β -lactam disk with boronic acid is $>$ or $=$ 5mm larger than that around a disk containing β -lactam substrate alone (Tsakris *et al.* 2009).

Boronic Acid Synergy

Ertapenem + Boronic
Acid

*Klebsiella
pneumoniae*
ATCC BAA 1705



Ertapenem

Meropenem

Meropenem + Boronic
Acid

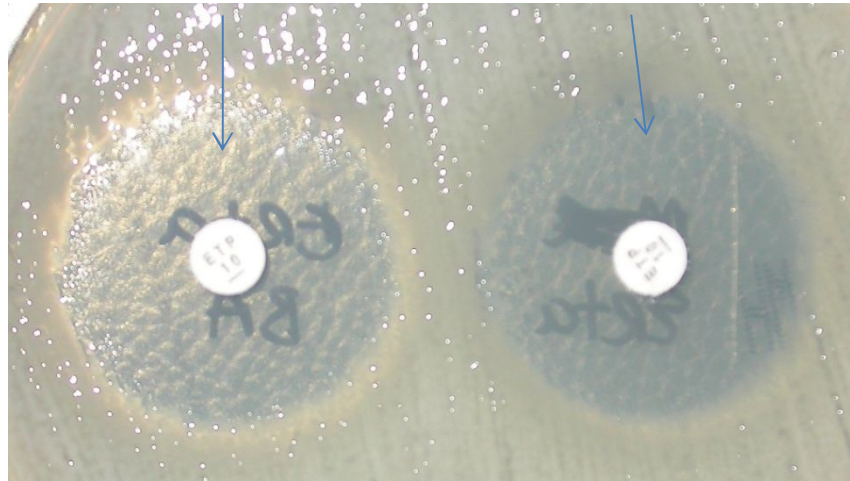
Boronic acid disk test

Klebsiella pneumoniae
ATCC BAA 1706

Negative control

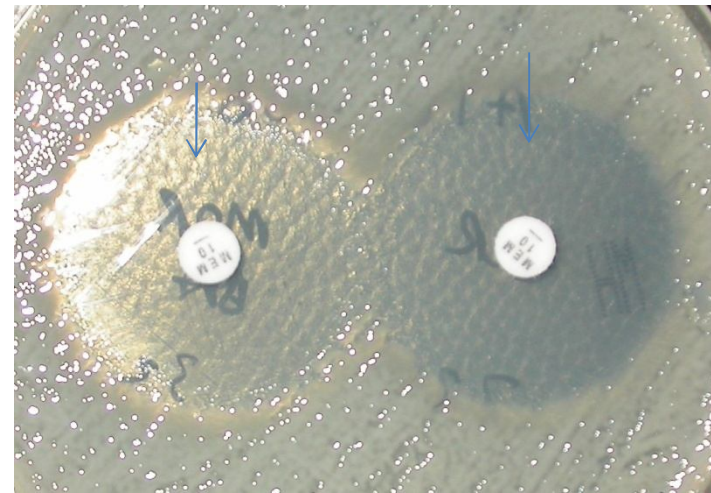
Ertapenem + Boronic
Acid

Ertapenem



Meropenem +
Boronic Acid

Meropenem



Laboratory Approach

- Organism I.D. & susceptibility breakpoint broth microtitre (Sensititre TREK)
- Additional susceptibility testing
 - confirmation of Meropenem resistance by Etest,
 - Colistin, Tigecycline Etests
 - no CLSI interpretative criteria
 - Report M.I.C.s , EUCAST used
 - Suggestive of carbapenemase production
- Genotypic investigation

3. Genotypic confirmation

- PCR assays can target different genes in single or multiplex formats
 - class A (KPC, IMI, NMC, SME),
 - class B (IMP, VIM, AIM, GIM, KHM, SIM and SPM)
 - class D (OXA-23-like, OXA-24-like, OXA-48-like, OXA-51-like and OXA-58-like)
- Gold standard
- Detect the presence of more than one enzyme in a single clinical isolate.
- Antimicrobial Susceptibility Testing reference laboratory, Health Protection Agency (HPA), Colindale.
- Antimicrobial Resistance and Microbial Ecology Group in the School of Medicine, NUI, Galway.

KPC positives to date

- 1st isolate 2009 in sputum.
- 16 patients identified with KPC producing isolates were detected between Jan 2010 to date.
- 1 patient positive for two KPC positive isolates from different bacteria- *E.coli*, *K. pneumoniae*.
- 16 isolates identified as *Klebsiella pneumoniae* and one isolate as *E.coli*.
- KPC's isolated from urine, sputum, blood cultures, rectal swabs, drain fluids, CAPD fluid.
- January - December 2010: 5 KPC positive patients
 - Long term care facility- urine
 - Another hospital region - urine
 - Surgical outpatient - urine
 - Medical ward – sputum
 - Private outpatient dialysis clinic- urine (Screening begins)

CDC recommended Screening method- TSB broth enrichment



KPC positive isolate:
Meropenem Zone ≤ 23 mm

Rectal swab
Incubated 24 hrs in TSB broth with
meropenem disc
Aliquot plated McConkey agar.

Hospital Outbreak

- **Index case** – January 2011- blood culture
- 70y male history UC, open wounds.
- Admitted from St Johns Hospital to 3A(medical ward) to ICU, to **HDU** to 1D and readmitted to ICU.
- Blood culture identified as ? KPC and commenced treatment for KPC pneumonia and was isolated with deep clean around his bed space.
 - On i.v. piperacillin/tazobactam for LRTI, changed to i.v. tigecycline AND nebulised Colistin M.I.C. = 1mg/L.
 - 30 days later Colistin M.I.C. = 16mg/L

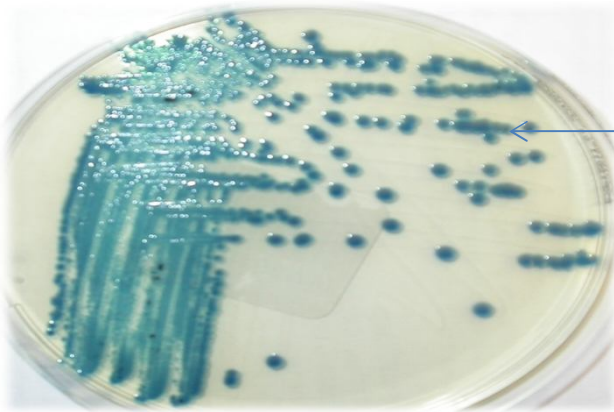
Patient 2

- Patient recently in HDU MWRH - GI haemorrhage
 - HDU
 - 3A
 - Self discharged
- Re-presented Dublin hospital with further GI bleed.
- Admitted to ICU.
- Line tip - *Klebsiella pneumoniae* 30/1/2011
 - Subsequently confirmed as KPC.

- Outbreak declared-7/2/11
- Alert sent to all staff advising of screening policy for KPC for critical care areas; HDU & ICU
 - Admission/ Weekly/ Discharge screens.
 - Inpatient contacts of patient 1& 2 screened.
 - Screened surgical wards 1B

CHROMagar™ KPC

- Detection of gram-negative bacteria expressing a reduced susceptibility to antibiotics of the carbapenem family. KPC, OXA, MBL (incl. NDM-1).
- Faster turnaround time.
- No pre-enrichment step. Direct plating of the sample is possible.
- Requires only 18-24 hours of incubation.



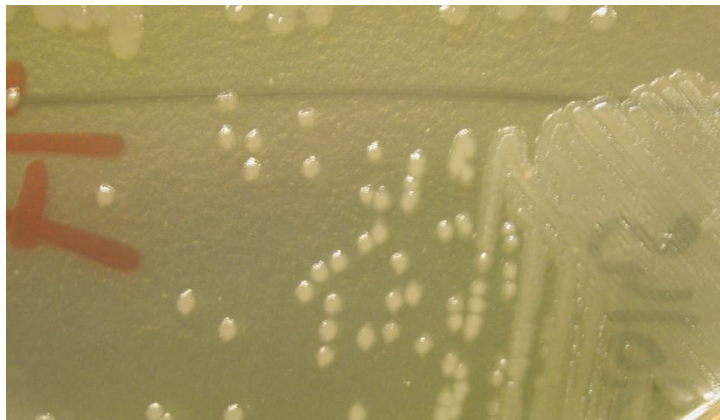
CHROMagar™ KPC
K. pneumoniae KPC positive
metallic blue colonies

CHROMagar™ KPC



E.coli CarbapenemR
→ dark pink to reddish

Klebsiella, *Enterobacter*,
Citrobacter CarbapenemR
→ Metallic blue



Pseudomonas CarbapenemR
→ Cream, translucent

CarbapenemS strains - inhibited

(Panagea *et al.* 2010).

Detection of CRE from rectal Swabs using 2 methods

	N	McConkey + Meropenem Disk	Chromagar KPC
	621	Negative	Negative
	15	Positive	Positive
	1	Negative	Positive
	1	Positive	Negative
	1	False Positive	False Positive
Total	639		

Rectal swabs from Ennis, Nenagh, St. John's, MWRH.

17 positives.

15 positive for both methods (grew metallic blue on CHROMagar KPC and zone ≤ 23 on McConkey method).

1 false positive on both- AmpC positive *Enterobacter cloacae* (boronic acid positive and Hodge negative).

CHROMagar™ KPC versus Enrichment

Method	Sensitivity	Specificity
CHROMagar KPC	94.44%	99.84%
CDC protocol	94.44%	99.84%

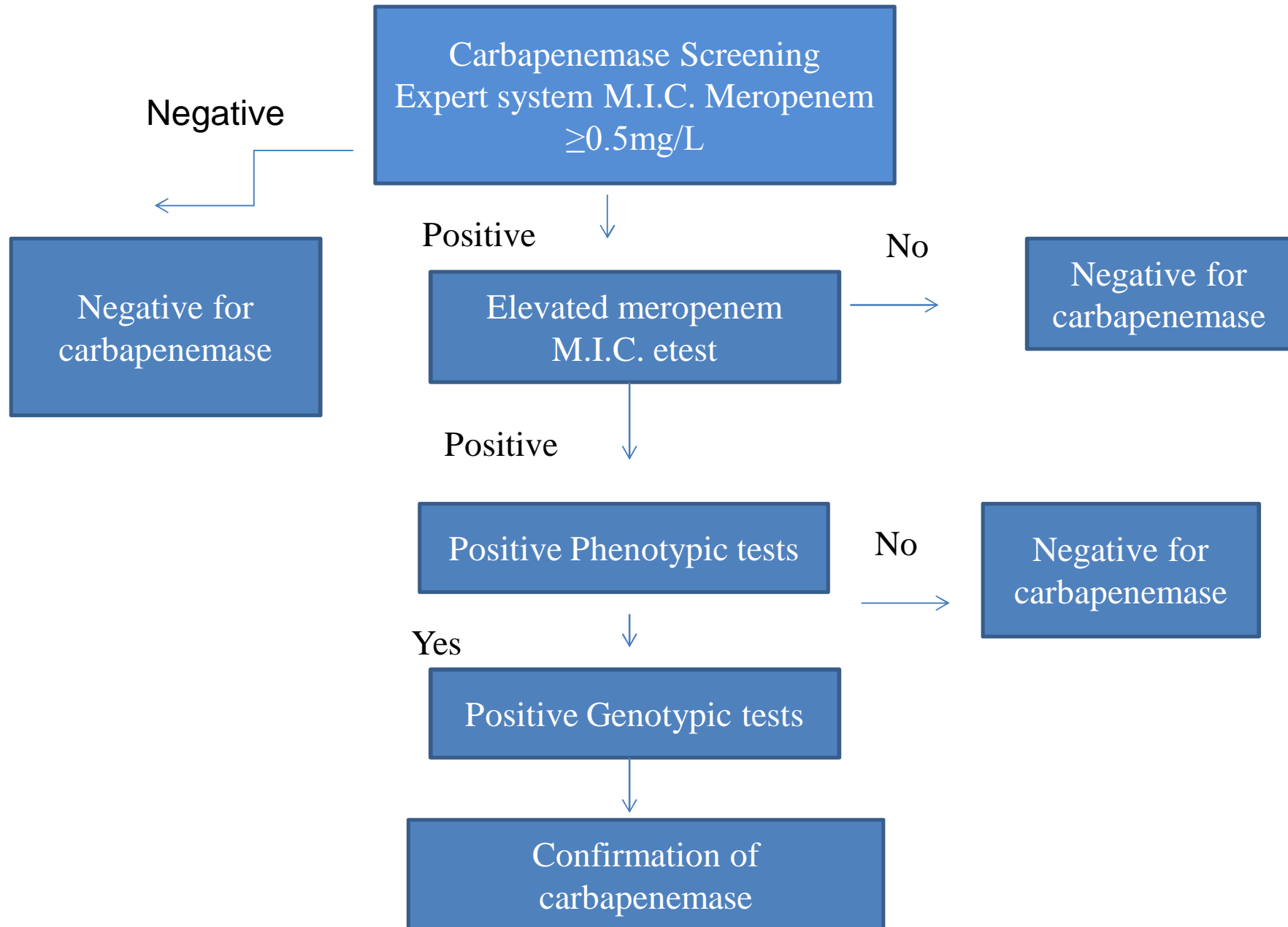
- KPC Agar - different carbapenemases present in single sample indistinguishable on agar.
- The lower limit of detection for CHROMagar KPC - was 1×10^2 CFU/mL.
- Previous studies performed by Landman *et al.* 2005 and studies performed at the CDC suggest that the lower limit of detection for CDC protocol method is between ranges from 1×10^2 CFU/ mL to 1×10^6 CFU/ mL.

Outcomes to date

- Current screening protocol
 - All ICU/HDU screened for KPCs on admission, weekly and discharge
 - Contacts of new positive case screened
 - All dialysis patients on 3 monthly schedule
 - In-house patients if moving to another hospital
 - Parallel methodology once monthly
- Implemented meropenem 0.5mg/L screening well on Automated Sensititre panels.
- Alert system set up to flag reduced carbapenem MIC or resistant MIC.
- Flagged isolates – phenotypic testing

- Sensitivity of MHT = 100%
- Sensitivity of Boronic Acid Test = 100%
- Meropenem MICs differed among the KPC isolates, ranging from 0.5 to >32mg/L.
- *E.coli* isolate had MIC 0.5mg/L. This tests sensitive under both CLSI and EUCAST guidelines.
- High carbapenem MIC is not always present.
- Can miss potential carbapenemase production
- KPC agar (ESBL, *Enterobacter*, *K. oxytoca*).
 - Meropenem M.I.C. >0.5.

Summary Flow Chart



Conclusion

- Prevalence of carbapenemase producing *Enterobacteriaceae* in Ireland is currently unknown.



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