Comparison of molecular techninques for detection of CPE.

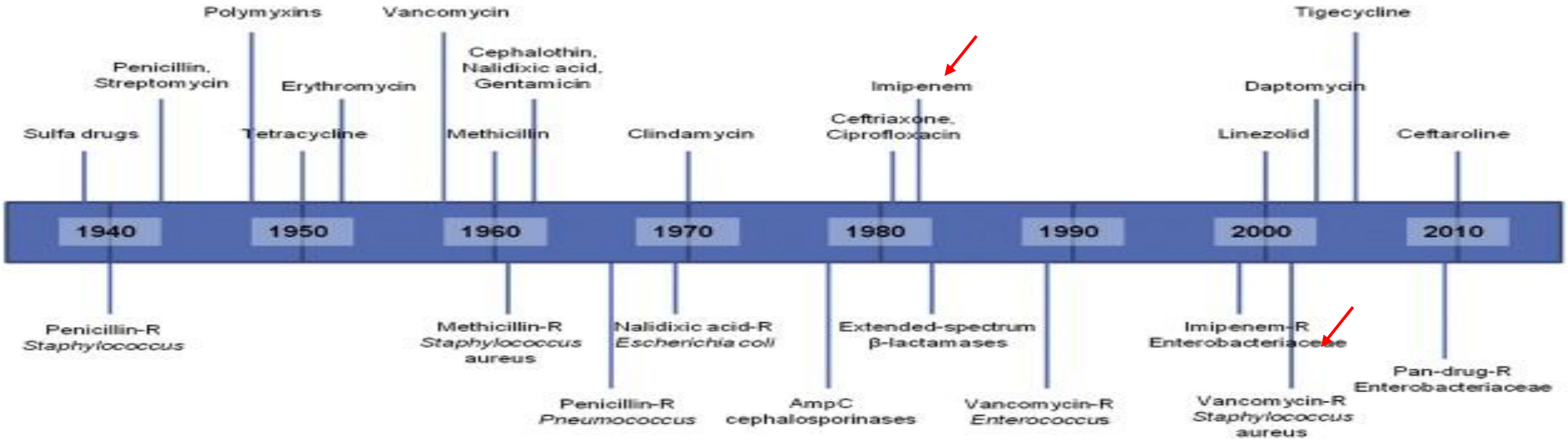
Kate Byrne (MMUH), Denise Drudy (DIT), Deirdre Keating (SVUH), Orla Donoghue (SVUH), Emer Coyle (SVUH) and Dr. Susan Fitzgerald (SVUH).

Carbapenems

- B-lactams constitute 50% of the worldwide use of antimicrobials.
- Carbapenems are deemed the most effective broad-spectrum antimicrobial.
- Carbapenems can withstand the hydrolysing activity of ESBLs and AmpC enzymes.
- Carbapenems are a reliable, last resort treatment option in cases where resistance to 4^{th} generation cephalosporins has emerged.

(Meletis, 2016).

Carbapenem resistant Enterobacteriaceae



- Imipenem was introduced in 1985.
- Within 13 years, imipenem resistant Enterobacteriaceae emerged.
- Carbapenem resistance is recognised internationally as one of the most pressing aspects of the growing public health threat posed by antimicrobial resistance.
- Carbapenem resistance can be brought about by ESBL or AmpC enzyme activity often coupled with porin loss or efflux pump activity.

(Ventola., 2015)

Carbapenemases

- Enzymes detected in the 1980's.
- Produced by Enterobacteriaceae.
- Clinically relevant carbapenemases produced by Enterobacteriaceae belong to Ambler Class A, B and D. These three classes contain the 'Big Five'.
- Genes encoding these enzymes are mostly plasmid-mediated and associated with various mobile genetic element, enhancing their mobility and spread in hospital and community setting.

Ambler Group	Mediates Resistance to	Examples	Inhibited by:
A	Carbapenems, penicillins, cephalosporins, aztreonam	**KPC**, GES, IMI, SME, NMC	Boronic Acid
B Metallo-β-lactamases	Carbapenems, penicillins, cephalosporins, not aztreonam	**IMP, NDM, VIM,** SIM, GIM, SPM	EDTA & Dipicolonic Acid
D	Carbapenems, penicillins, low level resistance to cephalosporins and aztreonam	**OXA-48**, OXA-23,-51,-24,-58	Avibactam

(Herra, 2018)

Clinical Implications of CPE

1. Sporadic outbreaks or endemic situations.
2. High mortality. ~50%
3. Carriage has a strong link to infection. ECDC ~89% link.
4. Rapid dissemination.
5. Community and hospital transmission.

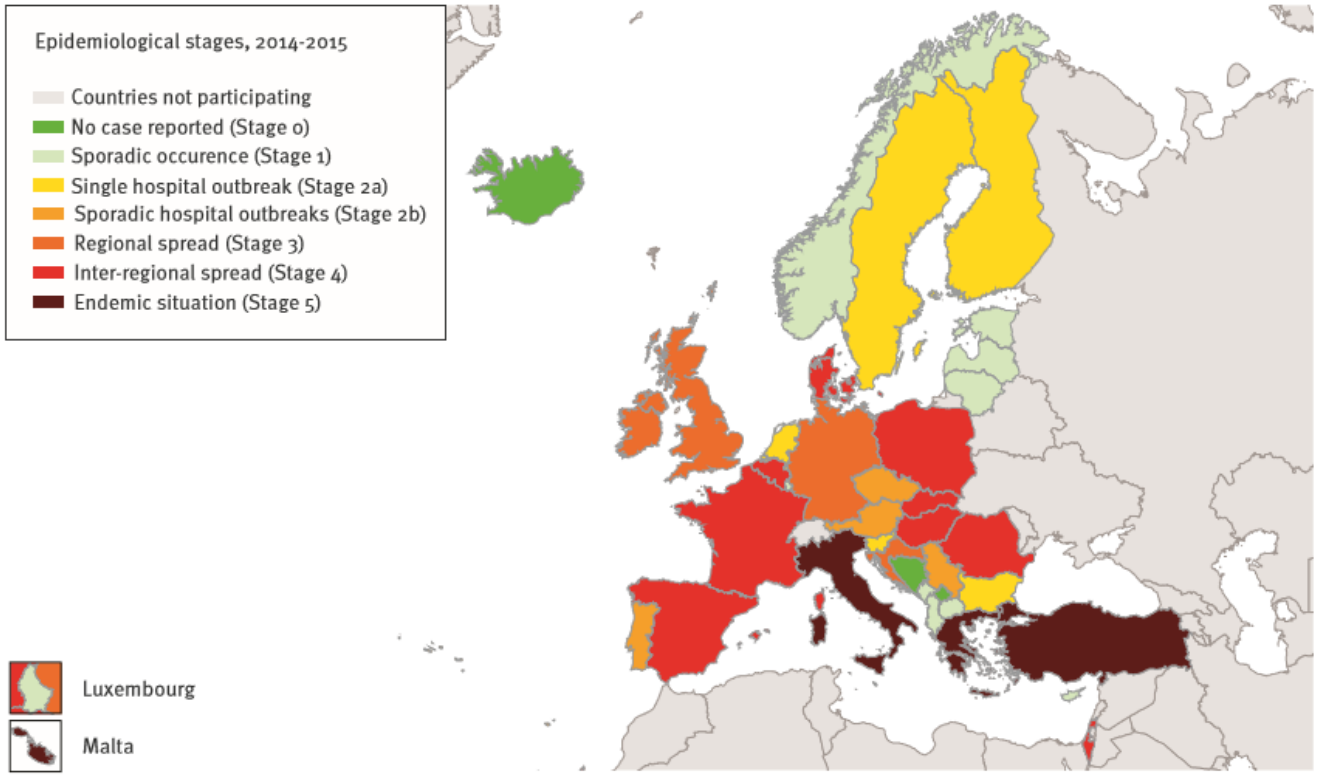
Prevention of spread relies on the early detection of carriers and implementation of infection control procedures.

ECDC, (2016)

Epidemiology of CPE in Europe

FIGURE 1

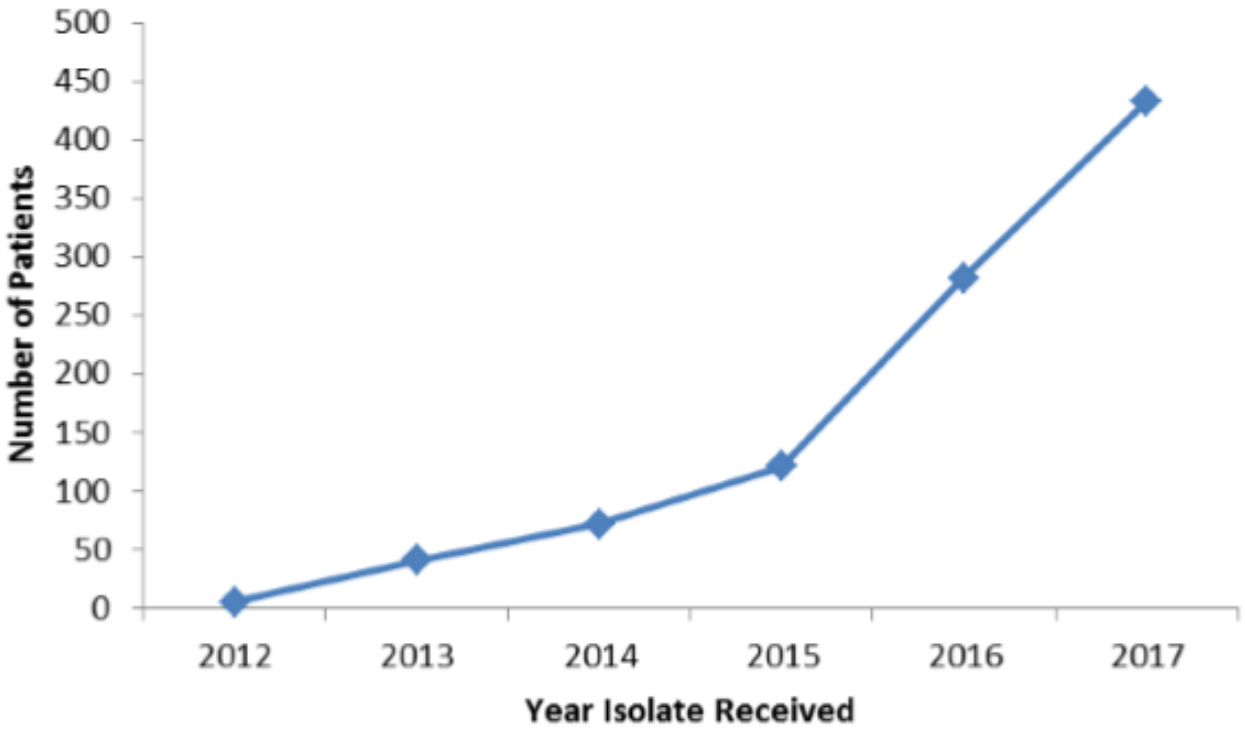
Occurrence of carbapenemase-producing *Enterobacteriaceae* based on self-assessment by national experts, 38 European countries, May 2015



- KPC producing Enterobacteriaceae had the widest dissemination in Europe (endemic in Greece and Italy).
- Oxa-48 carbapenemases were endemic in Turkey and Malta.
- Seven countries reported regional and interregional spread of NDM.
- VIM-4 producing Enterobacteriaceae were deemed the predominant CPE in Hungary and responsible for hospital outbreaks in Denmark.
- IMP remained rare throughout Europe.

(Albiger et al., 2015)

Epidemiology of CPE in Ireland

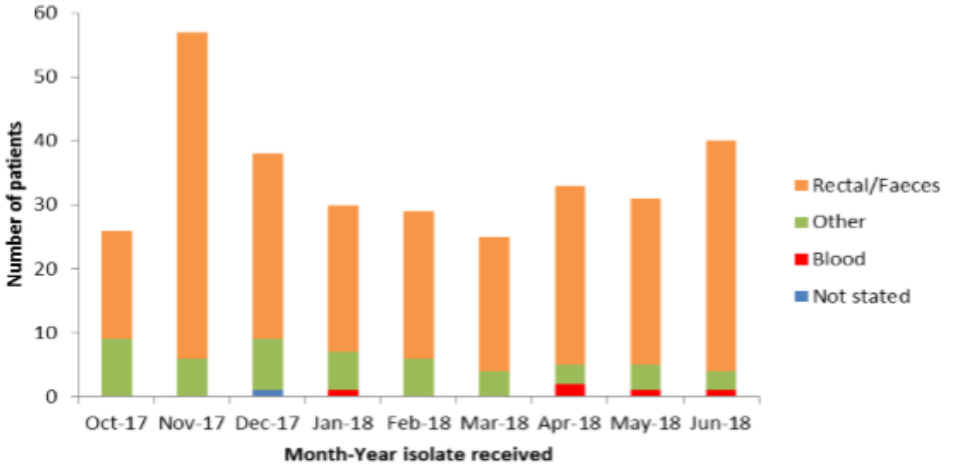


Annual numbers of patients with CPE newly-confirmed: 2012-2017

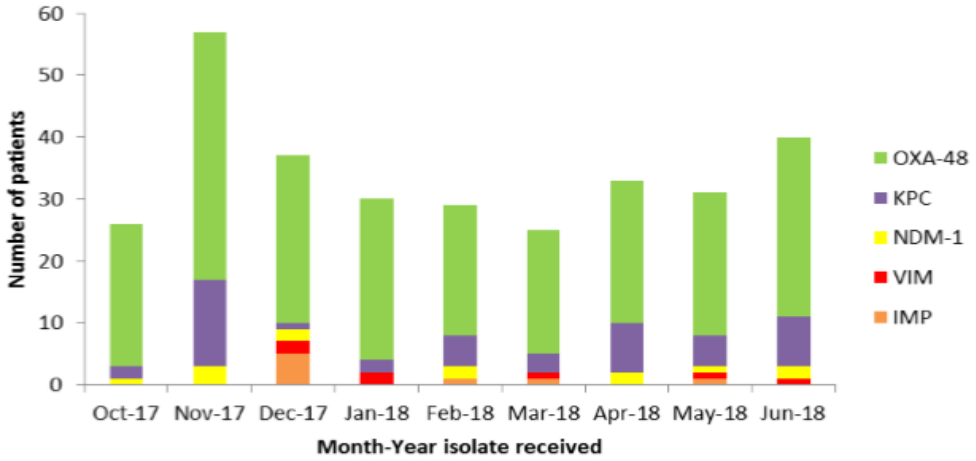
- In 2017, 433 patients with newly identified CPE were detected versus 282 in 2016, 87 in 2014 and 50 in 2013.
- In July 2018, 59 newly identified CPE were detected in comparison with 40 in June 2018.
- There were 15 new CPE outbreaks created in 2017 versus five in 2016.
- True increase in incidence **Vs** better detection systems and enhanced screening programmes.

(HPSC, 2018)

Monthly number of patients with CPE newly-confirmed by NCPERLS



Monthly number of patients with CPE newly-confirmed by specimen type.

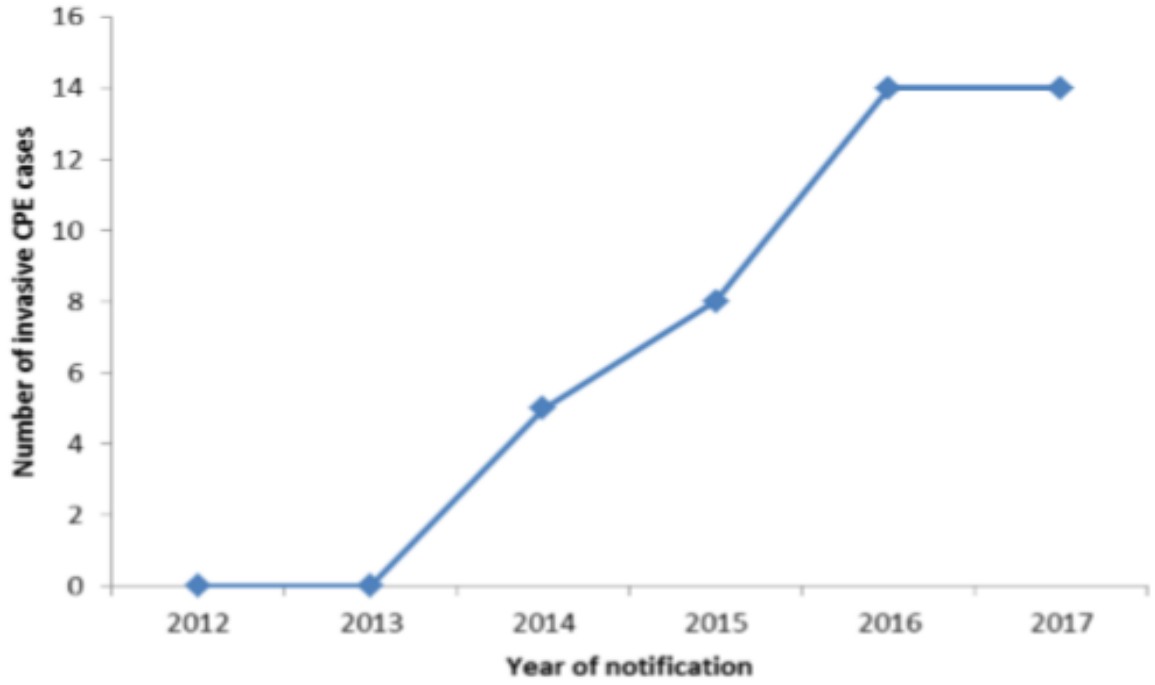


Monthly numbers of patients with CPE newly-confirmed by carbapenemase type.

- OXA-48 is the predominant carbapenemase in Ireland.
- Increase in the detection of KPC carbapenemases has been observed since Q2 2018.
- Of 59 patients in July 2018, 93% were newly-confirmed carriers detected on CPE screening (rectal swab/faeces.

(HPSC, 2018)

Invasive CPE infection in Ireland



Annual notification of invasive CPE infection 2012-2017

- One case of invasive infection was notified in June 2018, and no cases notified in July 2018.

(HPSC, 2018)

CPE screening

Who must be screened?

The following patients **must** be screened for CPE in acute hospitals.

a. All contacts of a patient with CPE. Where such contacts have been discharged prior to their identification as a contact, their record should be marked to ensure screening on next admission.[2 3]
b. All admissions to critical care areas (Intensive Care Units, High Dependency Units, Neonatal Intensive Care Units[4]), on admission and weekly thereafter.
c. All admissions to haematology and transplant wards on admission and weekly thereafter.
d. All patients who have received cancer chemotherapy in the previous 12 months on admission.
e. All patients who were transferred from any other hospital in Ireland or elsewhere.
f. All patients who have been inpatients in any hospital in Ireland or elsewhere any time in the previous twelve months. Any hospital includes previous admissions to the hospital to which they are now being admitted. [2 4]
g. Renal dialysis patients at first dialysis in a unit, periodically during dialysis treatment (at intervals of not less than six months), and on return from dialysis elsewhere.
h. All patients who normally reside in a long term care facility.

This level of screening should be implemented in all hospitals from **1st March 2018**.

- All Irish microbiology laboratories are required to report information on CPE cases (infection/colonisation) to the HPSC aided by the CPERLS.

(CPE Expert Group, 2018)

Laboratory screening

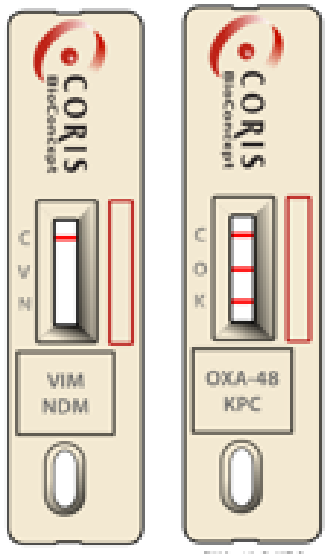
Laboratory screening for CPE should at a minimum mean plating of rectal swabs/faeces on one of the accepted CPE chromogenic agars. Access to rapid methods for direct testing of selected samples and/or for rapid confirmation of suspect CPE from agar plates should be available. Where screening is based on an initial molecular method those samples that test positive should be cultured to attempt to isolate the organism. It is accepted that in some instances it will not be possible to confirm a molecular result by culture. If the molecular test used is a CE (European Conformity) marked product or a well validated in-house method, this result is generally sufficient to designate the patient as CPE colonised though consideration should be given to culturing subsequent samples to obtain culture confirmation whenever possible.

Where capacity to perform screening does not exist in each individual hospital laboratory, hospital groups may consider providing the testing from one centralised

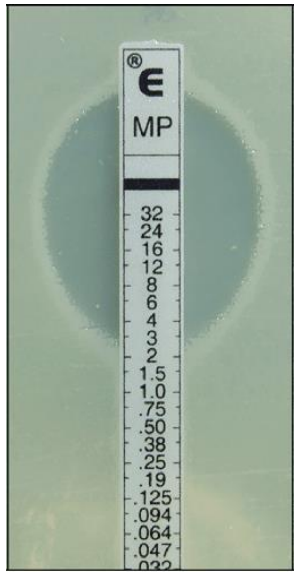
[5] Rescreening of known CPE positive patients on readmission is not essential for infection prevention and control purposes if the patient is managed as CPE positive but it may be of value to assess if they have acquired additional CPE variants. It may also have a value in providing assurance regarding the capacity of the screening system in place to detect CPE. However, it is important to ensure that patients are not considered cleared of CPE because of a single "CPE not detected"/ "CPE negative" rectal swab/faecal sample result.

(CPE Expert Group, 2018)

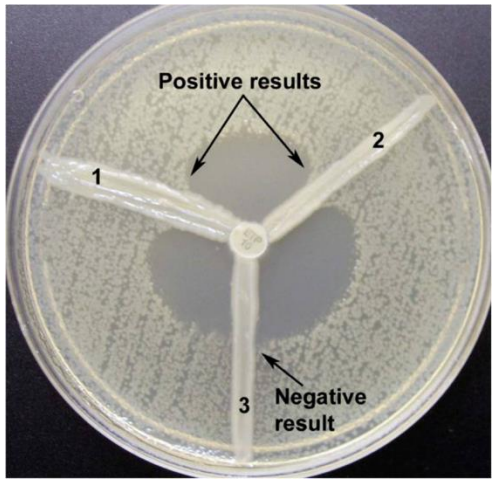
Culture based CPE detection kits.



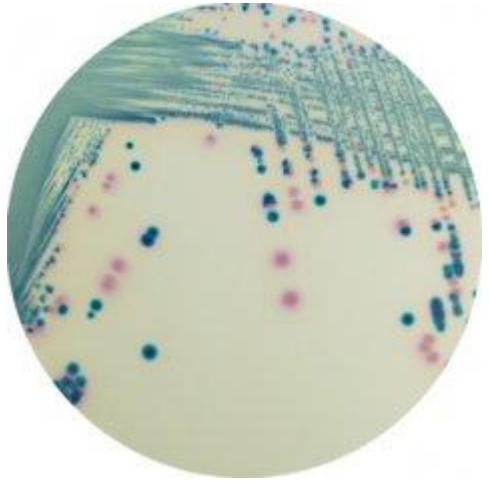
RESIST-4 O.K.N.V



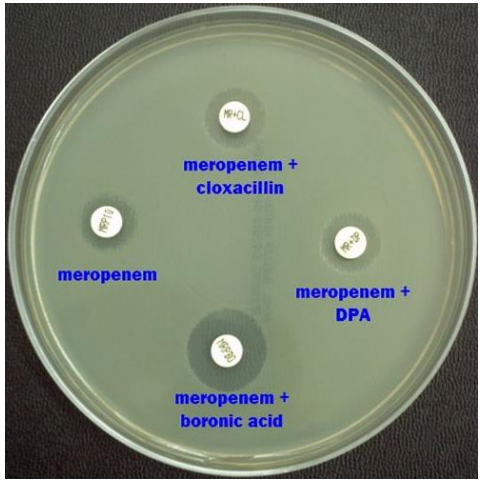
E-Test



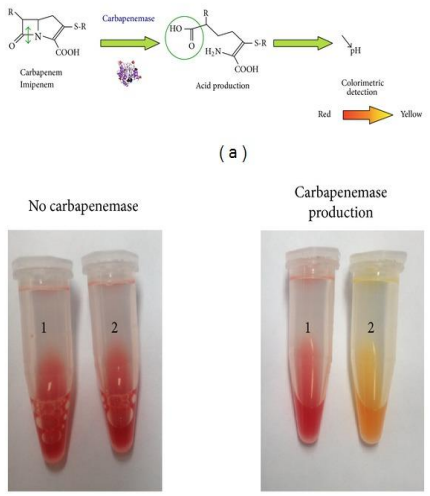
Modified Hodge Test



CHROMagar™ mSuperCARBA™



KPC/MBL confirmation kit



Rapid B-Carba test

- Prolonged incubation 24-72hrs.
- Indicate presence of carbapenemase Vs distinguishing carbapenemase type.
- Require cultured isolate.
- Subjectivity

Molecular techniques for the detection of CPE

- Increased sensitivity and specificity.
- Improved turn around time
- Prevent/identify an outbreak.
- Infection control interventions. i.e. Standard precautions, patient isolation and cohorting.
- Prompts enhanced antimicrobial stewardship.

EntericBio realtime CPE assay

Newly released molecular platform designed for the in-vitro diagnostic testing and qualitative detection of Enterobacteriaceae produced carbapenemase genes.

- Samples: Isolates/rectal/faecal swabs
- Throughput= 46
- Automation: Set-up and detection
- TAT= Approx <3.5hrs
- Hands on time 30 minutes
- Targets covered: KPC, OXA-48, NDM, VIM, IMP, GES
- Other assay: Enterics, C.diff, Parasites
- CE marked

(Serosep, 2018)

EntericBio Realtime CPE assay

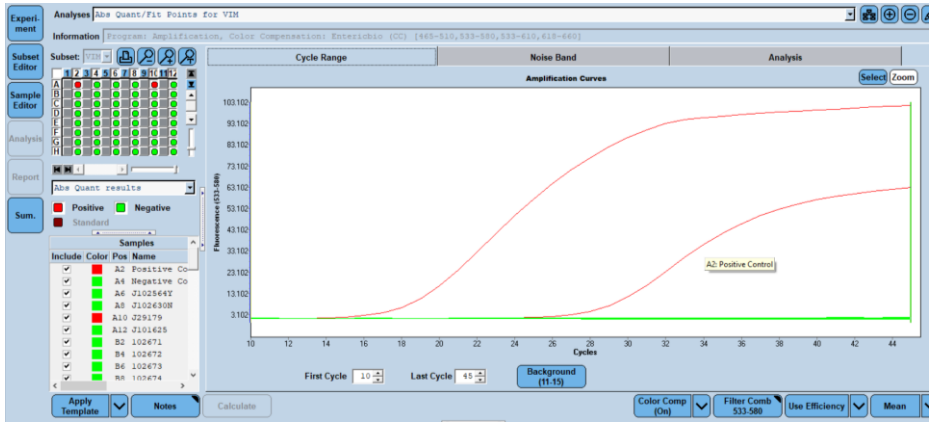
Innoculate SPS

Rapid heat extraction

Automated sample transfer

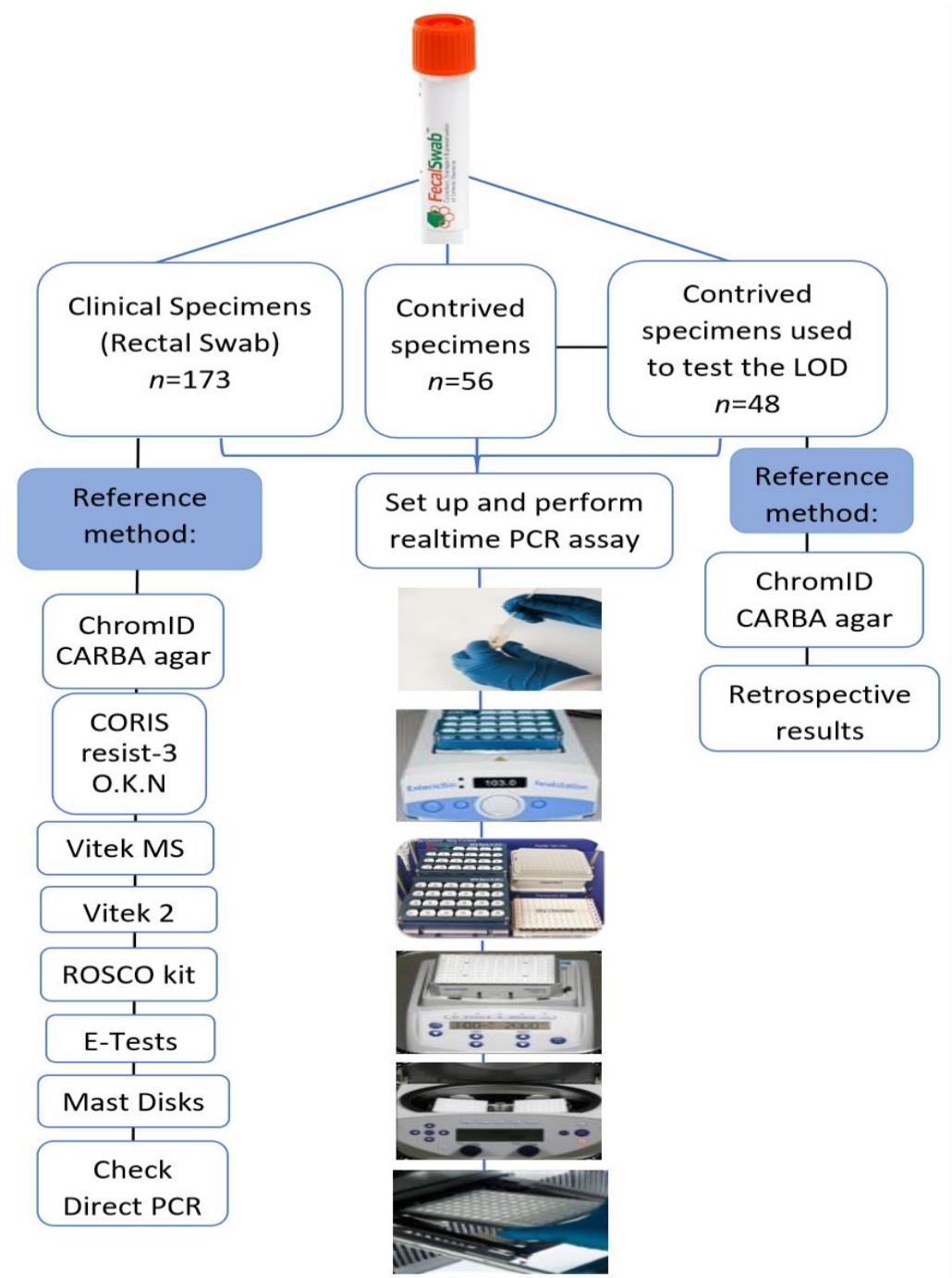
Mix test strips

Amplification/detection



Graph interpretation

Study Design



Results

EntericBio realtime® CPE assay result by target for clinical and contrived specimens.

EntericBio realitme CPE assay	Clinical Specimens n=173	Contrived Specimens n= 56	All Specimens n=229
KPC	0	4	4
OXA-48	1	15	16
NDM	0	5	5
VIM	0	4	4
IMP	1	3	4
GES	0	2	2
KPC/IMP	0	1	1
OXA-48 + IMP	0	1	1
OXA-48 + NDM	0	1	1
OXA-181	0	1	1
OXA-181 + NDM	0	1	1
KPC+OXA-48	0	1	1
Negative	171	17	188

Positive

The EntericBio realtime® CPE assay produced two false positive results. A false positive OXA-48 (Ct 25.52) was detected in a contrived specimen and a false positive IMP (weak positive, no Ct) was detected in a clinical specimen.

Assay	Sensitivity (%(95% CI))	Specificity (%(95% CI))	PPV (%(95% CI))	NPV (%)
EntericBio Realtime CPE	100 (90.9-100)	98.95(96.25-99.87)	95.1(83.1-98.7)	100

- Overall performance of the EntericBio realtime® CPE assay for clinical specimens (n=173) and contrived specimens (n=56).

Discrepant testing results of clinical and contrived specimens.

Reference Method			Discrepant analysis		
ChromID Carba	Retrospective analysis	Targets detected by EB CPE assay	Repeat original SPS	Repeat Spike	Outcome
No growth	NA	IMP	Neg	Neg	FP
Coliform	KPC	KPC + OXA-48	OXA-48	OXA-48	I

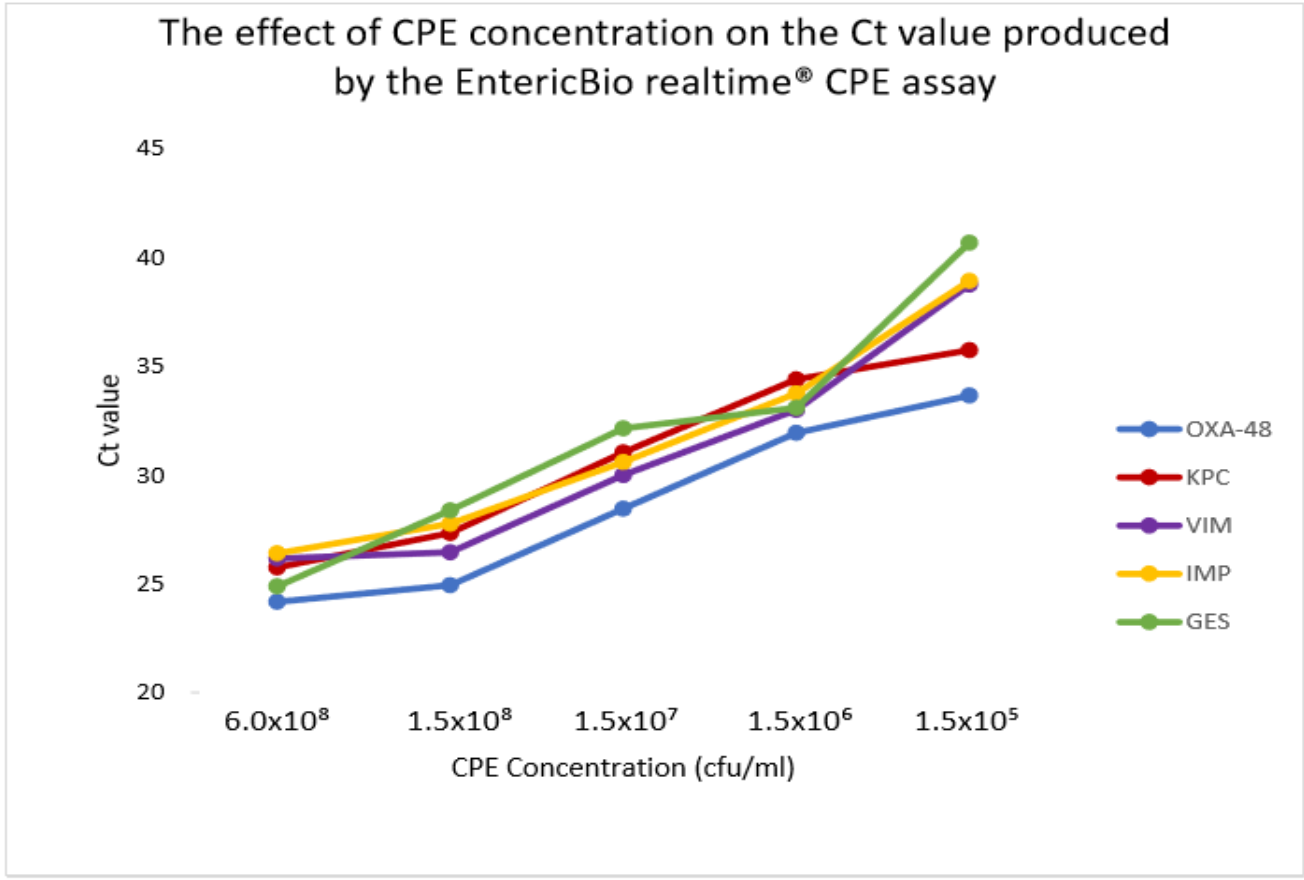
FP; False positive, I; Indeterminate

Summary of the EntericBio realtime® CPE assay performance with carbapenemase contrived samples (n=48) prepared at a concentration range of $1.5x10^8$-$1.5x10^5$cfu/ml.

Carbapenemase concentration (cfu/ml)	Sensitivity (%(95% CI))	PPV (%)
$1.5x10^8$	100 (73.5-100)	100
$1.5x10^7$	100 (73.5-100)	100
$1.5x10^6$	100 (73.5-100)	100
$1.5x10^5$	83.3 (51.6-97.91)	100

- False negative NDM and VIM result at $1.5x10^5$ cfu/ml.

Results produced by the rectal swabs contrived at a concentration range ($1.5x10^8$-$1.5x10^5$ cfu/ml) used to test the LOD, highlight that there is an indirect relationship between the target gene concentration and its corresponding Ct value.



Limitations of the validation/assay

- Lack of recovery of carbapenemase producing Enterobacteriaceae in the clinical cohort.
- Rectal swabs were obtained from patients on the same designated wards, thus repeat swabs were obtained and included in the study.
- The reference method for clinical and contrived specimens differed.
- The assay does not target IMI carbapenemases.
- Assay does not distinguish between viable and non-viable DNA in specimens.
- Qualitative system.
- Fluorescent leakage (corrected with colour compensation).

Evaluation of the new EntericBio CPE real-time PCR assay for the detection of Carbapenemase-Producing *Enterobacteriaceae* from rectal swabs.

E. Phelan, C. Fanning, P. Mulhare, Dr. M. Hickey
Microbiology Department, University Hospital Waterford, Ireland.

Introduction

The recently published Irish National screening policy[1] for Carbapenemase-Producing *Enterobacteriaceae* (CPE) will add workload pressures to already at capacity microbiology laboratories.

Increasingly, laboratories are exploring the strategic and economical feasibility of molecular solutions to meet screening demands for CPE.

In addition to potential improved laboratory testing strategies, molecular assays offer same-day results, and as such, their use may positively impact patient management.

In this study we evaluated the EntericBio CPE realtime PCR assay (Serosep, Ireland) for the detection of CPE from rectal swabs. We compared the assay to our routine algorithm for the detection of CPE from rectal swabs. The EntericBio system offers direct, extraction-free, automated molecular testing.

The assay targets the *bla*NDM, *bla*KPC, *bla*IMP, *bla*VIM, *bla*GES and *bla*OXA-48-like genes.

Methods

- Ethical approval was obtained to allow for the inclusion of stool samples sequentially submitted routinely to the diagnostic laboratory from patients >60 years old.
- Rectal swabs (n=232) were simulated by inoculating FaecalSwab™ (Copan, Italy) collection devices with these stool samples.
- Each sample was vortexed for 30 seconds and processed on the EntericBio CPE assay according to manufacturer's instructions. See Figure 1.
- As comparison, culture to CHROMagar mSuperCARBA™ (CHROMagar, France) was performed in parallel.
- Culture plates were incubated for 18-24 hrs in air @ 35+/-1°C.

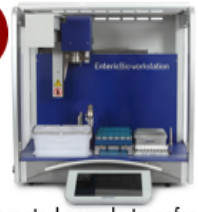
Methods

- Suspicious colonies were identified using MALDI-TOF-MS (Bruker, Germany).
- The presence of carbapenemase genes in all *Enterobacteriaceae* identified was confirmed with Xpert Carba-R (Cepheid, USA).
- Spiked samples (n=30) were also processed by both methods.
- Five well-characterised isolates harbouring carbapenemase genes from each of the six targets on the EntericBio CPE assay were used.
- A 200µl aliquot of a 2.0 McFarland suspension of organism was inoculated into a FaecalSwab™ device that had also been inoculated with a confirmed negative stool sample.
- Two external quality assurance panels were also processed on the EntericBio assay (QCMD panel 2016 & 2017).

Figure 1: EntericBio ® CPE Assay Method

1. 
Sample Preparation –Inoculation of SPS

2. 
Heat samples @ 103°C for 30 minutes

3. 
Automated sample transfer by EntericBio Workstation

4. 
Load onto Lightcycler® for amplification/detection

Results

		EntericBio Pos	EntericBio Neg
Culture	Pos	6[a]	0
	Neg	0	226

[a] CPE prevalence observed in the study was 2.6%, all isolates were OXA-48-like positive.

Sensitivity 100%

Specificity 100%

A total of 4.4% (n=10) grew *Enterobacteriaceae* on CHROMagar and required additional testing with Xpert Carba-R before being reported as negative.

All spiked samples gave expected results. The presence of *bla*GES could not be confirmed using the Xpert Carba-R.

Satisfactory results were achieved for each of the two EQA panels.

There was no evidence of sample inhibition with the EntericBio CPE assay.

Average time to result → Culture +/- confirmation: 18-26 hrs
Average time to result → EntericBio CPE Assay: <3hrs

Conclusions

The excellent performance of the EntericBio CPE assay for the direct detection of CPE from simulated rectal swabs allows us to consider it as a candidate in future CPE screening algorithms.

It represents an economically feasible, fast (< 3hr), easy to use solution to manage the expected increase in CPE screening.

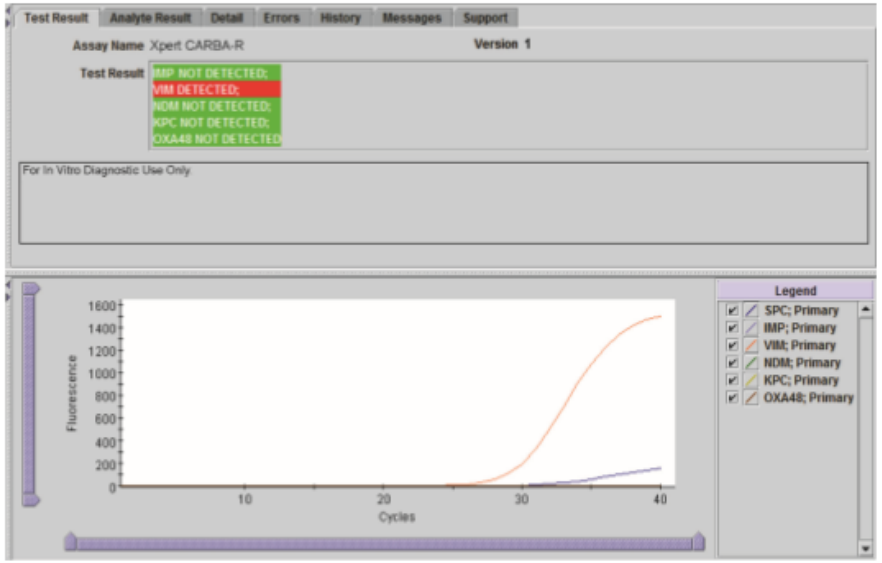
The inclusion of *bla*GES is advantageous as it is a relevant additional target [2,3] not routinely incorporated in our current algorithm. As the global threat of CPE evolves, employing comprehensive detection strategies is of paramount importance.

References

1. Requirements for Screening of Patients for Carbapenemase-Producing Enterobacteriales (CPE) in the Acute Hospital Sector, Ireland, CPE Expert Group, February 2018
2. Detection of GES-5 Carbapenemase in *Klebsiella pneumoniae*, a Newcomer in France, AAC, 2017
3. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. JID, 2017

Email: elaine.phelan@hse.ie

Cepheid GeneXpert Carba-R



- Samples: Isolates/rectal swabs
- Throughput= Low (Dependent on GeneXpert module).
- Automation: Semi automated
- TAT= Approx 55-60 minutes
- Hands on time <5 minutes
- Targets covered: KPC, OXA-48, NDM,VIM,IMP
- Other assay: Flu, C.diff, MTB/RIF and CT/NG.
- CE marked

(Cepheid, 2016)

Check Direct

- Samples: Isolates/rectal swabs
- Throughput= 48-96
- Automation: Manual/automated extraction, manual setup, PCR
- TAT= Approx 2hrs
- Targets covered: KPC,OXA-48,VIM/NDM (NO IMP)
- Other assays: Enterics
- CE marked

(Check Points, 2017)

Check-Direct CPE for BD MAX™

- Samples: Isolates/rectal swabs
- Throughput= 24
- Automation: Semi automated (pipetting involved)
- TAT= Approx 2-3hrs
- Targets covered: KPC,OXA-48,VIM/NDM (NO IMP)
- Other assays: Enterics, C.diff, Parasites.
- CE marked

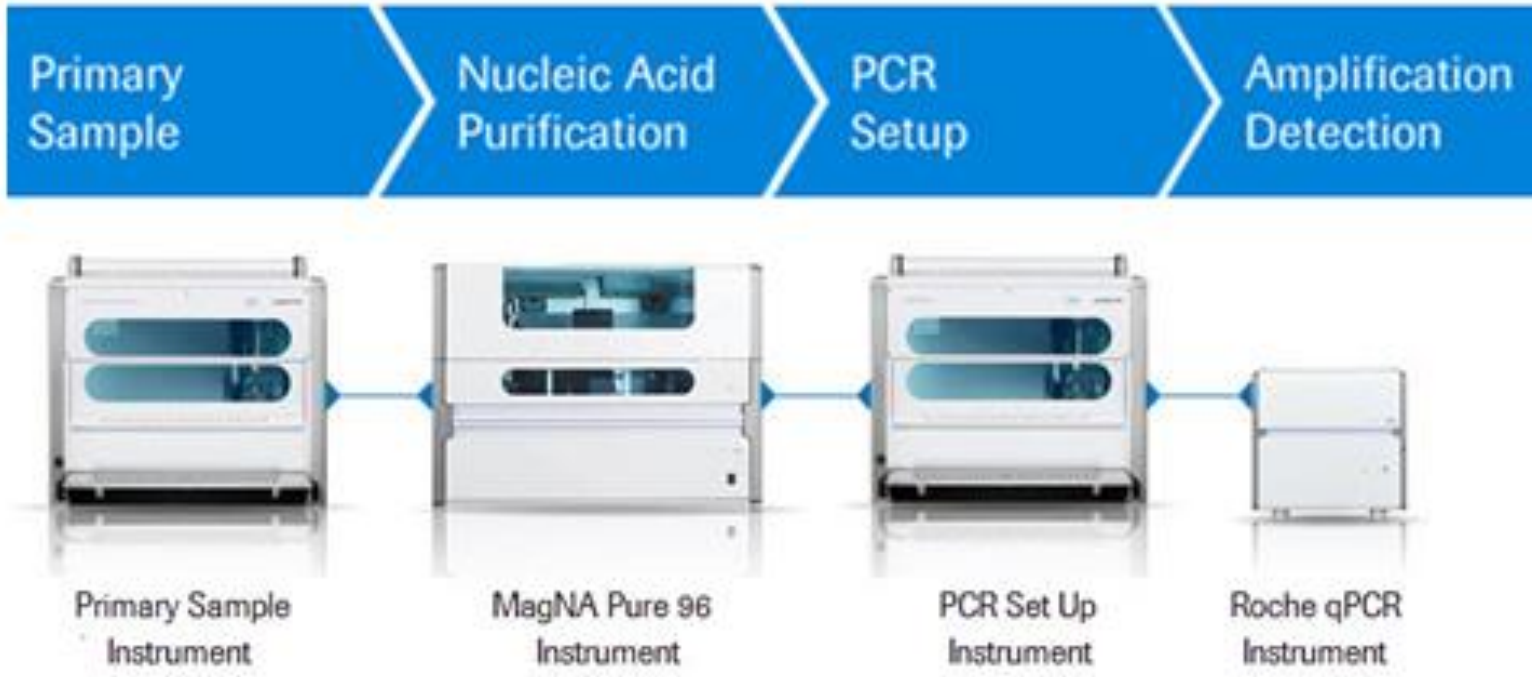
(Check Points, 2017)

Lightmix Modular CPE assay

- Samples: Isolates/rectal swabs
- Throughput= 96-384
- Automation: Sample prep, extraction, plate set up, PCR.
- TAT: 4 hrs (new run after 1.5hrs)
- Targets covered: VIM, NDM, OXA-48, OXA-23,KPC,GES, MCR-1, IMP
- Other assays: Flu, norovirus, enterics.
- CE marked

(Roche Diagnostics, 2018)

Flow Flex


Primary Sample
Nucleic Acid Purification
PCR Setup
Amplification Detection
Primary Sample Instrument
MagNA Pure 96 Instrument
PCR Set Up Instrument
Roche qPCR Instrument

Amplidiag® CarbaR+MCR

- Samples: Isolates/rectal swabs
- Throughput= 48-144
- Automation: Amplidiag easy system workflow (extraction, setup, RT-PCR)
- TAT: 4 hrs
- Targets covered: KPC, NDM, VIM, OXA-48/OXA-181, ISAbal-OXA-51, OXA-23, OXA-40, OXA-58, IMP, MCR-1 & MCR-2, GES
- Other assays: C.diff, H.pylori, Enterics, parasites, VRE
- CE marked April 2018

(Amplidiag, 2018)

Comparison of molecular techniques

KIT	Isolates	Samples	Throughput	Automation	Time	Target coverage	Singular/batch
EntericBio CPE assay	Yes	Rectal swabs	48	Yes	<3.5hrs	OXA-48, KPC, NDM, VIM, IMP, GES	Batch
LightMix modular CPE	Yes	Yes	96-384	Yes	4hrs	VIM,NDM,OXA-48, OXA-23, KPC, GES, MCR-1, IMP	Batch
Check Direct CPE	Yes	Yes	48/96	Yes/manual	2hrs	KPC, OXA-48, VIM/NDM	Batch
Check Direct CPE for BD MAX	Yes	Yes	24	Semi	2-3hrs	KPC, OXA-48, VIM/NDM	Batch
GeneXpert Carba-R	Yes	Yes	Low	Semi	1hr	KPC, OXA-48, NDM, VIM, IMP	Singular

Choosing a molecular technique for CPE detection

- CPE prevalence
- Sample volume
- Sample type
- Cost
- Platform

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