# A LABORATORY RESPONSE TO A CPE OUTBREAK


- How concerned do we need to be about CPE?
- Emergence of CPE & establishment of outbreak
- Accessing suitable detection methodologies
- Evaluation and validation of molecular detection methods
- Challenges faced
- Outcomes


In a time before COVID-19.....
COVID-19 TEST
We tested for other things....

# WHY ARE CARABAPENEMASE PRODUCING ENTEROBACTERALES SUCH A HEALTHCARE CONCERN?

- CPE target our last resort antibiotic – carbapenems
- Worryingly high Mortality Rate
  - Invasive CPE 40% (Doi et al, 2015)
  - KPC >50% Mortality
  - NDM 18-67% mortality rate (Nordmann,et al, 2011)
- GNB's propensity to cause Infection Control issues in health care setting.
- Rate & ease of dissemination in the modern world

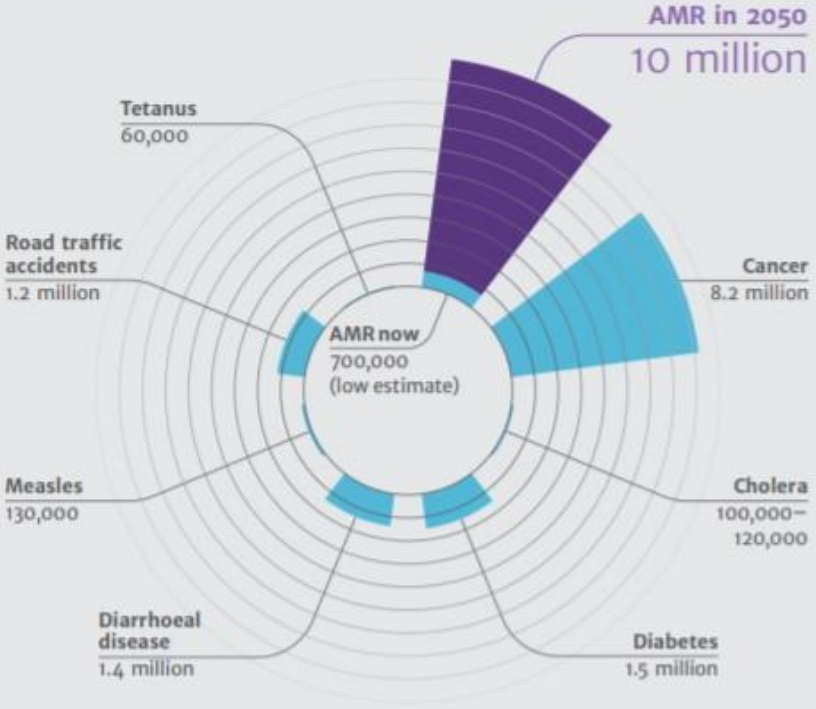
Review on Antimicrobial Resistance

*Tackling drug-resistant infections globally*

# Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations

**The Review on Antimicrobial Resistance**
**Chaired by Jim O'Neill**

## Deaths attributable to AMR every year compared to other major causes of death

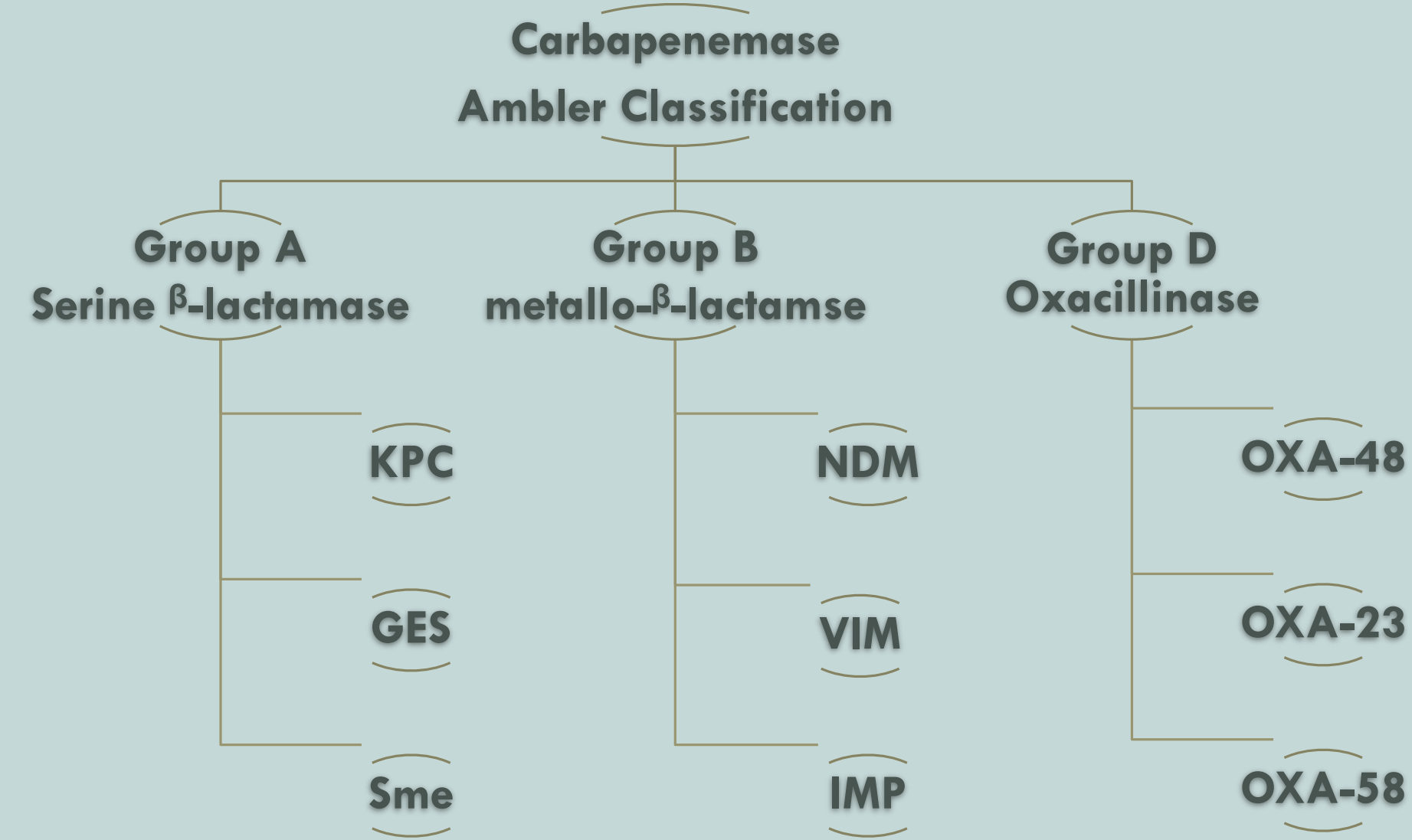



Sources

Diabetes
Cancer
Cholera
Diarrhoeal disease
Measles
Road traffic accidents
Tetanus

# CARBAPENEMASE PRODUCING ENTEROBACTERALES - A GLOBAL CONCERN

- In 2016 the WHO declare Antimicrobial Resistance a Global health concern
  - Cited carbapenem resistance in Enterobacterales as being of critical importance




# AMBLER CLASSIFICATION OF β-LACTAMASES

# "THE BIG FIVE CPE"

- KPC - Klebsiella pneumoniae Carbapenemase
  - Plasmid mediated resistance
  - Epidemic strain K. pneumoniae (ST) 258, on transposon Tn4401
- NDM - New Delhi Metallo-β-lactamase
  - Plasmid mediate & endemic in Indian subcontinent
  - Discovered in Sweden in 2008 + identified world wide within 2 years.
- VIM - Verona integron-encoded metallo-β-lactamase
  - Integron associated - Frequently isolated in Pseudomonas spp.
- IMP - Imipenemase
  - Usually in Class I integrons – incorporated into plasmids
- OXA- 48 Oxacillinase 48
  - Plasmid mediated
  - Discovered in Turkey 2003, Outbreaks in Europe in late 2000's, Irish hospitals since 2013
  - Weak carbapenem hydrolysis, susceptible to broad spectrum cephalosporins

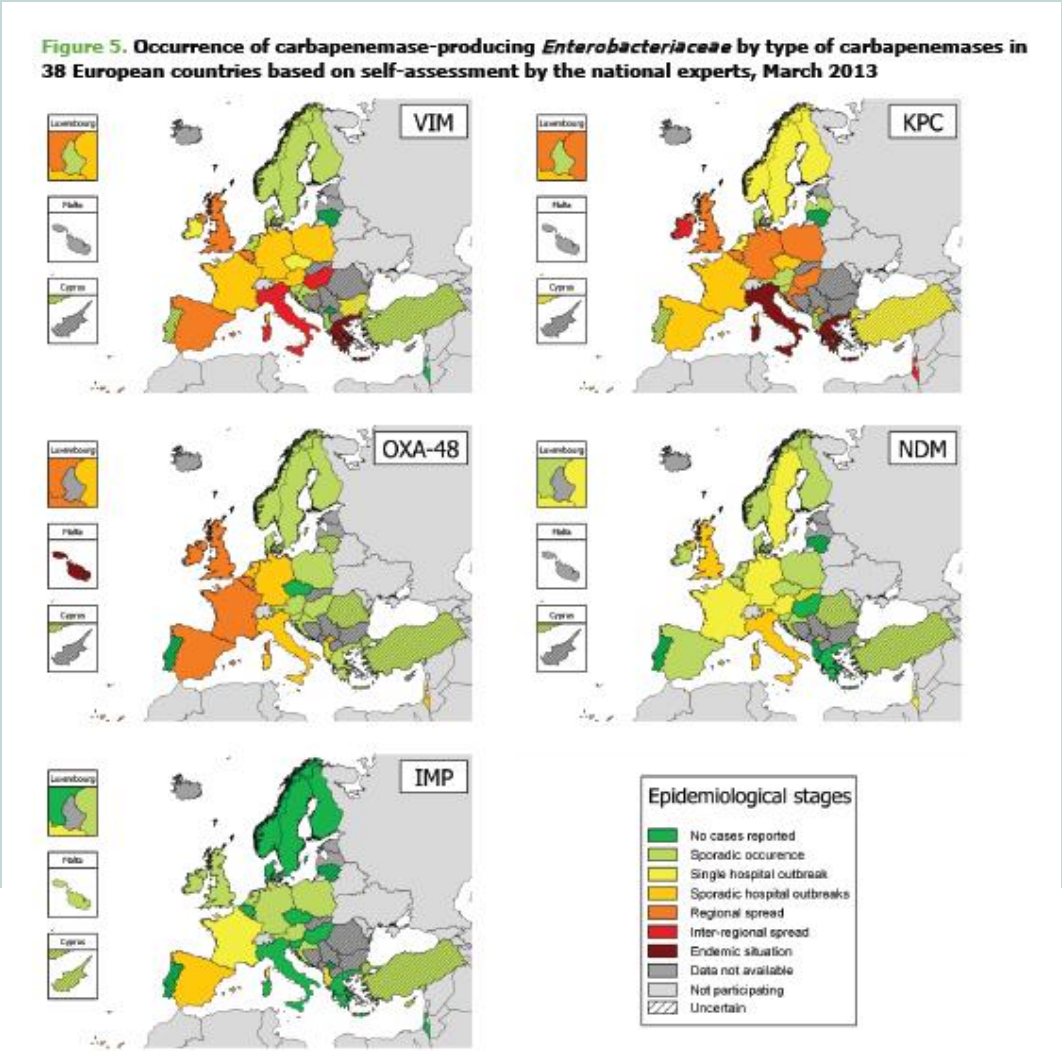
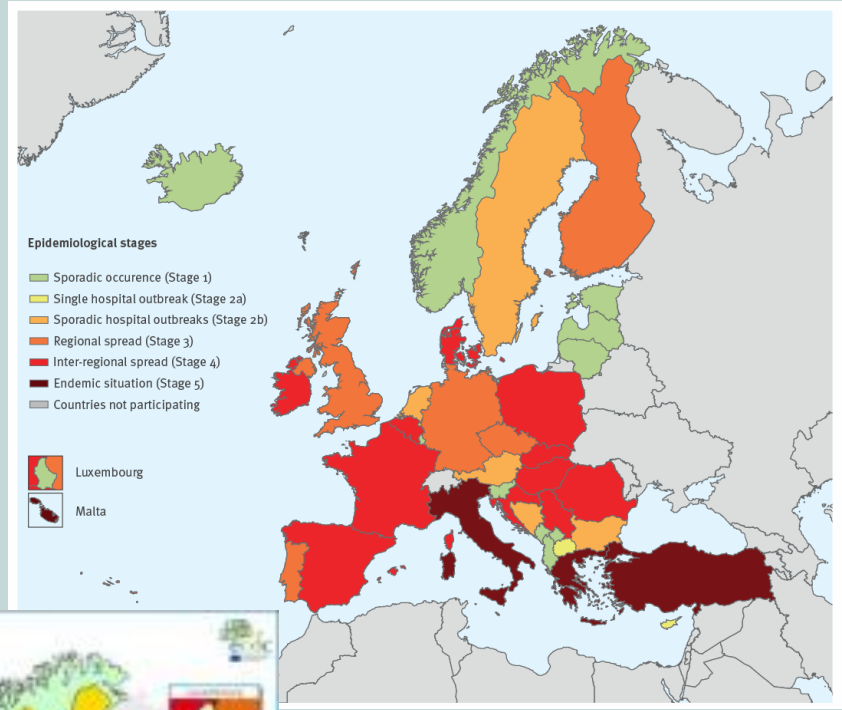



**Figure 5. Occurrence of carbapenemase-producing *Enterobacteriaceae* by type of carbapenemases in 38 European countries based on self-assessment by the national experts, March 2013**

***KPC:*** Klebsiella pneumoniae *carbapenemase-producing* Enterobacteriaceae*; NDM: New Delhi metallo-beta-lactamase; OXA-48: carbapenem-hydrolysing oxacillinase-48; VIM: Verona integron-encoded metallo-beta-lactamase. In some countries, the epidemiological stage might not represent the exact extent of the spread of CPE as it is a subjective judgment by national experts.. Results presented here reflect the uncertainty at the time of the survey.*

Fig : Occurrence of carbapenemase producing *Enterobacteriaceae* in 38 EU countries based on self assessment by the national experts, March 2013

HTTP://ECDC.EUROPA.EU/EN/PUBLICATIONS/PUBLICATIONS/ANTIMICROBIAL-RESISTANCE-CARBAPENEMASE-PRODUCING-BACTERIA-EUROPE.PDF

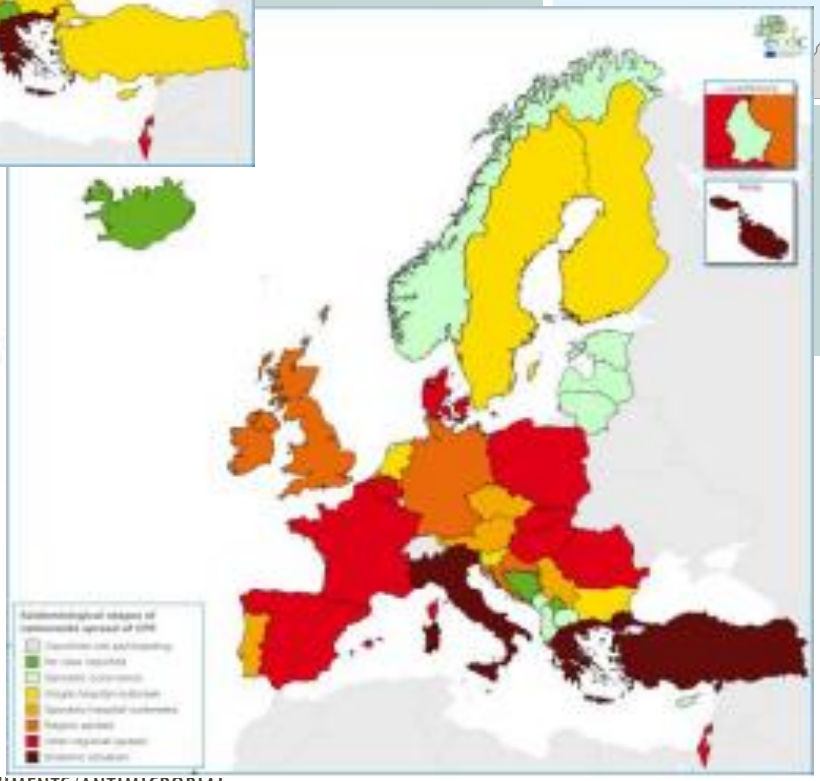



Fig :Occurrence of CRE using an epidemiological scale of nationwide spread in 38 European countries, 2013 (left), 2015 (centre) and 2018 ( top right).

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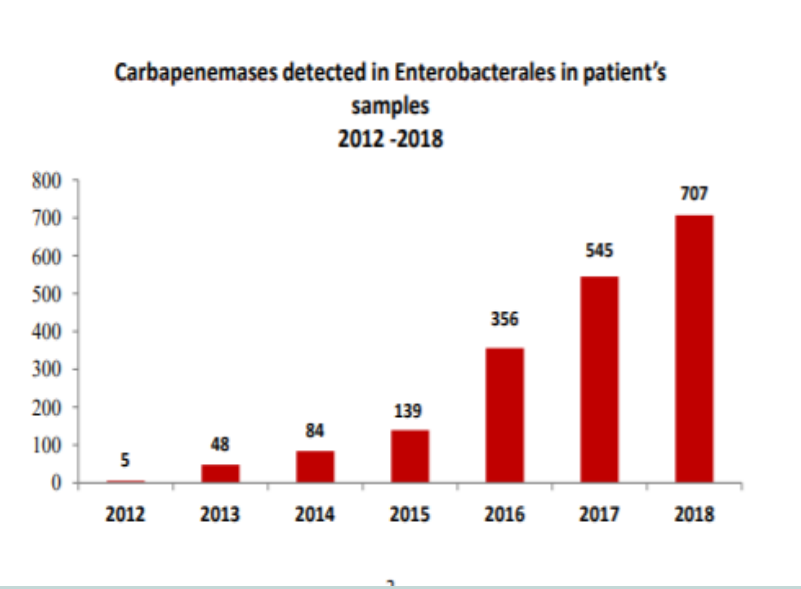


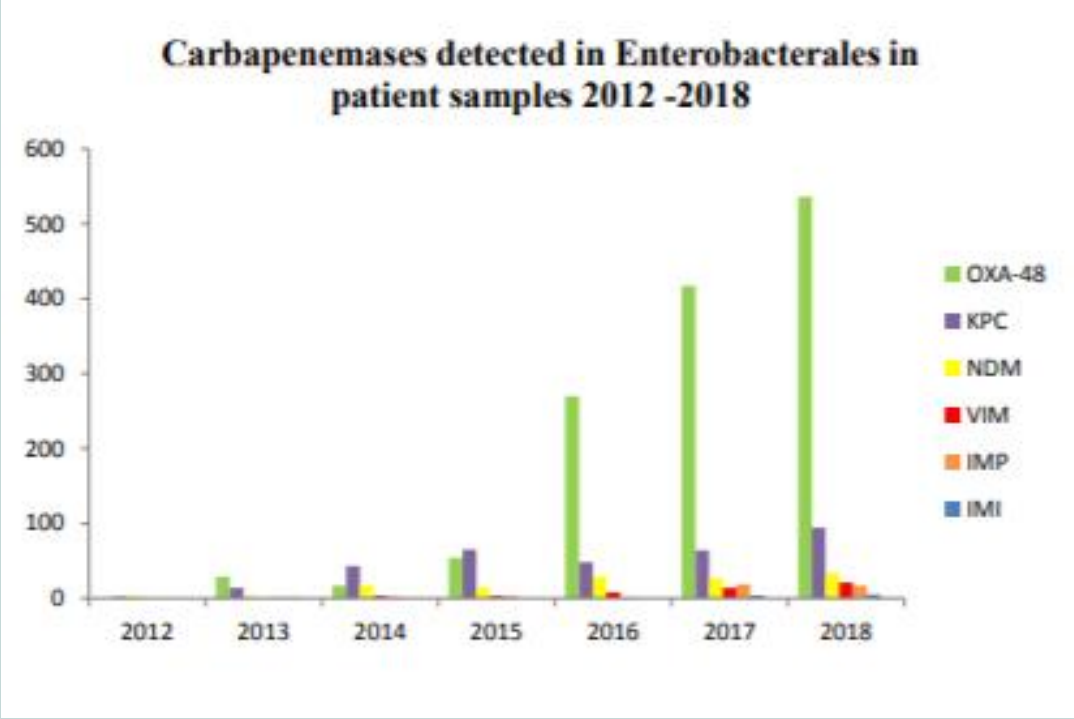


# CPE PREVALENCE IN IRELAND

Data from CPE Reference laboratory encompasses clinical and screening isolates.

ANNUAL REPORT 2018 National Carbapenemase Producing Enterobacterales (CPE) Reference Laboratory Service

themedicalindependent

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Ireland facing up to critical fight on CPE

Catherine Reilly | 07 Nov 2017 | 0 Comment(s)

# OXA-48 OUTBREAK | 2015

# TALLAGHT UNIVERSITY HOSPITAL

- Treats over 410,000 patients per year
- 562 beds, 12 theatres, 14 critical care beds
- Staff of nearly 3000
- Over 20 medical and surgical specialities.
  - A regional orthopaedic trauma centre
  - National urology centre & dialysis services centre
- **Microbiology laboratory**
- Staff of 19 - 15 medical scientists, 3 laboratory aides and 1 porter.
- Small molecular laboratory established 2015

# EMERGENCE OF OXA-48 OUTBREAK

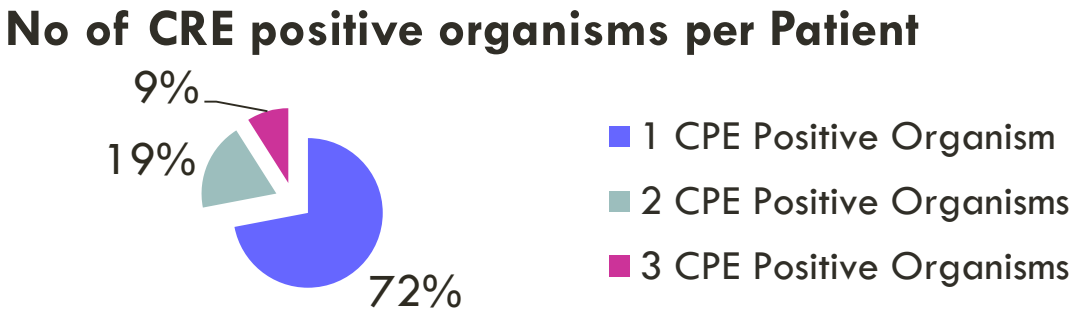
- Small, presumably contained outbreak in 2015
  - July 2015 – 1 case of OXA-48 on ward
    - Patient had no history of exposure to OXA-48
- Late November 2 more OXA-48 cases detected on same ward
  - Early December further 2 OXA-48 cases detected on same ward
  - No new cases identified in following months
- Several months later further cases were detected, many of which were linked back to the initial 5 cases or the ward

# CHALLENGES IN TACKLING OUTBREAK

| | |
|---|---|
| ○ Delayed time to positivity increased number of contacts generated | ➢ Resulted in ever increasing numbers of contacts generated |
| ○ CPE screening 3 wks post exposure | ➢ Minimum of 4 wks screen post exposure required |
| ○ Long T.A.T's for results & larger volumes of CPE screens required | ➢ Increased the burden on already stretched lab service<br>➢ Increased time to detection hindered patient management response, & generated greater numbers of contacts |
| ○ Environmental sources | ➢ Biofilm formation and colonisation of drains in certain areas |

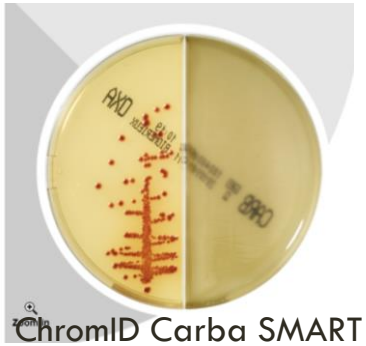
# CHALLENGES IN TACKLING OUTBREAK

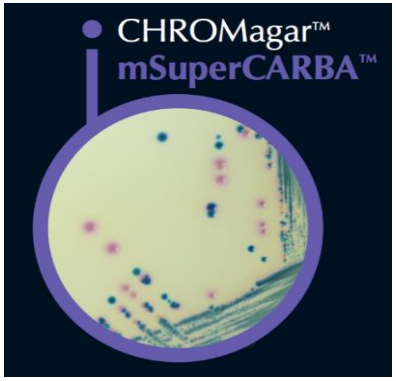
- Lack of OXA-48 specific detection methods
  - Traditional methods struggled to detect CPE presence. OXA-48 has no known inhibitor.
- Weak Carbapenemase activity of OXA-48
  - Carbapenem MIC didn't readily detect isolates. EUCAST & CLSI adjusted breakpoints accordingly
- Possibility of variant
  - Variants emerging across the world
- OXA-48 promiscuity
  - Plasmid based *bla*$_{OXA-48}$



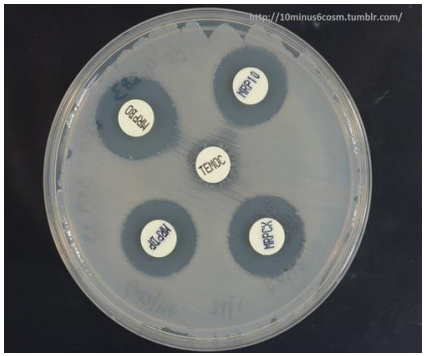
# AVAILABLE DETECTION METHODS

- CPE selective agar
  - Some agars display poor sensitivity, particularly for weak carbapenemases.
- Identification & sensitivity testing
  - Carbaepenem MIC
- Confirmatory CPE testing
  - Modified Hodge
  - Roscos
  - Lateral flow devices


ChromID Carba SMART







# CPE DETECTION PROTOCOL IN TUH

**Figure 1.7.1-1: CPE protocol pre-OXA-48 outbreak**

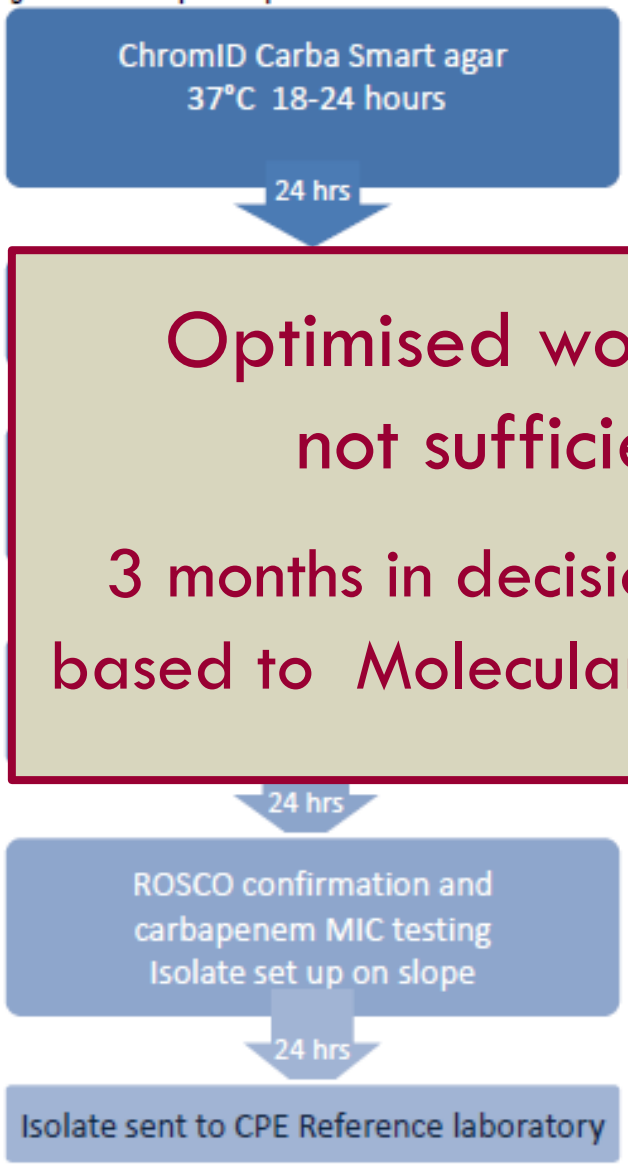



**Figure 1.7.1-2: Outbreak optimised CPE detection protocol**

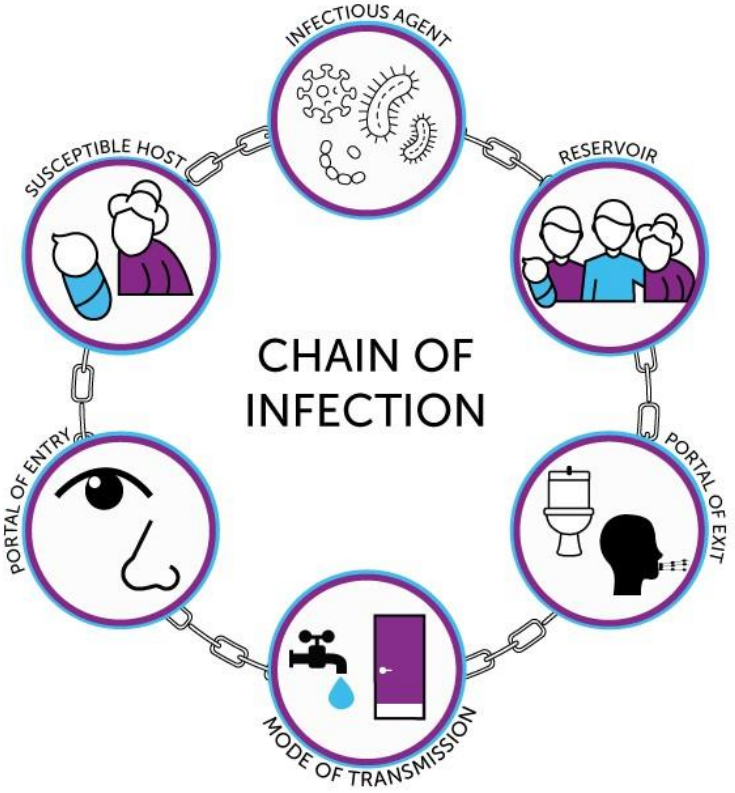

Optimised work flow + **7 day service**
not sufficient to slow outbreak

3 months in decision made to move from Culture based to Molecular based CPE screening methods

# WHY WE DID THIS PROJECT

**To break the chain of infection**

- To provide faster patient results,
- To facilitate higher sample through put
- To increase detection sensitivity



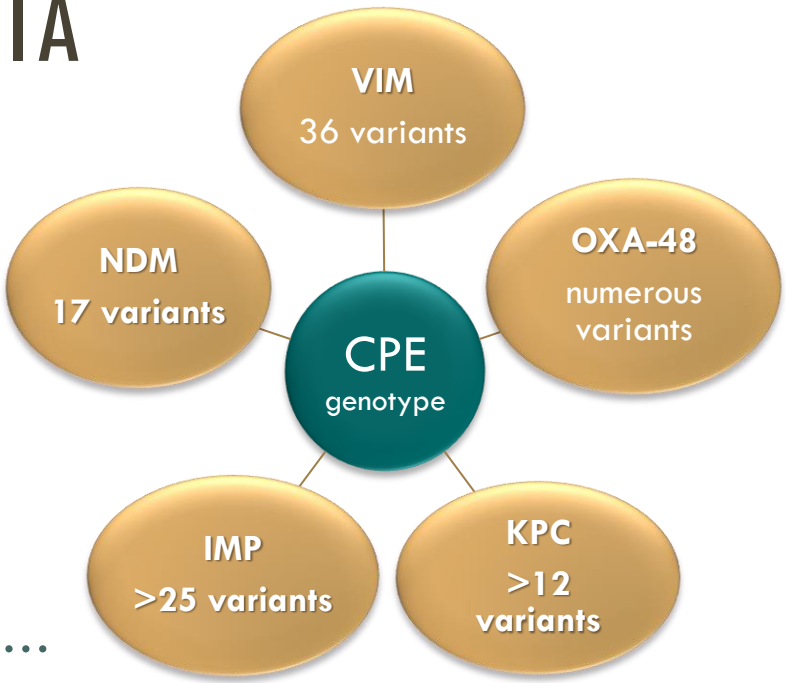
# AUTOMATED TESTING PLATFORM

## FLOWFLEX platform (ROCHE)

- 96+ samples processed per run
  - extracts 96 samples in 1hour
- Automated Platform
- Open System
  - – any PCR assay can be used
- Linking software
  - Automatic file transfer between systems
- Interpretation software
  - Centralised data analysis

# ASSAY SELECTION CRITERIA

- Specificity of the method
  - Assays can only detect what they are designed to detect.
- Numerous variants within each CPE group
  - Both phenotypic and genotypic
  - 'Big 5' have 122 described variants so far… difficult to detect all.




- System needs to be adaptable
  - Responsive to evolving epidemiological distribution Worldwide, Regional and National.
  - Rapid dissemination of NDM & OXA-48 highlights the importance of surveillance

# OXA-48-LIKE VARIANTS DETECTION

## Variants displaying (weak) Carbapenemase activity

- OXA-181
  - First variant to emerge Plasmid InX3
  - Represents 19% of OXA-48 genes detected in the UK 2007-2014 (Findley et al, 2017)
- OXA-162 Turkish strain
- OXA-232
- OXA-370
- OXA-204 North Africa
- OXA-244
- OXA-245

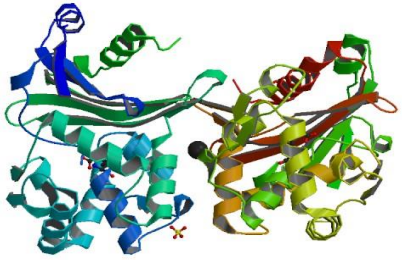
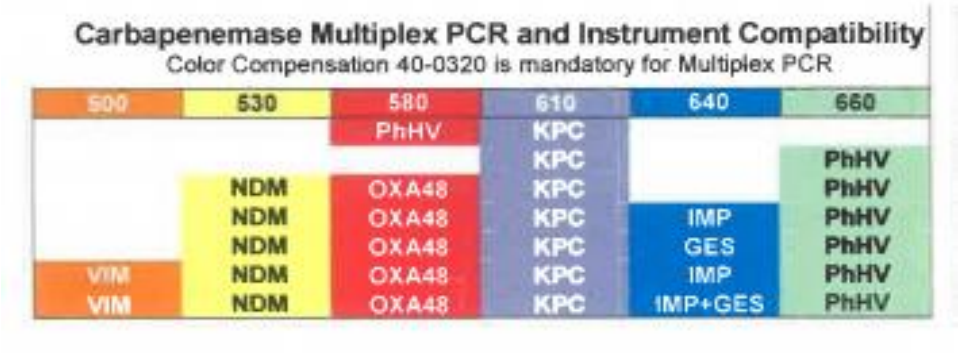


Fig. Crystalline structure of OXA-181

- Failure to detect this variant lead to an outbreak in a French hospital of OXA-181, while expensive molecular methods were being used (Findlay et al., 2015)
- Assay bioinformatic revised to detect OXA-181 - 97% sensitivity, 98% specificity (Tato 2016)

# LIGHTMIX CPE ASSAY, TIBMOL BIOL

## Building block' style assay




- Assay designed on universal assay parameters
- Add desired targets based on local epidemiology up to hexaplex conformation

| Target | Gene | Variants detected |
|---|---|---|
| OXA-48 | $bla_{OXA-48}$ | OXA-48, 181, 162, 163, 244, 245, 247, 204, 232 |
| KPC | $bla_{KPC}$ | 1- 11 KPC variants |
| NDM | $bla_{NDM}$ | 1 -13, 16, 17 NDM variants |
| VIM | $bla_{VIM}$ | 1 – 36 VIM variants |
| IMP | $bla_{IMP}$ | 1 – 17 IMP variants |

# CPE VALIDATION STUDY PLAN

| | |
|---|---|
| Assay Specificity & Sensitivity | • Detection analysis of 44 CPE positive organisms, and 5 non-CPE organisms |
| Limit of detection | • Analytical sensitivity of representative CPE targets |
| OXA-48-like investigation | • OXA-48-like variant detection across numerous CPE detection methods |
| Clinical evaluation | • 213 rectal swabs analysed on traditional culture screening methods and LightMix modular CPE screening methods. |

# SPECIFICITY AND SENSITIVITY ANALYSIS

| Target | Total | Organism |
|---|---|---|
| VIM | 5 | *K. oxytoca, E. cloacae, E. coli, P. aeruginosa* x2 |
| NDM | 6 | *E. coli* x3, *K. pneumoniae* x3 |
| KPC | 5 | *Citrobacter freundii, K. pneumoniae* x3, *Raoultella ornithinolytica* |
| IMP | 3 | *K. oxytoca* x2, *E. cloacae* |
| OXA-48 (incl variant strains) | 25 | *K. pneumoniae* x7, *K. oxytoca* x6, *E. coli* x5, *E. cloacae* x2, *Serratia marcescens* x2, *Citrobacter koseri* |
| Total CPE | 44 | |
| | | |
| Total non-CRE isolates | 5 | |

- Assay specificity 71%, and sensitivity of 98%.
  - Low specificity due to 2 detection of 2 VIM positive Pseudomonas sp, and low no of true negative isolates tested
- PPV 95%, NPV 83%.
  - Failed to detect an IMP – most likely due to lose of plasmid in freeze thawing

# LIGHTMIX MODULAR CPE

| CPE target | LightMix modular CPE assay | | | | GeneXpert CarbaR assay | ChromID Carba SMART agar |
|---|---|---|---|---|---|---|
| | Phosphate buffered Saline solution | | Simulated faecal solution | | Simulated faecal solution | Simulated faecal solution |
| | LoD | Efficiency | LoD | Efficiency | LoD | LoD |
| IMP | $10^3$ | 0.9902 | $10^3$ | 0.9989 | $10^2$ | $10^4$ |
| VIM | $10^2$ | 0.9911 | $10^2$ | 0.9985 | $10^3$ | $10^4$ |
| NDM | $10^3$ | 0.9974 | $10^1$ | 0.9800 | $10^2$ | $10^3$ |
| KPC | $10^2$ | 0.9853 | $10^2$ | 0.9988 | $10^2$ | $10^2$ |
| OXA-48 | $10^2$ | 0.9991 | $10^2$ | 0.9993 | $10^2$ | $10^6$ |
| OXA-181 | $10^3$ | 0.9907 | $10^3$ | 0.9979 | $10^3$ | $10^4$ |

# OXA-48-LIKE VARIANT ISOLATES ANALYSED ON THE LIGHTMIX MODULAR CPE ASSAY VALIDATION

Table of OXA-48 like variants analysed for the assay validation study in Tallaght University hospital

| OXA-48-like variant | Organism | Predominant activity |
|---|---|---|
| OXA-162 #1 | *Citrobacter koseri* | Carbapenemase |
| OXA-162 #2 | *Klebsiella pneumoniae* | Carbapenemase |
| OXA-204 | *Klebsiella pneumoniae* | Carbapenemase |
| OXA-232 | *Escherichia coli* | Carbapenemase |
| OXA-244 | *Escherichia coli* | Carbapenemase |
| OXA-163 | *Enterobacter cloacae* | Extended-spectrum cephalosporinase |
| OXA-405 | *Serratia marcescens* | Extended-spectrum cephalosporinase |

# RESULTS OF OXA-48-LIKE VARIANTS STUDY

| OXA-48-like variant | Growth on ChromID Carba Smart agar | | MIC strip value | | R.E.S.I.S.T – O.K.N (OXA-48, KPC, NDM) | KPC, MBL & OXA-48 Confirm (ROSCO) | LightMix modular CPE assay (LoD) | GeneXpert Carba-R (LoD) |
|---|---|---|---|---|---|---|---|---|
| | DAY 1 | TSB enrichment | Meropenem (mg/L) | Ertapenem (mg/L) | | | | |
| OXA-163 † | Neg[B] | Pos | 3 | 6 | Neg | Neg | $10^2$ | $10^2$ |
| OXA-405 † | Neg | Neg | 0.19 | 0.38 | Neg | Neg | $10^2$ | $10^3$ |
| OXA-162 | Pos[A] | Pos | 0.25 | 0.5 | Pos | Pos | $10^2$ | $10^3$ |
| OXA-162 #2 | Pos | Pos | 0.25 | 0.5 | Pos | Pos | $10^1$ | $10^2$ |
| OXA-204 | Pos | Pos | 0.5 | 1.5 | Pos | Pos | $10^2$ | $10^3$ |
| OXA-232 | Pos[C] | Pos[C] | 0.094 | 0.25 | Pos | Pos | $10^2$ | $10^3$ |
| OXA-244 | Pos[C] | Neg | 0.5 | 1.5 | Pos | Pos | $10^2$ | $10^3$ |

[A]= mixed growth by two, [B]=Growth on Carba side and not on OXA-48 side of ChromID Carba SMART agar (BioMerieux), [C]=single colony growth on ChromID Carba SMART agar (BioMerieux) confluent on Columbia sheep blood agar (Fanin), †= cephalosporinase activity

# OXA-48 VARIANTS STUDY RESULTS

- Molecular methods - increased analytical sensitivity for CPE detection compared to culture screening methods
- LightMix modular CPE assay Limit of Detection is comparable to that of Gene Xpert Carba-R.
- Culture based CPE screening methods had difficulty detecting all OXA-48-like variants
  - MIC values were all below the breakpoints for carbapenem, with several close to the E-coff values.
  - Both ROSCO and R.E.S.I.S.T-O.K.N.V accurately detected all OXA-48-like carbapenemases
    - However, these confirmation methods are dependent on screening agar / MIC tests identifying query CPE isolates!
- Molecular methods detected all OXA-48-like variant analysed as part of this study, regardless of enzymatic activity spectrum

- Initially samples presented with very low CP values
  - Clear cut positives – easy to recover on culture
- Higher CP values proved more difficult
  - Extend incubation of CPE agar
  - Use enrichment TSB broth
  - Sub to CPE agar and MacConkey, extend incubation if required

## Retrospective analysis of CPE detected using LightMix CPE assay

| Total no of CPE detected on LightMix CPE assay | No of CPE recovered on culture Day 1 | No of CPE recovered via enrichment | No of non-culturable CPE confirmed by NCPERL |
|---|---|---|---|
| 93 | 59 | 33 | 1 |
| 100% | 63% | 37% | |

Table: Retrospective analysis of molecular CPE result January to December 2017

# LESSONS LEARNED


- High CT values & confirmatory methods
  - High incidence of low copy number positives
  - What do you do with high CT samples???
  - Criteria needed to define results
- Viscosity of the media
  - Viscous gel like media – unsuitable for automated pipetting robot
- Monitoring of controls
  - UNG successfully validated for use in assay
  - Positive controls multiplexed into 2 wells

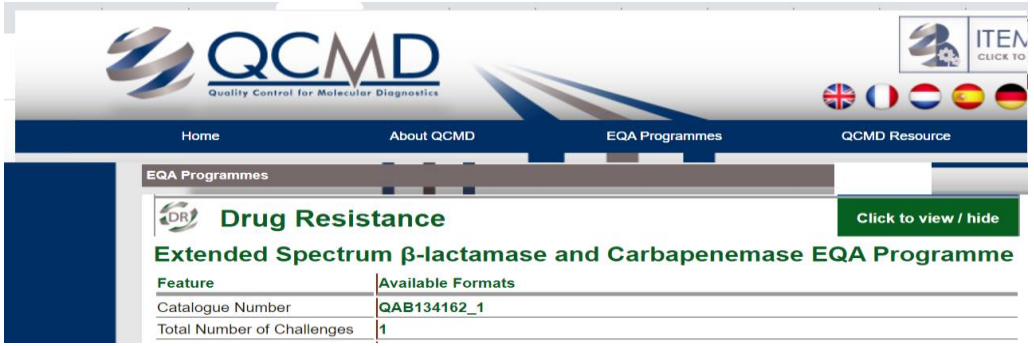
    PTC1 = VIM, KPC, IMP

    PTC2 = NDM, OXA-48
  - Once in use noticed IMP control CP values were sometimes deteriorating
  - External controls ran – IMP was detected
  - Validation most likely not representative of routine testing.

# Third party controls

- Third party controls & EQA Schemes
  - Cultureed and mocked up third party controls in-house
    - Using standard strains / positive strains in-house
  - In-lieu of EQA schemes
    - Interlaboratory quality control
    - contact with CPE reference lab/HPSC
    - monitor national/EU epidemiology
    - & review emerging literature


# EQA scheme



**Microbiology Laboratory**

*Tallaght University Hospital*

# THANK YOU TO

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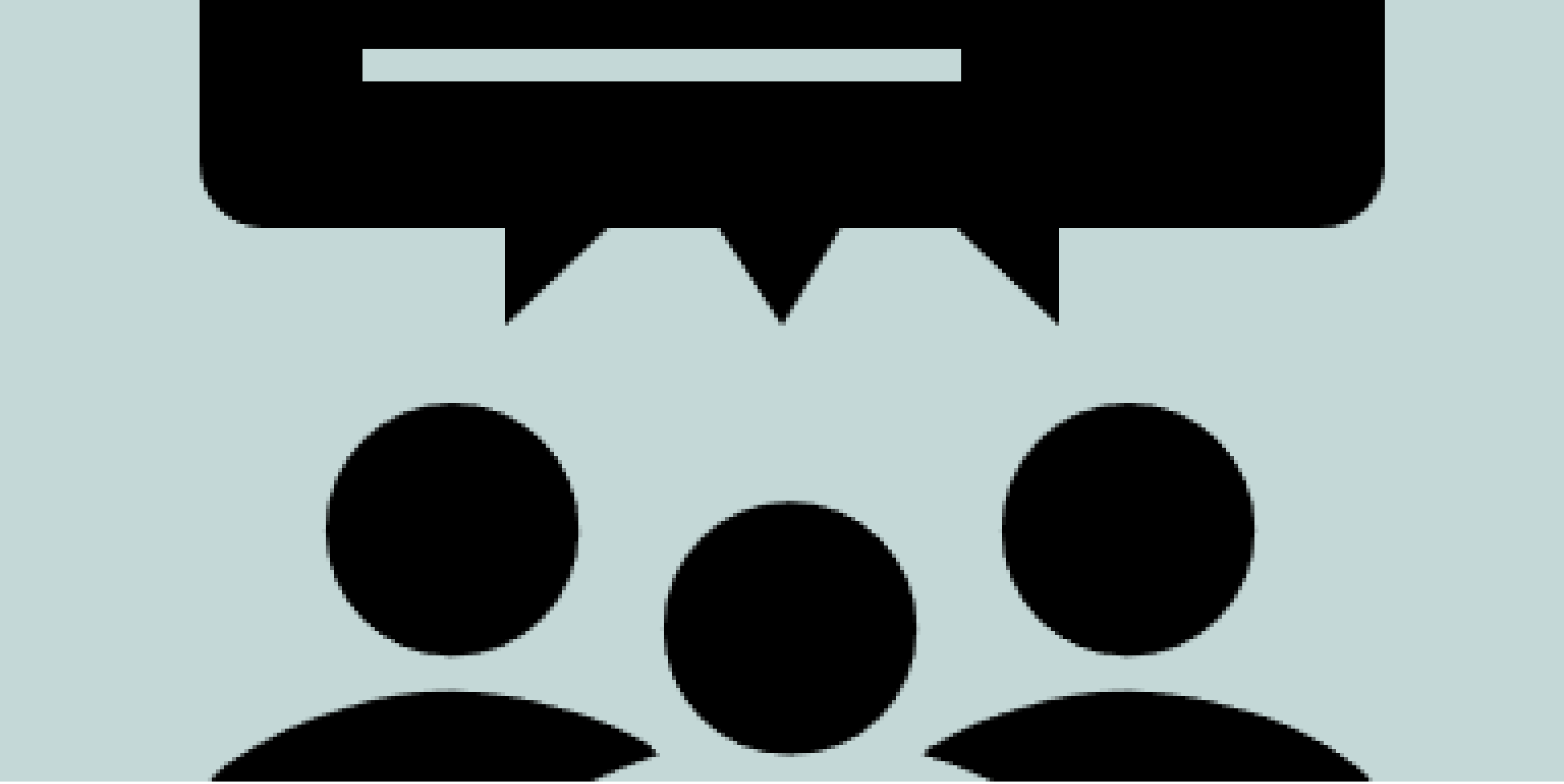
DR SUSANNAH FROST

The Irish Laboratory Awards 2018
WINNER
Laboratory Team of the Year

The Irish Laboratory Awards 2018
WINNER
Medical Laboratory of the Year



# QUESTIONS?