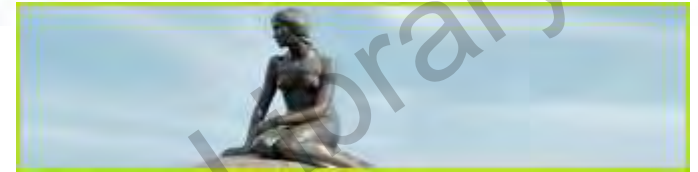


How to screen for carriage of carbapenem-resistant bacteria?



Annarita Mazzariol

University of Verona

Talking about...

CRE

Carbapenem

Resistant

Enterobacteria

CRA

Carbapenem

Resistant

A. baumannii

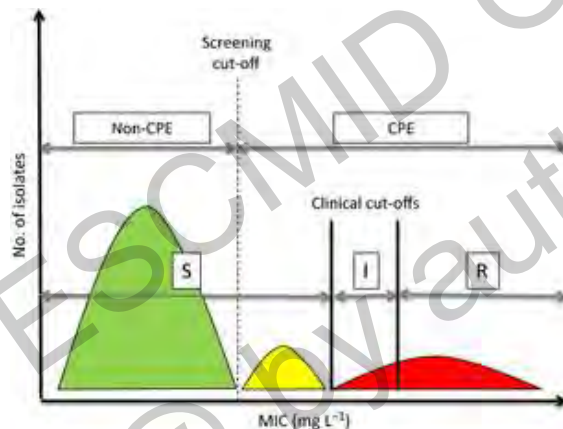
P. aeruginosa

Multi-Drug-Resistant

Carbapenems MICs range

Carbapenems MICs of carbapenemases producers may have value categorized as susceptible by EUCAST

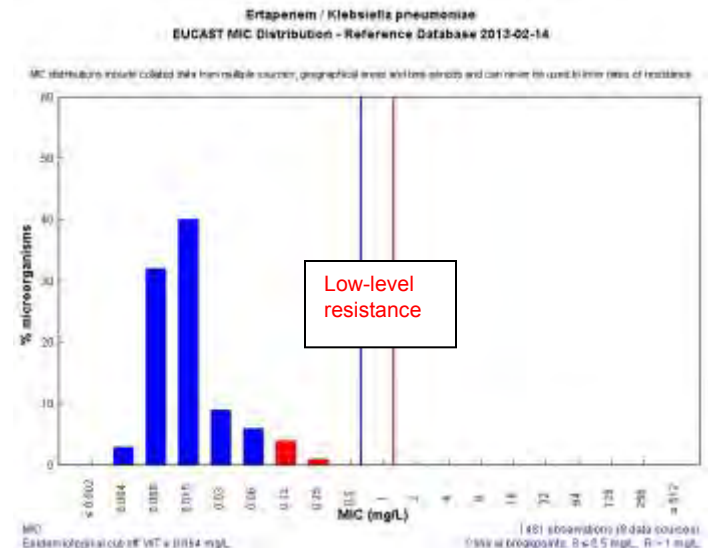
Carbapenemase	MIC ranges (mg/L)		
	Imipenem	Meropenem	Ertapenem
KPC	0.5 → 64	1 → 64	0.5 → 64
MBL	0.5 → 64	0.25 → 64	0.5 → 64
OXA-48	1 → 64	0.5 → 64	0.25 → 64



Tängdén T, Giske CG.

Journal of Internal Medicine

Volume 277, Issue 5, pages 501-512, 27 JAN 2015



Molecular methods

PCR based methods

• In house methods

Single PCR

Multiplex PCR

RT PCR

• Commercial methods

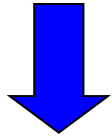
Microarray

RT-PCR

Low resource setting?

Phenotypic methods

Culture based



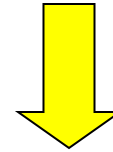
Cromogenic culture media

Modified Hodge test MHT

Inhibitors based test

Carbapenem Inactivation
Method

Enzymatic activity based



Carba NP test

Blue carba test

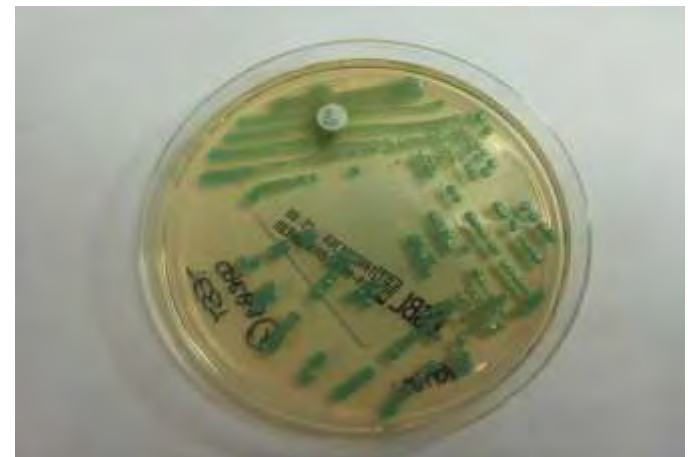
Hydrolysis on UV
spectrophotometer

Hydrolysis on Maldi-toff

Cromogenic culture media

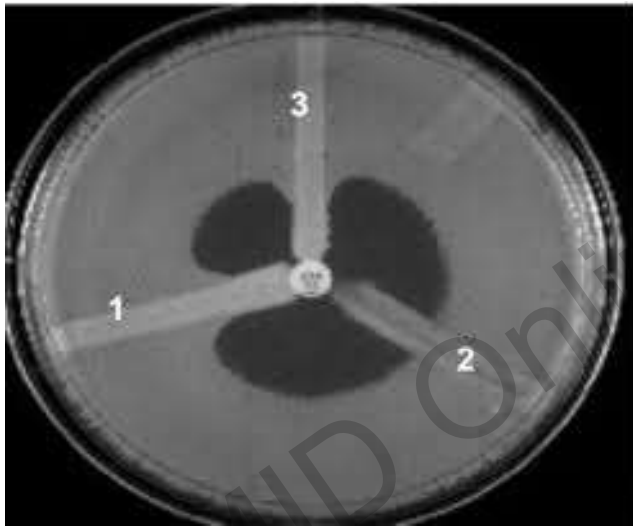
Selective plate

- Presumptive identification by color
- Resistant selection by antibiotic of different enzymes
 - ESBL
 - Carbapenemase
 - OXA-48



Phenotypic culture based test

Modified Hodge test

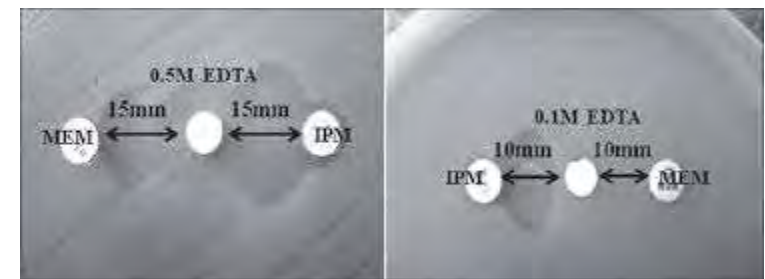
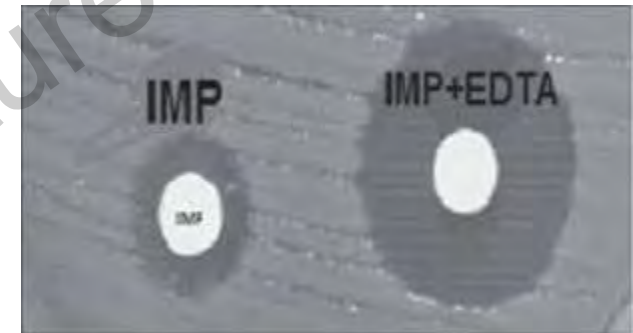


- Limited to test Enterobacteriaceae
- Time consuming: +24 h
- False positive: impermeability or ESBL
- False negative: in case of MBL

Phenotypic culture based test

Inhibitor based test: disk or strips

- Class A carbapenemase:
Boronic acid compounds
- Class B carbapenemase:
EDTA
Dipicolinic acid
- Class D carbapenemase:
Not inhibitors



Phenotypic culture based test

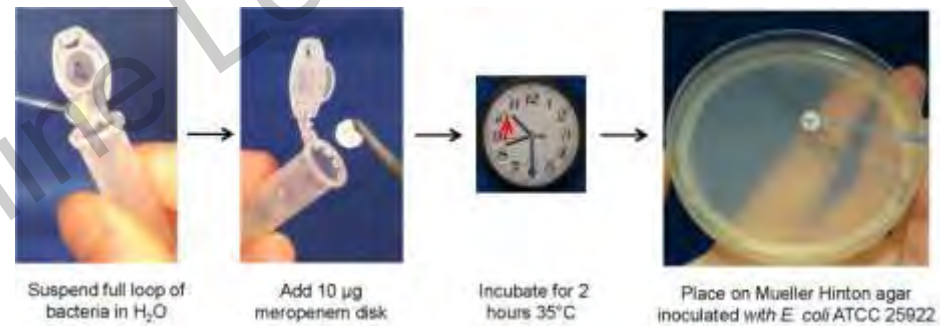
Carbapenem Inactivation Method (CIM)

Perform in one day

not very fast

High sensitivity and specificity

Detection also of OXA-48

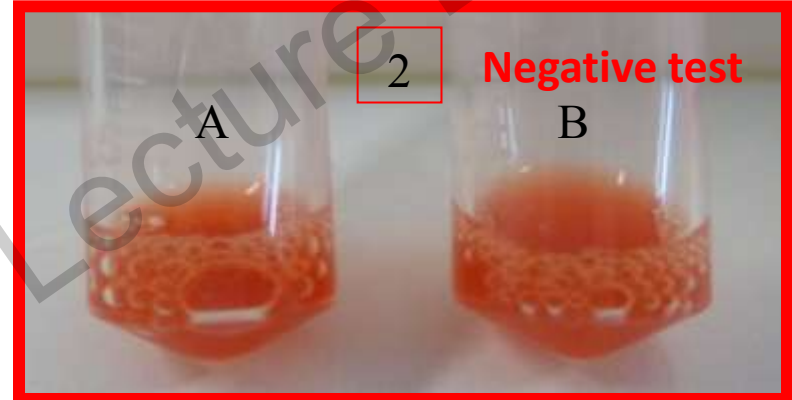
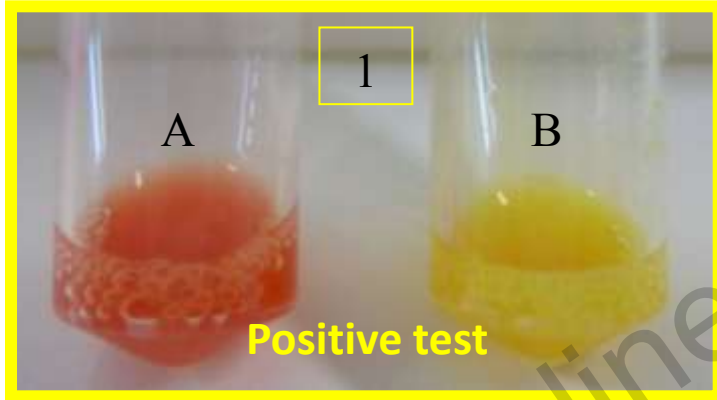


CARBA NP TEST



ESBL NDP TEST

Enzymes extraction → Add antibiotics and pH indicator → Incubation and reading



~~Carbapenemases producer strains~~

~~Antibiotic (Imipenem)~~

cefotaxime

~~Beta-lactams ring hydrolysis~~

Medium pH changes

Yellow color

Positive test

Negative test

Red color

Phenotypic enzyme detection test: colorimetric

CarbaNP test

- Easy to perform
- Fast results (few minutes – max 2 h)
- Detection of all carbapenemase also unknown
- Low cost
- Critical points
 - Colony must be fresh
 - Amount of bacteria
 - Dedicated test for *A. baumannii*

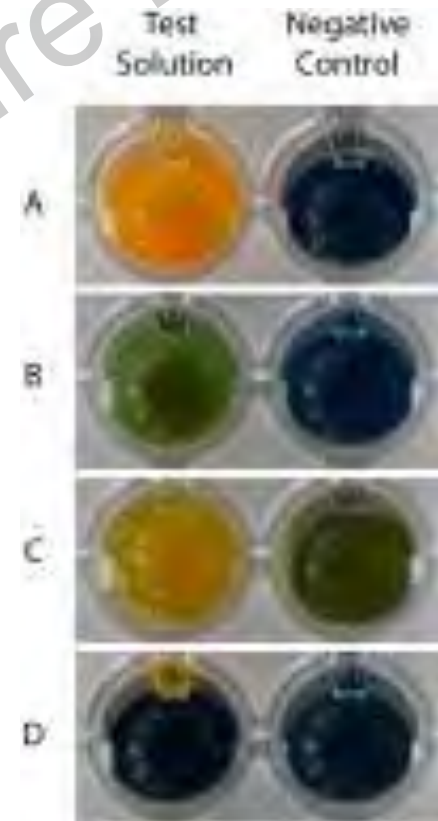
Home-made
or
commercial



CarbAcineto Test

Phenotypic enzyme detection test: colorimetric Blue carba test

- Work as CarbaNP test
 - Easy to perform
 - Fast results
 - Low cost
 - Detection of all carbapenemase
- It differ from CarbaNP
 - Bromothymol blue as indicator
 - No need buffer extraction
 - No need different protocol for *A. baumannii*



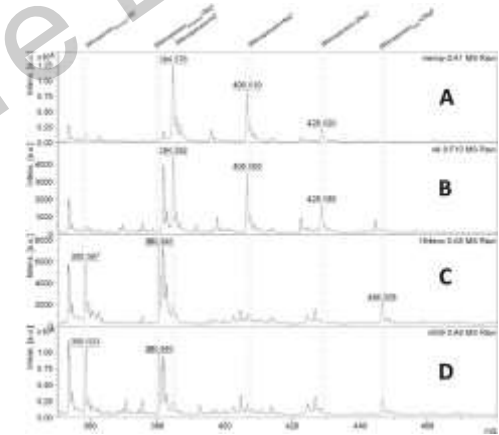
Home-made
or
commercial

Phenotypic enzyme detection methods: instruments UV-spectrophotometer and Maldi-Toff

Can performed in one day but not very fast

Need expertise

Need technical time



Hrabak et al. JCM 2012;50:2441

All carbapenemase detected

High sensibility and specificity

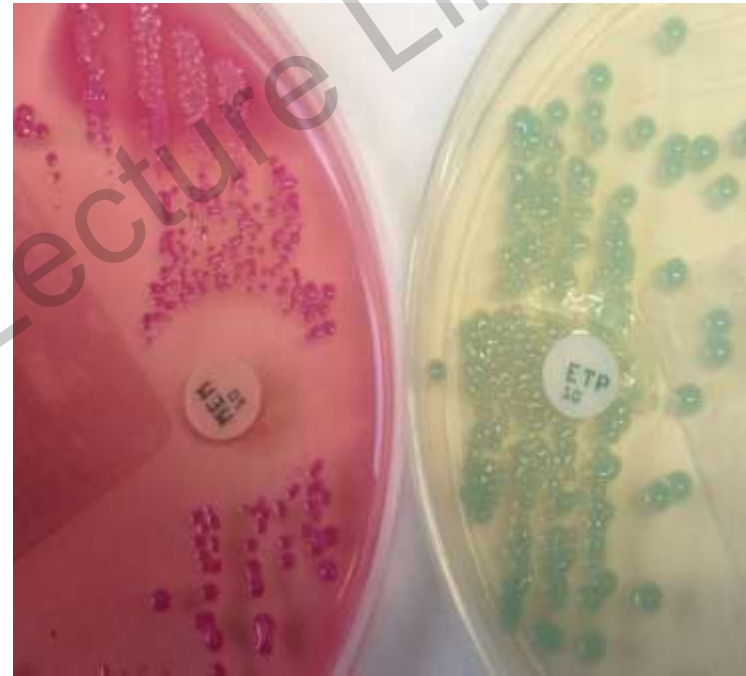
How we work...

K. pneumoniae carbapenemase producer different media

Streak rectal swab on

- ESBL detecting chromogenic media plus an ertapenem disk
- McConkey plus an meropenem disk

- ❖ OXA-48 strains may not grow on ESBL detecting chromogenic media: we check reduced susceptibility to MPM on McConkey
- ❖ *P. aeruginosa* is ertapenem resistant: we considered if is also Meropenem resistant on McConkey



How we work...

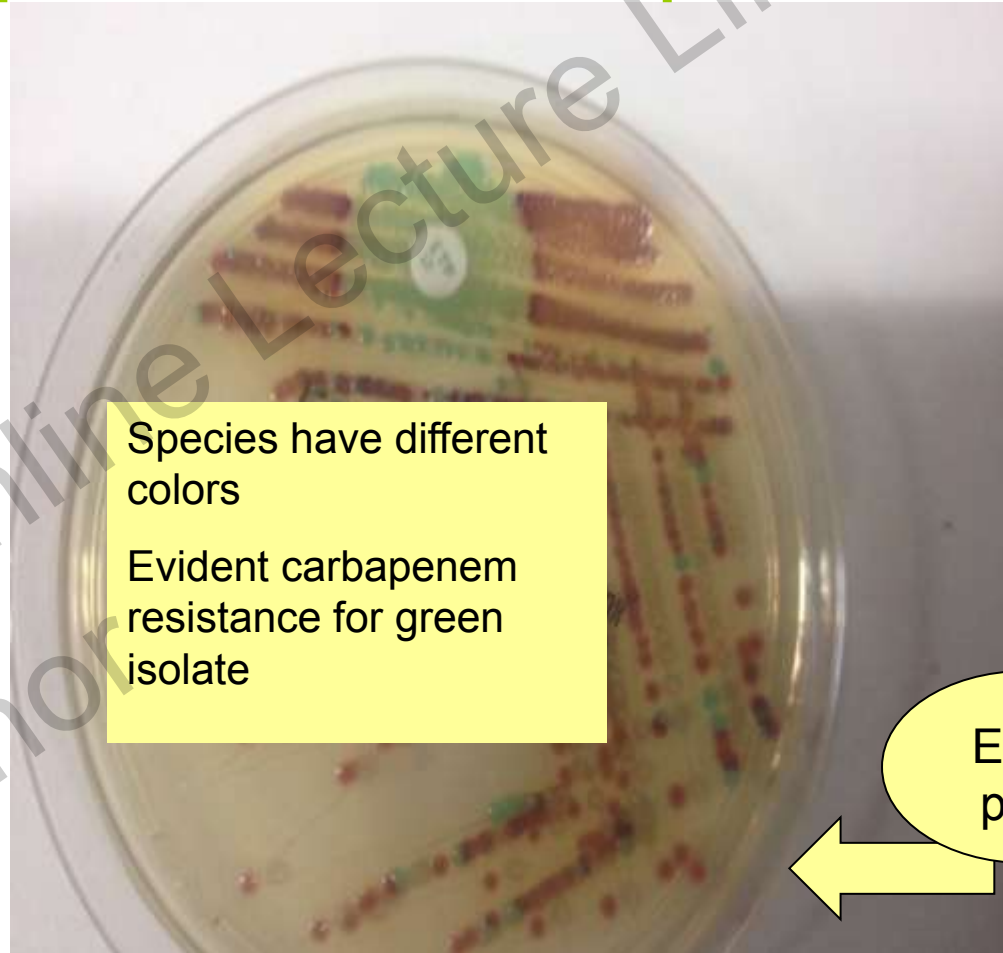
K. pneumoniae carbapenemase producer plus
E. coli ESBL producer in the same plate

We can check and
monitoring in the
main time also
ESBL producer

Species have different
colors

Evident carbapenem
resistance for green
isolate

ESBL
plate



How we work..

Flow-chart ESBL and carbapenemase producers

Growth on chromogenic media for ESBL detection plus ertapenem

disk (24 h)

Ertapenem R

Ertapenem S

TEST CARBA NP
Maldi-toff

Test ESBL-NDP
Maldi-toff

2 h

2 h

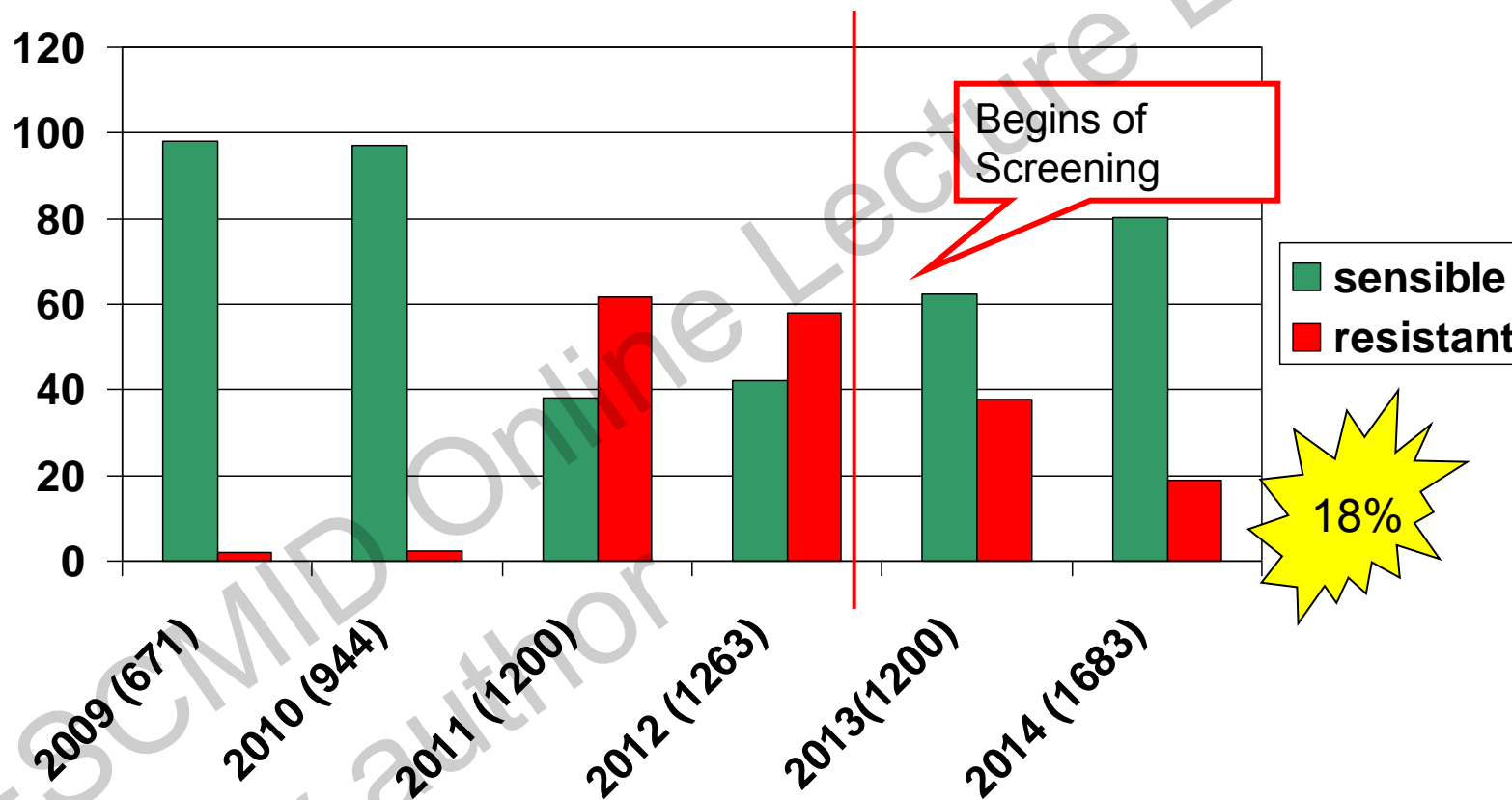
Carbapenemase producer
Use contact precautions

ESBL producer

Our results...

K. pneumoniae Meropenem resistant Verona 2009-2014

April 15, 2013



Molecular methods

• Advantages

– Speed \Rightarrow 1 to 4-5 h

– Used directly with clinical sample

Rectal swab

Other samples are going to be validate soon

To be use in specific situations

i.e. Check organ donors just before transplant

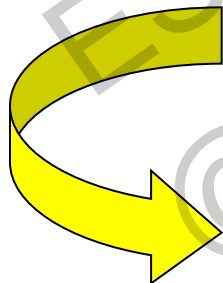
• Disadvantages

– High cost



– Detection of only known genes

– Need expertise



Phenotypic methods

- **Advantages**

- They can be combined to obtain a better performance
- Detection of unknown enzyme
- Not need expertise

- **LOW COST**

LARGE
SCREENING

USE IN LOW
RESOURCE
SETTING

- **Disadvantages**

- Miss epidemiology type enzyme in local setting
- Not apply directly to sample

Thank you very much for your attention

Thank to

Prof. Giuseppe Cornaglia

Thank to my MDR
screening team:

Mirta Bragantini
Franca Casotto
Anna Oliani
Martina Parisato

Thank to all the
student that work for
MDR screening
during these years:

Marta Piccoli
Giada Petrosino
Camilla Castagna
Elena Pilotto
Mattia Bocchese
Claudia Thoma"
Martina Bacchi
Annarita Centonze
Liliana Galia