



**Forum Risk Management**  
obiettivo sanità & salute

18

**21-24 NOVEMBRE 2023**  
**AREZZO FIERE E CONGRESSI**

**Gianluca Foglietta**  
Ospedale Pediatrico Bambin Gesù, Roma



**Applicazioni della Spettrometria di Massa e della  
Spettroscopia FTIR in microbiologia per la  
determinazione rapida di farmacoresistenza**

Gianluca Foglietta

Unit of Diagnostic Microbiology and Immunology,  
Bambino Gesù Children's Hospital,  
IRCCS - Rome (Italy)



La resistenza agli antibiotici, o antibiotico-resistenza, è un fenomeno naturale biologico di adattamento di alcuni microrganismi, che acquisiscono la capacità di sopravvivere o di crescere in presenza di una concentrazione di un agente antibatterico, che è generalmente sufficiente ad inibire o uccidere microrganismi della stessa specie.

Si parla di **resistenza intrinseca** quando è dovuta alla natura del microrganismo stesso, che non è mai stato sensibile a un particolare antimicrobico.

Si parla invece di **resistenza acquisita** quando il microrganismo, in precedenza sensibile a un particolare antimicrobico, sviluppa in un secondo tempo resistenza nei suoi confronti.

(Ministero della Salute)

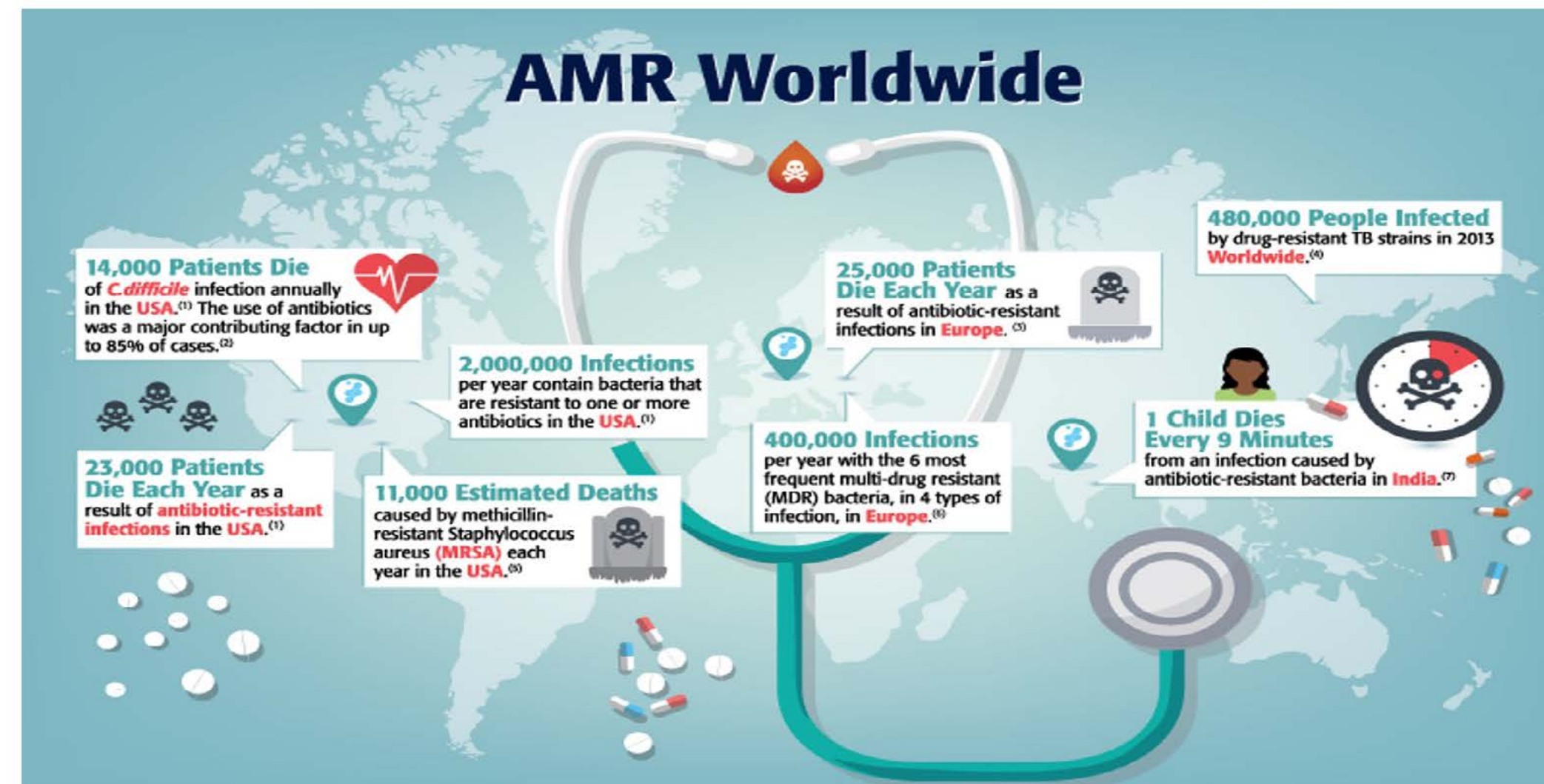




Antimicrobial resistance (AMR) is a global health and development threat. It requires urgent multisectoral action in order to achieve the Sustainable Development Goals (SDGs).

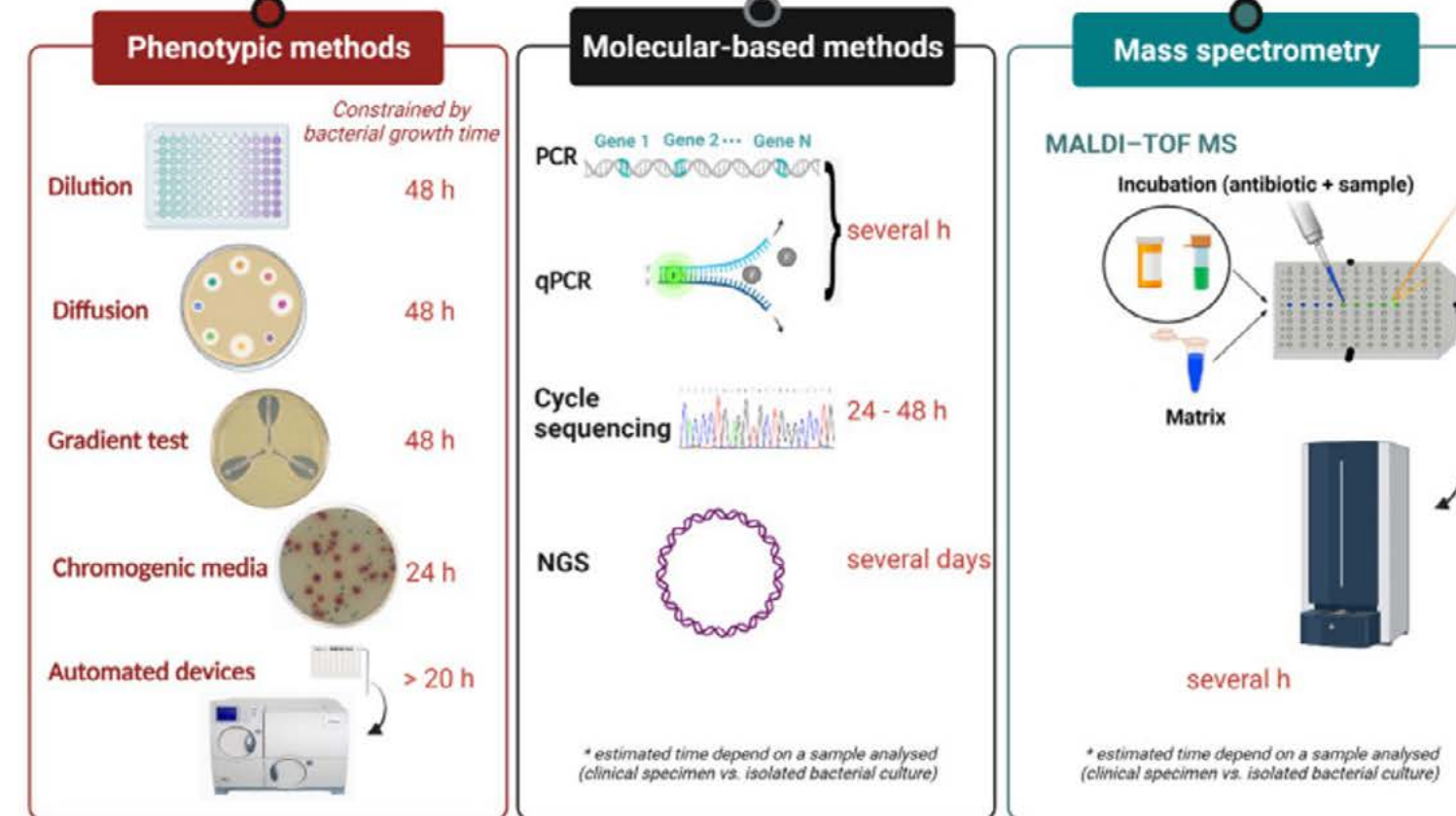
17 November 2021







**Methods of antimicrobial susceptibility testing**



Ina Gaijic et al.; Antibiotics 2022





**Table 1.** Advantages and disadvantages of the common methods of antimicrobial susceptibility testing.

Method	Advantage	Disadvantage	Comments
Broth dilution	Well-standardised Harmonised Commercially available tests are easy to perform	Time-consuming Individual mistakes	Quantitative **
Agar Dilution	Well-standardised Suitable for testing a large number of isolates	Time-consuming Limited concentration of antimicrobial agents	Quantitative Possible automation in part
Disk diffusion	Simple to perform Low cost Simple and fast interpretation The high number of test antibiotics per test High flexibility in antibiotic selection Detection of resistance patterns Mass use and the possibility of automatisation A number of a different use (AST, identification, screening, etc.) Detection of heteroresistant population or contamination	Time-consuming No MIC value The inability for some antibiotics to be tested	Qualitative *

Ina Gaijic et al.; Antibiotics 2022



Table 1. Cont.

Ina Gaijic et al.; Antibiotics 2022



Method	Advantage	Disadvantage	Comments
Gradient test	Convenient and flexible Simple to perform Does not require expertise Detection of resistance patterns	Relatively expensive Relatively long incubation	Quantitative
Automated systems	Simple to perform	Relatively expensive	Semi-quantitative ***
Chromogenic media	Mass use and the possibility of automation Simple to perform Simple and fast interpretation	Not completely susceptible and specific Time-consuming Limited spectra or single antibiotic Relatively expensive Screening only or required confirmatory identification No MIC value	Qualitative with no interpretation criteria (S, I, R)
MALDI-TOF MS	Rapid turnaround time Simple to perform Low sample volume requirements Low per-sample costs	High cost of the MALDI-TOF MS Need further optimisation for each species and antibiotic combination No MIC value	
Genetic methods	Rapid Highly accurate Sensitive Reproducible Increased ability to detect slow-growing or non-cultivable organisms	Limited spectra Limited throughput High cost	Qualitative Semi-quantitative
Genomic methods	Highly accurate Sensitive Increased ability to detect slow-growing or non-cultivable organisms	High cost Time-consuming Challenging interpretation of results	Qualitative





## Genotipo vs Fenotipo

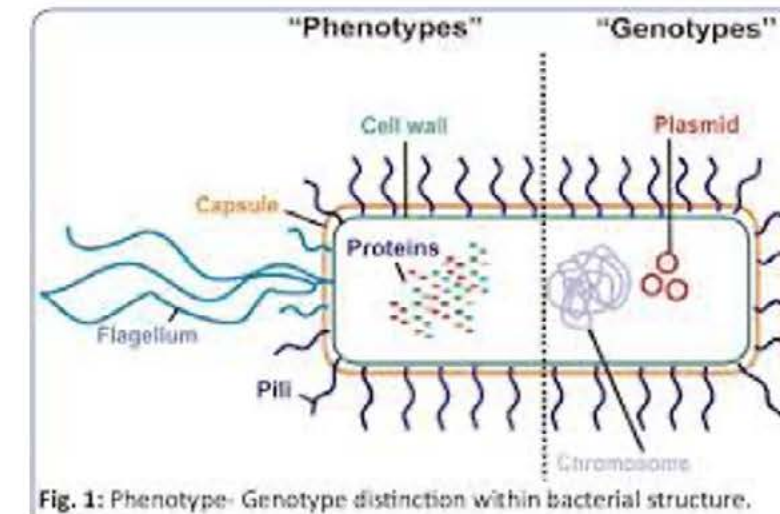
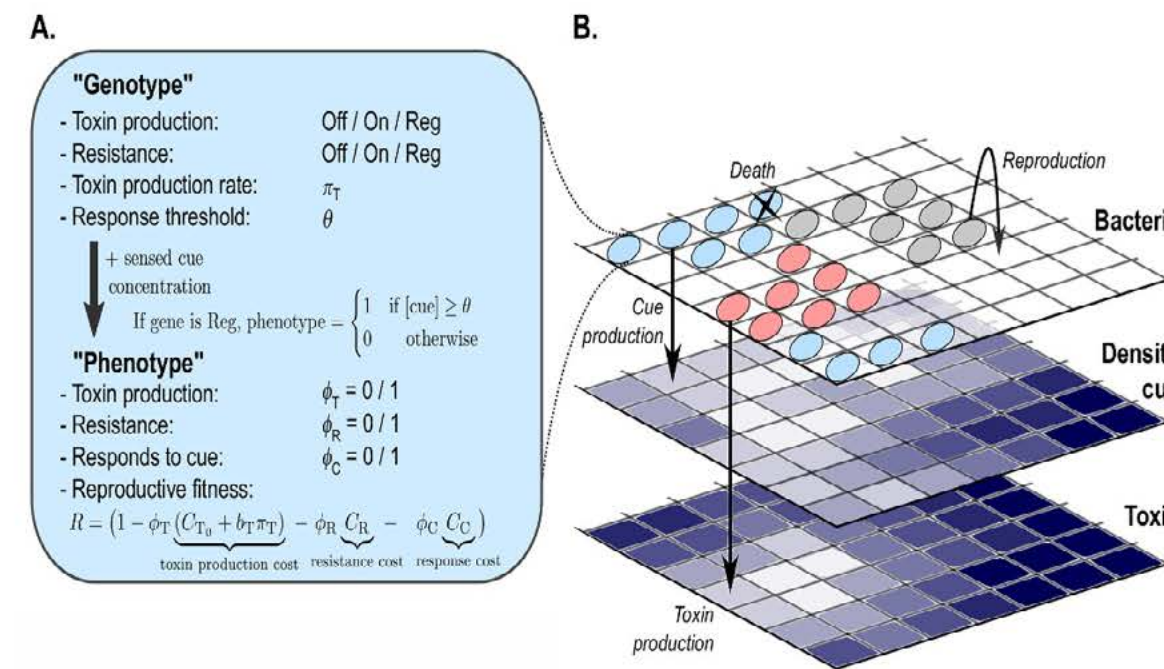
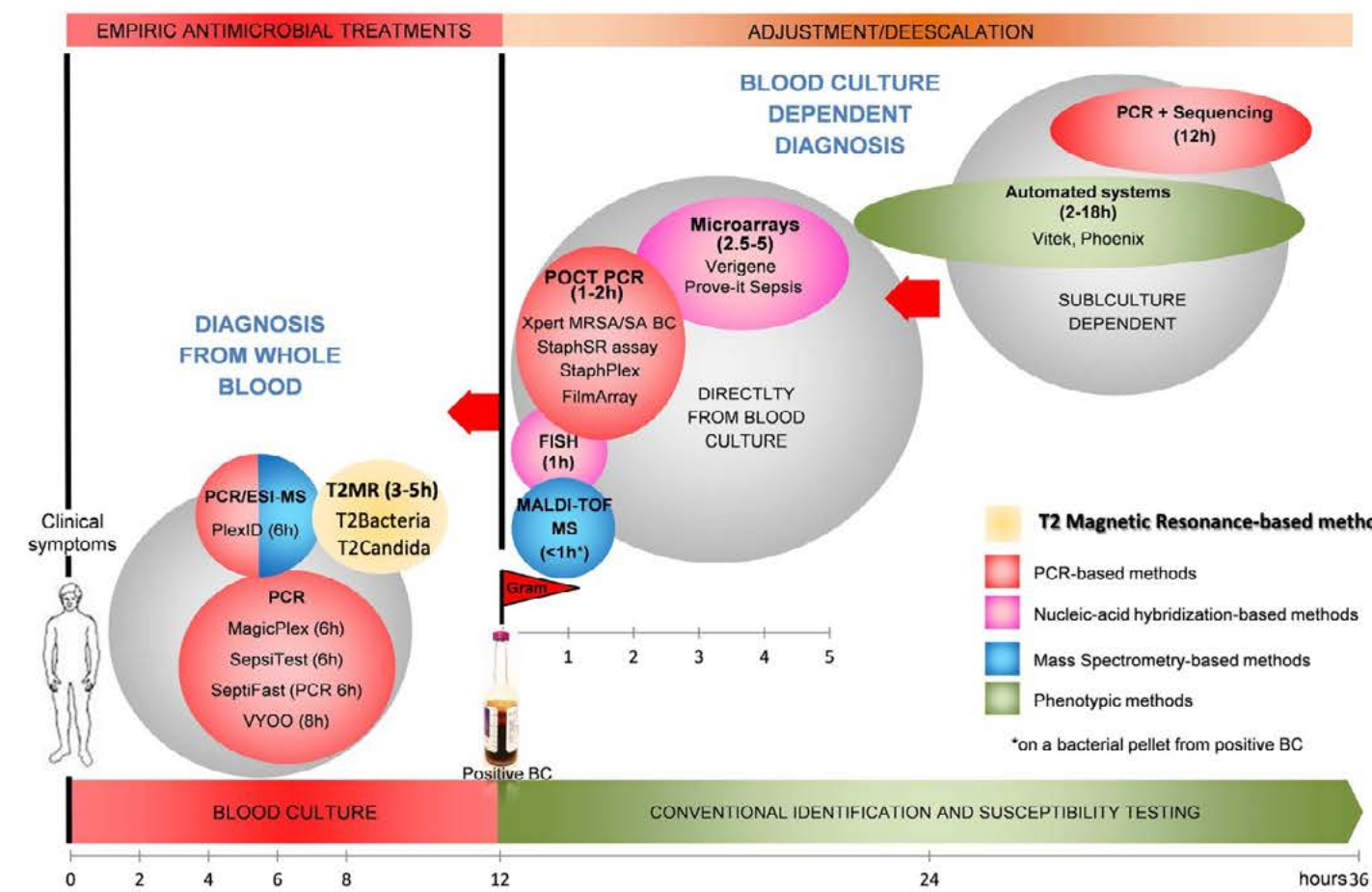


Fig. 1: Phenotype- Genotype distinction within bacterial structure.



**Gestione delle urgenze: Diagnosi rapida di sepsi**



Da 1 a 5 gg

**Tecnologie coltura-dipendenti**  
 Sensibilità e TAT dipendenti dall'emocoltura



3-5 ore

**Tecnologie coltura-indipendenti**  
 Risultati specie/target specifici  
 Consentono di impostare rapidamente una terapia mirata

Opota O, Jaton K, Greub G. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect. 2015

**Gestione delle urgenze: Diagnosi rapida di sepsi**  
Passato, presente e futuro

Technique	Target pathogen	Resistance typing	Turnaround time	Sensitivity	Specificity
<b>Culture-based technique</b>					
Blood culture	All culturable microbes	Yes	48-72	—	—
Automated identification	All culturable microbes	Yes	24-48	—	—
<b>Nucleic acid-based identifier</b>					
PNA-FISH	Differentiates between <i>Staphylococcus aureus</i> and CoNS; <i>Enterococcus faecalis</i> and <i>Enterococcus</i> species; <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , and <i>Pseudomonas aeruginosa</i> ; and <i>Candida</i> species	No	1.5-3	96-100%	96-100%
QuickFISH	<i>S. aureus</i> , CoNS, <i>E. faecalis</i> , other Enterococci, <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	No	<30 min <sup>a</sup>	96-100%	96-100%
MALDI-TOF	GP and GN bacteria, yeast, fungi, filamentous fungi, mycobacteria	In development	10-30 min <sup>a</sup>	—	—
GeneXpert MRSA/SA	<i>S. aureus</i>	<i>mecA</i> for methicillin resistance	<1 h <sup>a</sup>	98.3-100% for MSSA and MRSA	98.6-99.4% for MSSA and MRSA
Verigene gram-positive	<i>Staphylococcus</i> spp., <i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. lugdunensis</i> , <i>Streptococcus</i> spp., <i>S. pyogenes</i> , <i>S. agalactiae</i> , <i>S. anginosus</i> group, <i>S. pneumoniae</i> , <i>E. faecalis</i> , <i>E. faecium</i> , and <i>Listeria</i> spp.	<i>mecA</i> for methicillin resistance and <i>vanA/B</i> genes for vancomycin resistance	2.5 h <sup>a</sup>	92.6-100%	95.4-100%
Verigene gram-negative	9 bacterial targets including <i>E. coli</i> , <i>Shigella</i> spp., <i>K. pneumoniae</i> , <i>K. oxytoca</i> , <i>P. aeruginosa</i> , <i>Serratia marcescens</i> , <i>Acinetobacter</i> spp., <i>Proteus</i> spp., <i>Citrobacter</i> spp., <i>Enterobacter</i> spp.	KPC, NDM, CTX-M, VIM, IMP, OXA	2 h <sup>a</sup>	97.1%	99.5%
FilmArray	27 targets, including staphylococci, streptococci, <i>Enterococcus</i> , <i>Listeria</i> , <i>Acinetobacter</i> , <i>Neisseria meningitidis</i> , <i>P. aeruginosa</i> and members of the Enterobacteriaceae family, as well as <i>Candida</i> spp.	<i>mecA</i> , <i>vanA/B</i> , and <i>K. pneumoniae</i> carbapenemase (KPC) genes	1 h <sup>a</sup>	>90%	—
<b>Culture-independent diagnostic tests</b>					
SeptiFast	25 pathogens (10 GN, 9 GP, 6 fungi)	—	4-6 h	63-83%	83-95%
SepsiTest	>345 pathogens, 13 fungi	—	8-12 h	11-87%	83-96%
T2 MR <i>Candida</i>	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	In development	3-6 h	90%	98%

**MALDI ToF AST  
 Test**



**Colistin Resistance  
 in positive mode**



**Colistin Resistance  
 biomarkers**





## MALDI TOF AST Test

Department of International  
marketing  
Sorani

**Antibiotics**



**Gram negativi**

Amikacin; Gentamicin; Ampicillin/Sulbactam; Minocycline; Tigecycline; Aztreonam; Piperacillin/Tazobactam; Imipenem; Compound Sulfamethoxazole; Ceftriaxone; Cefuroxime; Ceftazidime/Avibactam; Cefoxitin; Cefepime; Meropenem; Levofloxacin; Polymyxin B; Cefoperazone/sulbactam; Ceftazidime.

**Gram positivi**

Ciprofloxacin; Tigecycline; Ampicillin; Rifampicin; Cefoxitin; Linezolid; Clindamycin; Gentamicin; High Concentration Streptomycin; High Concentration Gentamicin; Penicillin; Compound Sulfamethoxazole; Minocycline; Oxacillin; Teicoplanin; Moxifloxacin; Erythromycin; Levofloxacin; Vancomycin.



**Antibiotics**

**Gram negative  
bacterial antibiotics**

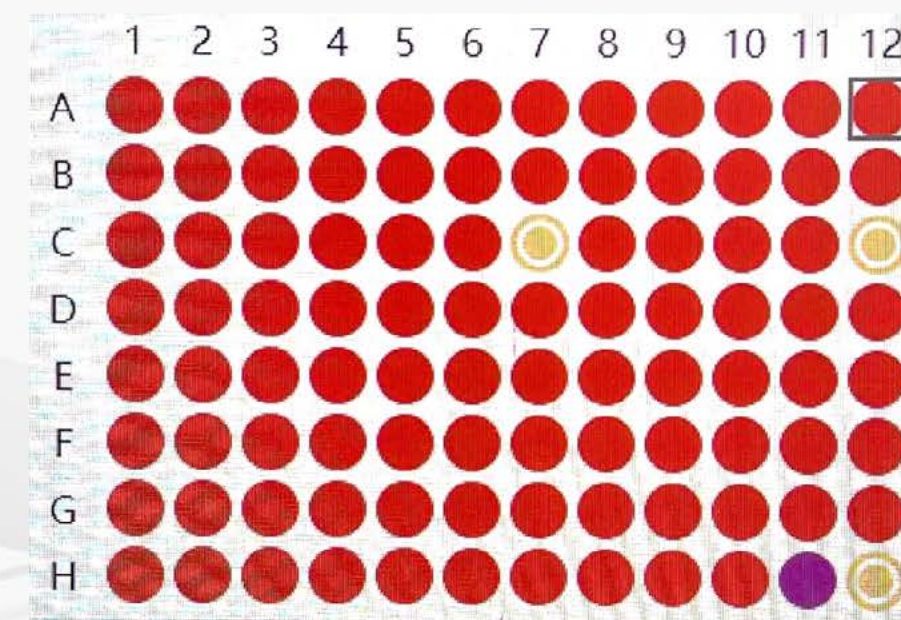
	1	2	3	4	5	6	7	8	9	10	11	12
A	AN	AN	AN	AN	GM	GM	GM	GM	GM	SAM	SAM	SAM
B	MI	MI	MI	MI	TGC	TGC	TGC	TGC	TGC	ATM	ATM	ATM
C	TZP	TZP	TZP	TZP	TZP	TZP	IPM	IPM	IPM	IPM	IPM	IPM
D	SXT	SXT	SXT	SXT	SXT	CRO	CRO	CRO	CRO	CRO	CRO	CRO
E	CXM	CXM	CXM	CXM	CXM	C-A	C-A	C-A	C-A	FOX	FOX	FOX
F	FEP	FEP	FEP	FEP	FEP	FEP	MEM	MEM	MEM	MEM	MEM	MEM
G	LEV	LEV	LEV	LEV	LEV	LEV	LEV	PB	PB	PB	PB	PB
H	CSL	CSL	CSL	CSL	CAZ	CAZ	CAZ	CAZ	CAZ	CAZ	CALIB	REF

**Gram positive  
bacterial antibiotics**

	1	2	3	4	5	6	7	8	9	10	11	12
A	CIP	CIP	CIP	CIP	TGC	TGC	TGC	TGC	AM	AM	AM	AM
B	RA	RA	RA	RA	FOX	FOX	FOX	FOX	LNZ	LNZ	LNZ	LNZ
C	CC	CC	CC	CC	CC	GM	GM	GM	GM	GM	ST	GM
D	P	P	P	P	P	P	P	P	SXT	SXT	SXT	SXT
E	MI	MI	MI	MI	MI	MI	OXA	OXA	OXA	OXA	OXA	OXA
F	TEC	TEC	TEC	TEC	TEC	TEC	MXF	MXF	MXF	MXF	MXF	MXF
G	E	E	E	E	E	E	LEV	LEV	LEV	LEV	LEV	LEV
H	VA	VA	VA	VA	VA	VA	VA	VA			CALIB	REF

## Interpretation of the results

Autobio 安图生物



If one antibiotic with different concentration all have mass spectrometry identification result, **marked green or yellow**, that means all the coated antibiotic concentration don't have inhibition to the sample -> **Resistance**.

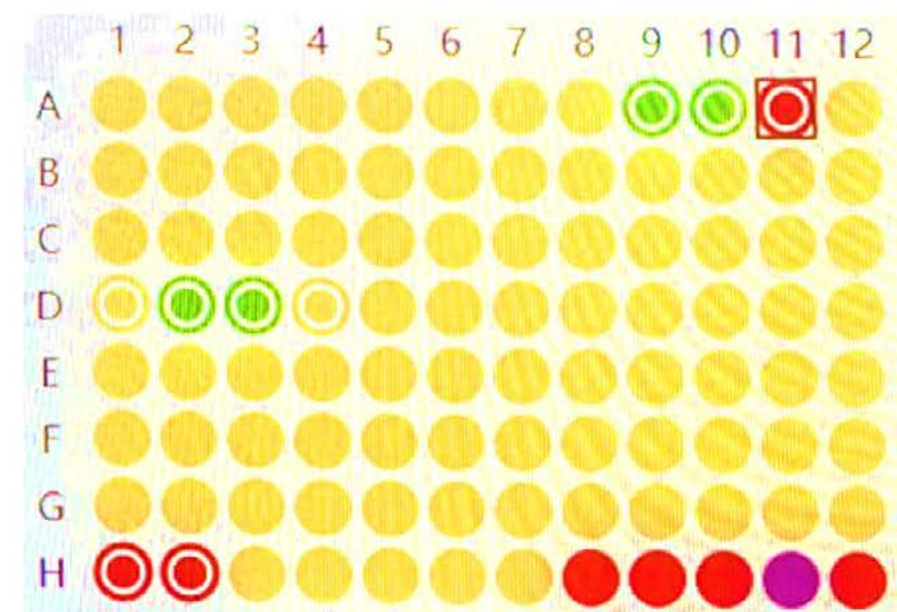
Recommend sampling these wells manually from high concentration to low concentration to make sure the result is correct.

When **the wells marked red** means on this well have no mass spectrometry identification result, corresponding antibiotic concentration has inhibition to the sample -> **Susceptible**





## Interpretation of the results



The reference spot H12 shows no result, that means the sample haven't cultured. No matter what other wells have or haven't result, stop using this plate.

After the software is ready, all the interpretations will automatically be done by the software and give report.





OPEN ACCESS

EDITED BY  
 Karsten Becker,  
 University Medicine Greifswald,  
 Germany

REVIEWED BY  
 Xiaogang Xu,  
 Fudan University,  
 China  
 Antonella Lupetti,  
 University of Pisa,  
 Italy

\*CORRESPONDENCE  
 Elena De Carolis  
 ✉ elena.decarolis@policlinicogemelli.it

<sup>†</sup>These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION  
 This article was submitted to  
 Antimicrobials, Resistance and Chemotherapy,  
 a section of the journal  
 Frontiers in Microbiology

RECEIVED 15 September 2022  
 ACCEPTED 01 February 2023  
 PUBLISHED 22 February 2023

**“CORE” a new assay for rapid identification of *Klebsiella pneumoniae* COListin RESistant strains by MALDI-TOF MS in positive-ion mode**

Gianluca Foglietta<sup>1†</sup>, Elena De Carolis<sup>2\*†</sup>, Giordana Mattana<sup>1</sup>, Manuela Onori<sup>1</sup>, Marilena Agosta<sup>1</sup>, Claudia Niccolai<sup>3</sup>, Vincenzo Di Pilato<sup>4</sup>, Gian Maria Rossolini<sup>3,5</sup>, Maurizio Sanguinetti<sup>2</sup>, Carlo Federico Perno<sup>1</sup> and Paola Bernaschi<sup>1</sup>

<sup>1</sup>Microbiology Unit and Diagnostic Immunology, Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy, <sup>2</sup>Microbiology Unit, Department of Laboratory Sciences and Infectious Diseases, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy, <sup>3</sup>Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy, <sup>4</sup>Department of Surgical Sciences and Integrated Diagnostics (DISCI), University of Genoa, Genoa, Italy, <sup>5</sup>Clinical Microbiology and Virology Unit, University Hospital Careggi, Florence, Italy

ABSTRACT

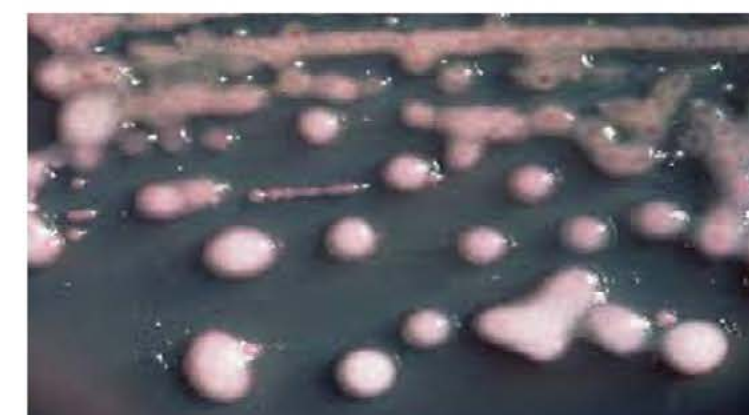
Due to the global spread of pan resistant organisms, colistin is actually considered as one of the last resort antibiotics against MDR and XDR bacterial infections. The emergence of colistin resistant strains has been observed worldwide in Gram negative bacteria, such as Enterobacteriaceae and especially in *K. pneumoniae*, in association with increased morbidity and mortality. This landscape implies the exploration of novel assays able to target colistin resistant strains rapidly.

In this study, we developed and evaluated a **new MALDI-TOF MS assay in positive ion mode that allows quantitative or qualitative discrimination between colistin susceptible (18) or resistant (32) *K. pneumoniae* strains in 3 h** by using the “Autof MS 1000” mass spectrometer. The proposed assay, if integrated in the diagnostic workflow, may be of help for the antimicrobial stewardship and the control of the spread of *K. pneumoniae* colistin resistant isolates in hospital settings.



### Strains collection

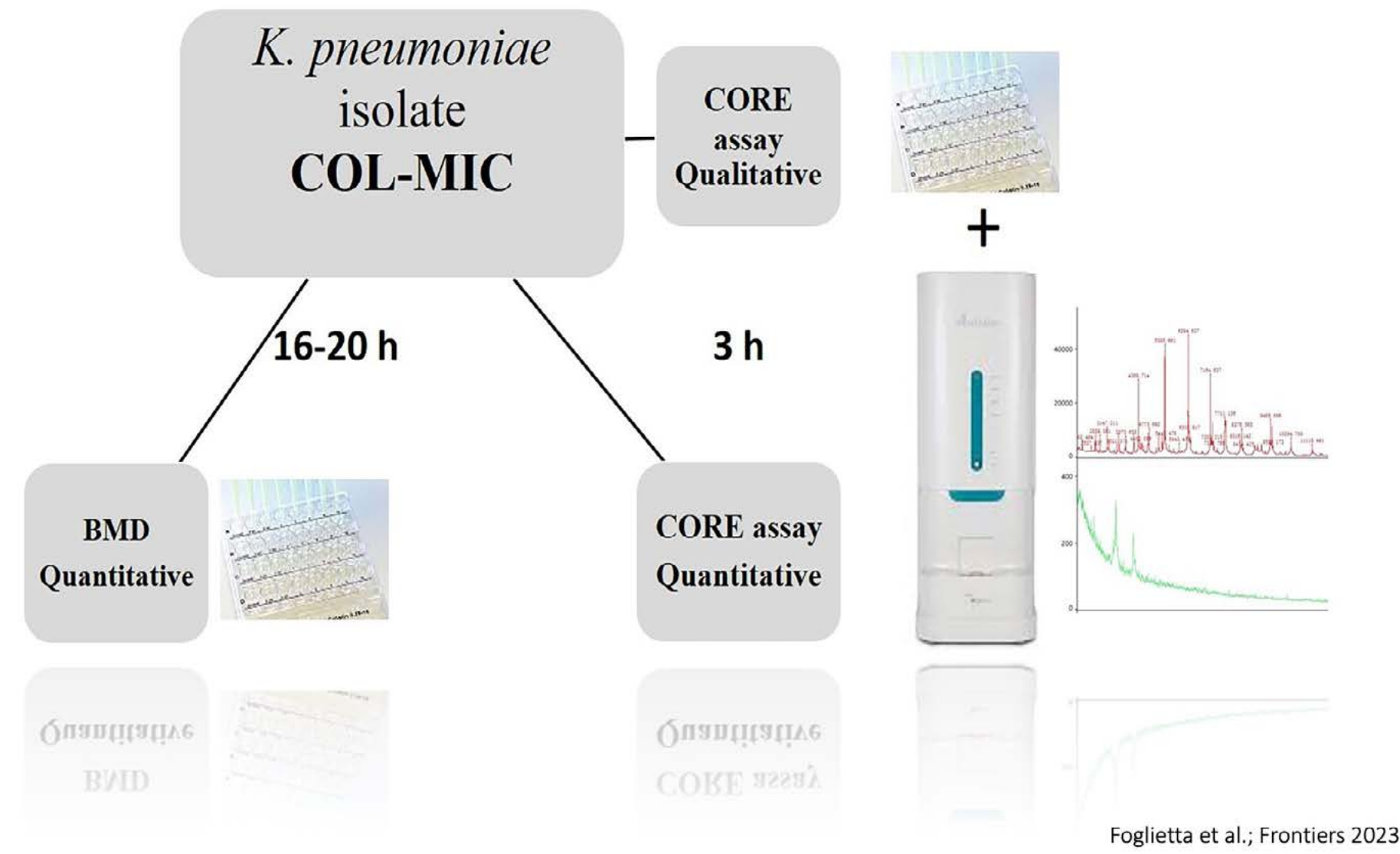
The study collection included 50 colistin-resistant ( $n = 32$ ) and-susceptible ( $n = 18$ ) *K. pneumoniae* isolates, cultured from blood ( $n = 33$ ), urine ( $n = 4$ ), rectal swabs ( $n = 8$ ), tracheal broncho-aspirates ( $n = 3$ ), cerebrospinal fluid ( $n = 1$ ) and wound swab ( $n = 1$ ).

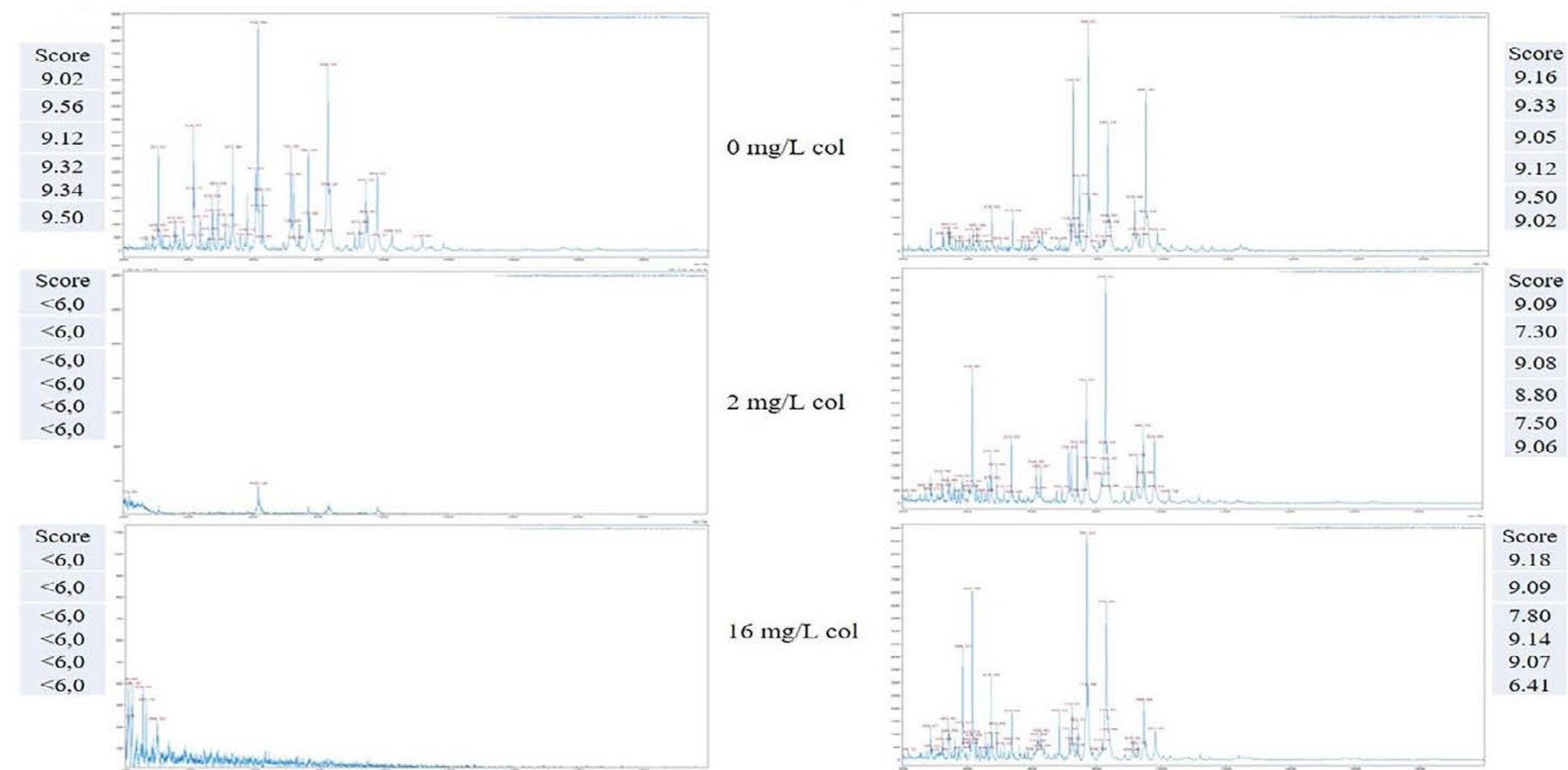


Within the study collection, 32 out of 50 *K. pneumoniae* isolates were previously characterized by whole-genome sequencing (WGS), and genetic alterations associated with colistin resistance were formerly investigated ([Cannatelli et al., 2014](#); [Di Pilato et al., 2021](#); [Boncompagni et al., 2022](#)). The remaining colistin resistant isolates ( $n = 18$ ) were screened for the presence of the most common *mcr* gene variants by Real-time PCR, including *mcr-1* and *mcr-2*, and additional *mcr* genes using specific primer/probes combinations ([Coppi et al., 2018](#); [Yang et al., 2018](#))

Foglietta et al.; Frontiers 2023







Foglietta et al.; Frontiers 2023

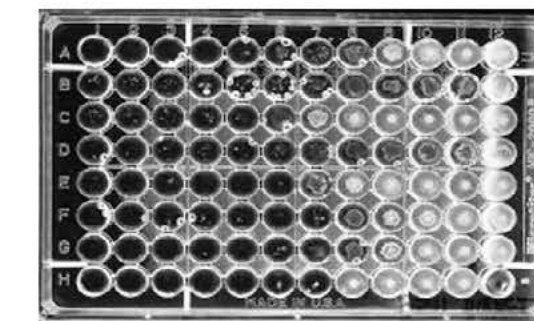


## Risultati

*K. pneumoniae*, 32 colR and 18 colS isolates, **were correctly classified in 3 h as resistant or susceptible by the CORE assay**, respectively. For what concerns the **quantitative CORE assay, 30 out of 32 colR**

*K. pneumoniae* agreed against BMD MIC values within **±1 dilution according to the modal MIC** results, whilst 7070100801 and B2 isolates obtained a MIC value 2 dilution higher (16 vs. 4 mg/L) using the quantitative CORE assay. Overall, an EA of 93.7% (30/32 *K. pneumoniae* resistant isolates) was reported following the 3 h incubation of the quantitative CORE assay.

Regarding the 18 colS *K. pneumoniae*, an EA of **83.3%** was calculated; in particular, 15 out of 18 isolates resulted concordant against the BMD assay within ±1 dilution



Foglietta et al.; Frontiers 2023



Sviluppo di una  
diagnostica rapida per la  
determinazione di  
biomarcatori di  
farmacoresistenza  
mediante Spettrometria  
MALDI ToF

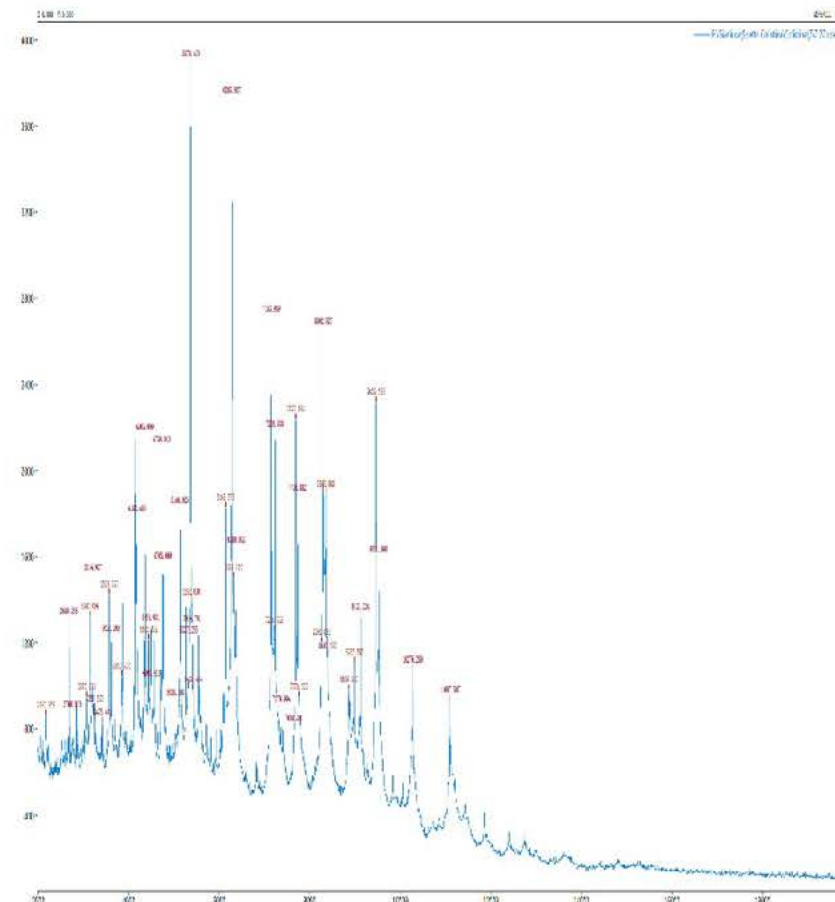


Implementazione clinica del MALDI-ToF MS nella determinazione rapida dell'antibiotico-resistenza



Trasformazione in file testo TXT degli spettri acquisiti (Spectrum).

Selezione manuale di ogni singolo spettro e passaggio in forma tabulare per trasferirli all'analisi bioinformatica.

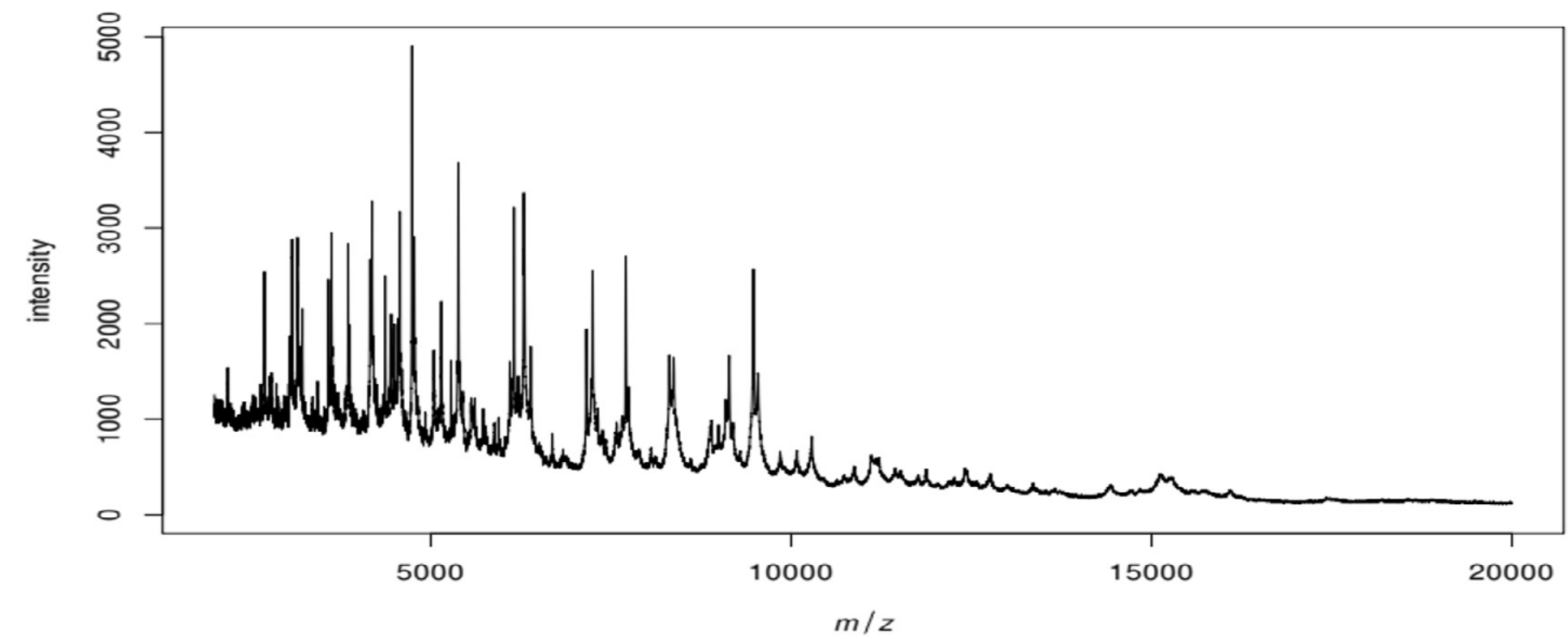


1998.915212	6110.000000
1999.261519	5903.000000
1999.607856	5742.000000
1999.954223	5645.000000
2000.300620	5423.000000
2000.647047	5258.000000
2000.993504	5066.000000
2001.339991	4957.000000
2001.686508	4844.000000
2002.033055	4836.000000
2004.806511	4467.000000
2004.112967	4688.000000
2004.459724	4616.000000
2004.806511	4467.000000
2005.153328	4331.000000
2005.500174	4528.000000
2005.847051	4588.000000
2006.193958	4624.000000
2006.540895	4737.000000
2006.887862	4622.000000
2007.234858	4421.000000
2007.581885	4386.000000
2007.928942	4476.000000
2008.276028	4547.000000
2008.623145	4474.000000
2008.970292	4475.000000
2009.317469	4397.000000
2009.664675	4473.000000
2010.011912	4477.000000
2010.359178	4420.000000



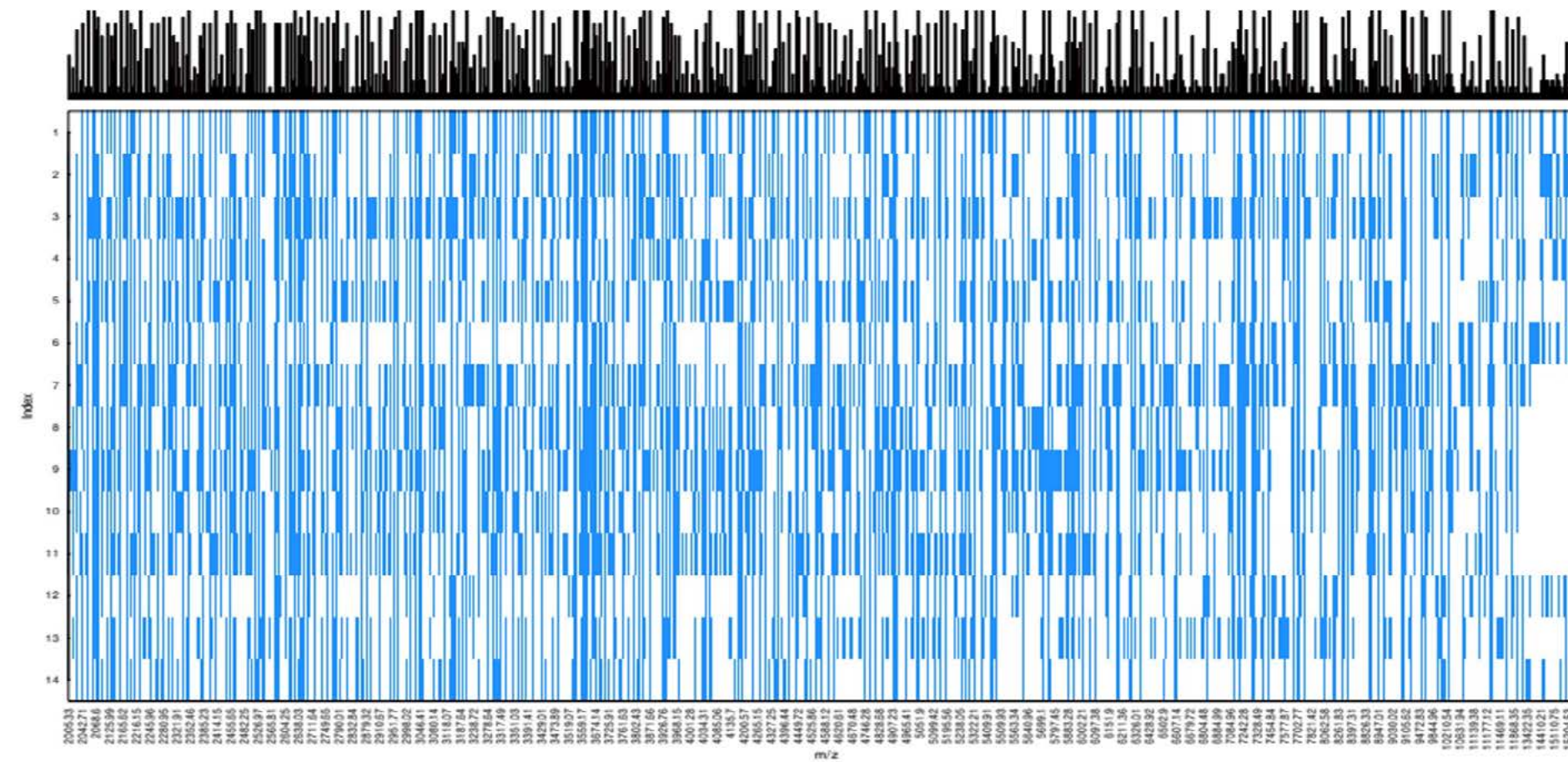


Raw spectrum

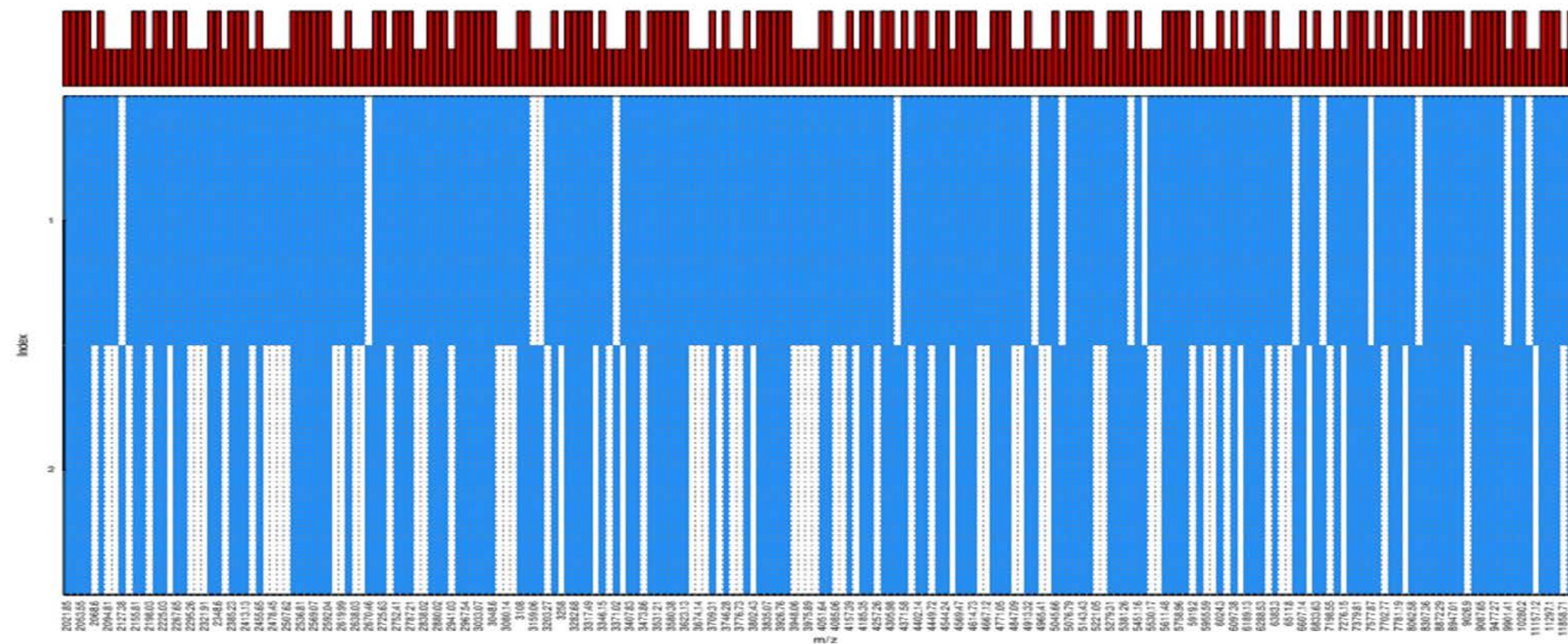


Gentile concessione Valentino Costabile

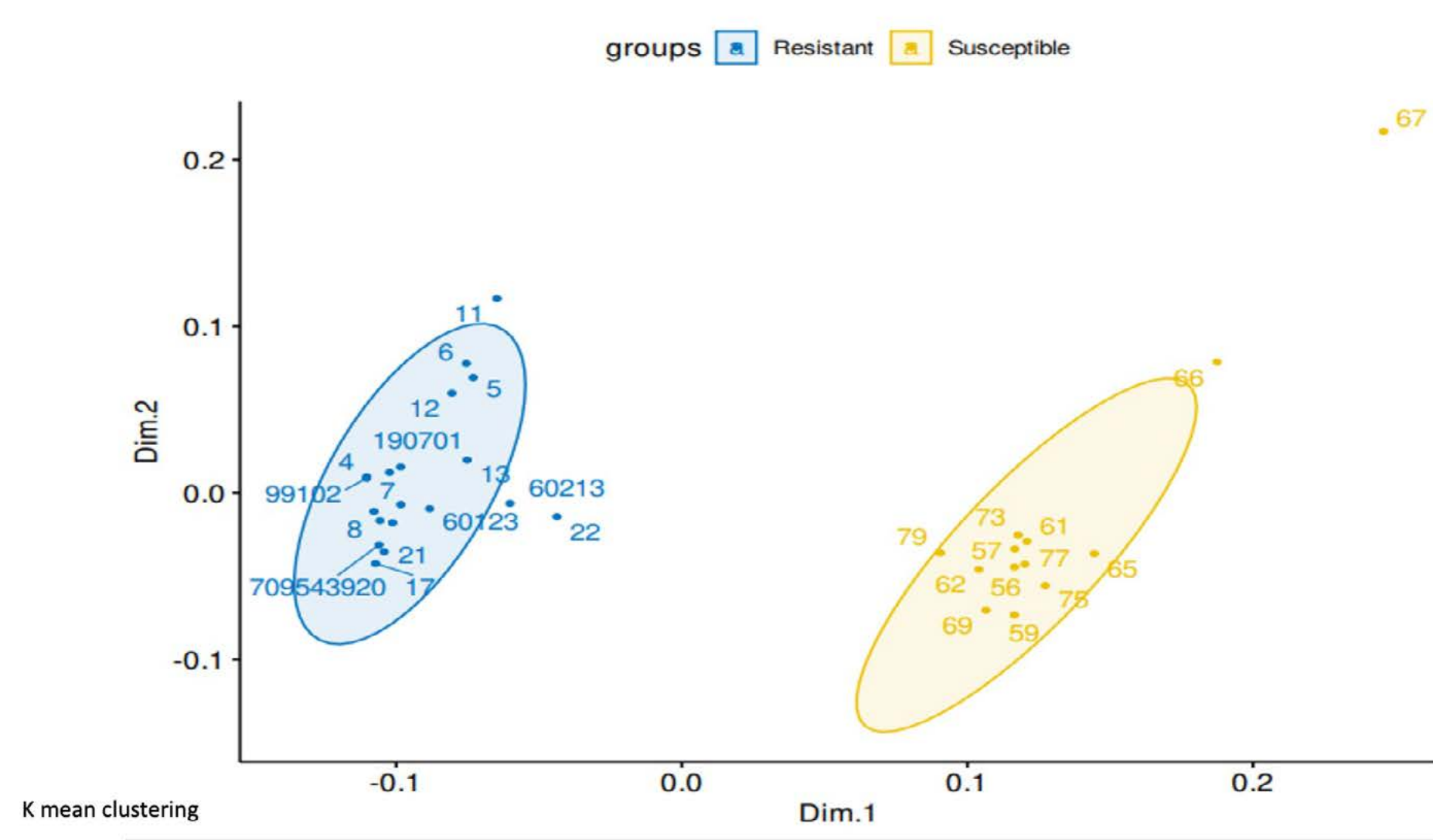
**Aligned peaks profile: preliminar comparison**



**Aligned filtered peaks profile: groups comparison (Resistant vs. Susceptible)**



Gentile concessione Valentino Costabile

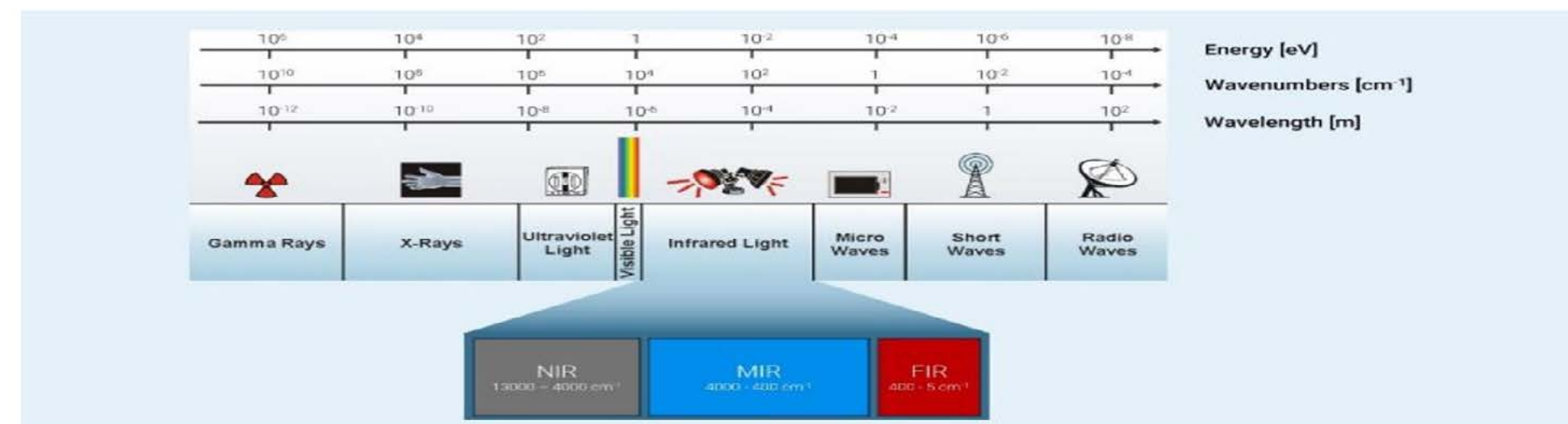


## Cosa è la Spettroscopia IR?



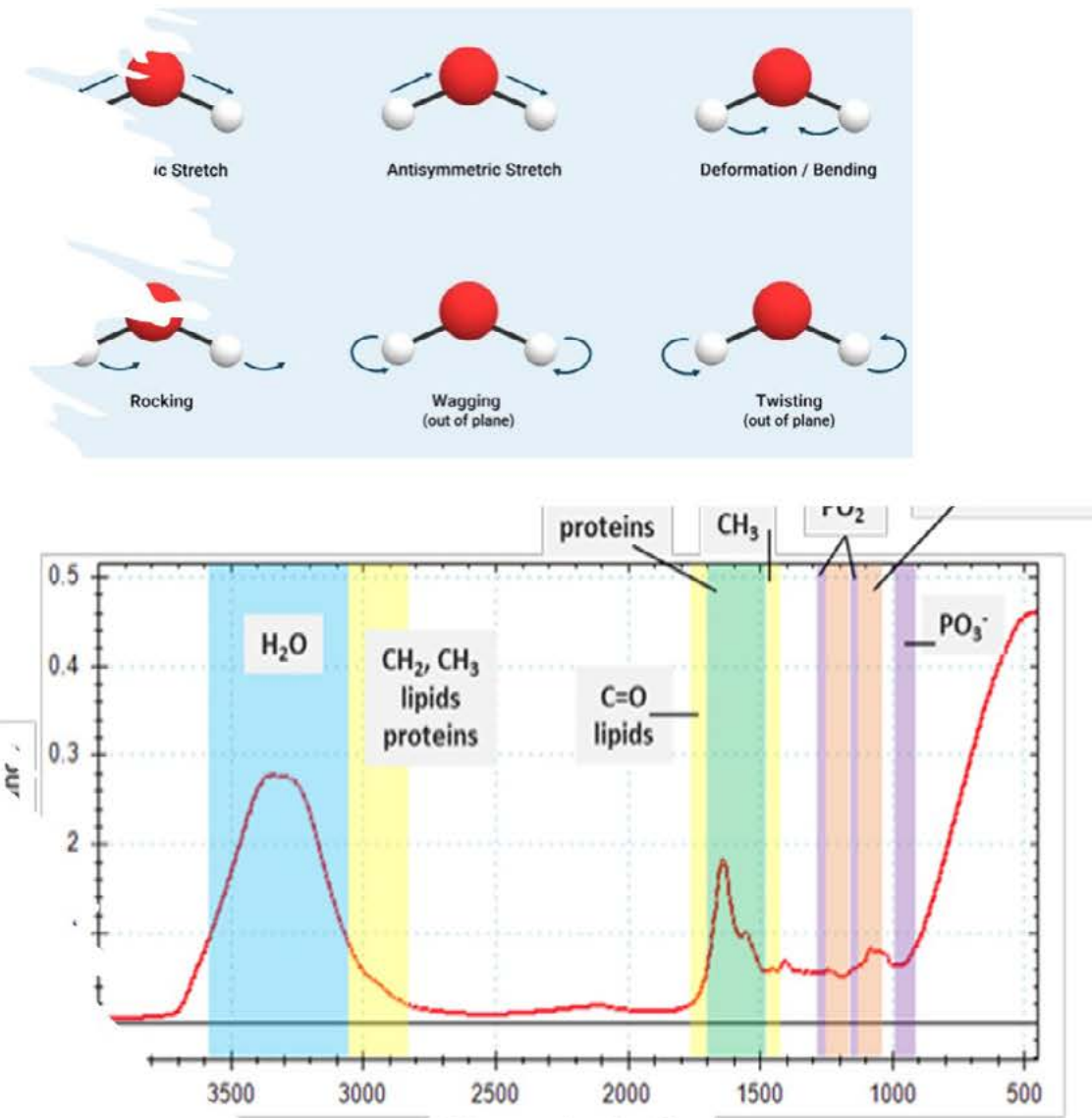
La spettroscopia infrarossa, o IR, è una tecnica di analisi chimica che sfrutta l'interazione tra la luce infrarossa e la materia. La luce infrarossa fa parte dello spettro elettromagnetico tra la luce visibile e le microonde, con lunghezze d'onda che vanno da 780 nm a 1 mm.

La luce infrarossa può essere ulteriormente suddivisa in tre categorie: Vicino infrarosso (NIR), medio infrarosso (MIR) e lontano infrarosso (FIR). Il NIR ha la lunghezza d'onda più corta e numero d'onda più alto, mentre il FIR ha la lunghezza d'onda più lunga con numeri d'onda più bassi. In genere, quando si parla di spettroscopia IR, il tipo di luce IR utilizzato è il MIR. La luce IR in questo intervallo è utile perché coincide con un'importante proprietà dei composti chimici: le loro vibrazioni.



## Le vibrazioni nell'IR

- Gli atomi dei composti chimici si muovono e vibrano costantemente in modi diversi. Anche in una molecola semplice come l'acqua ci sono sei modi diversi in cui la molecola può vibrare: in modo simmetrico, in modo asimmetrico, la deformazione o bending, rocking, la twisting e wagging.
- Ognuna di queste vibrazioni avviene a una frequenza diversa, unica per il legame chimico e il composto. Come già detto, queste frequenze corrispondono alle frequenze della luce nella regione MIR dello spettro elettromagnetico.

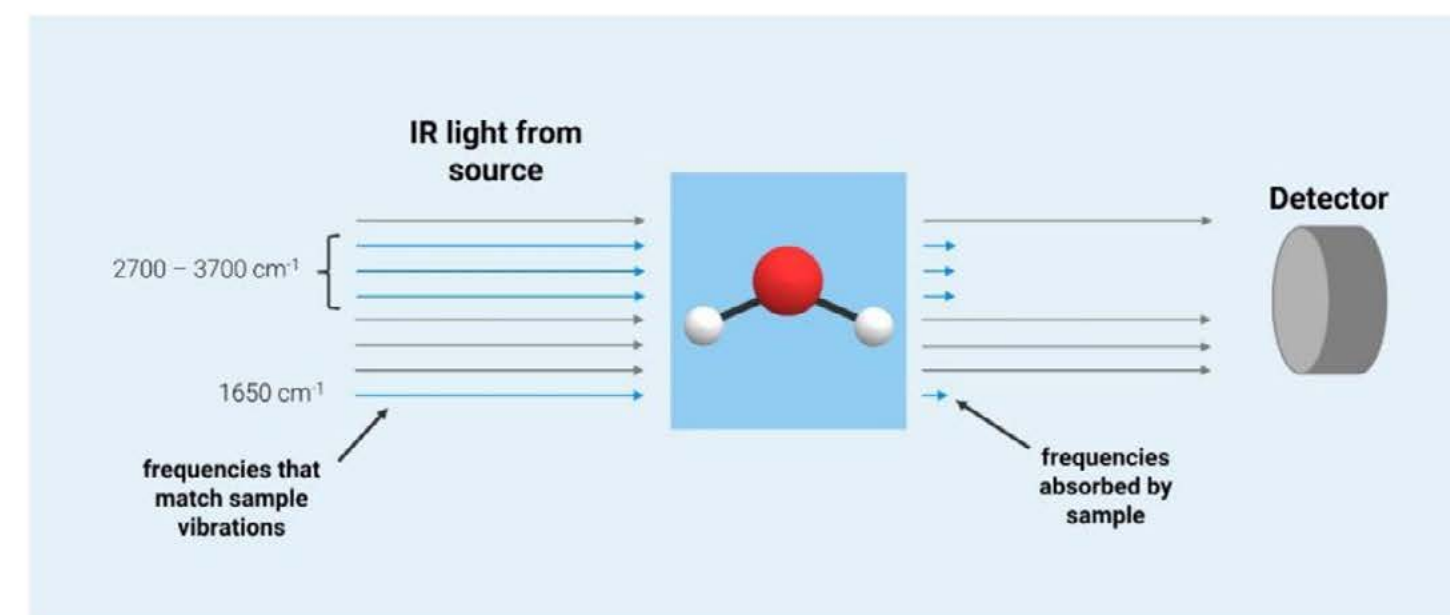


### Come determinare l'assorbimento IR

I composti chimici assorbono la luce IR che determina le vibrazioni nelle molecole delle loro strutture.

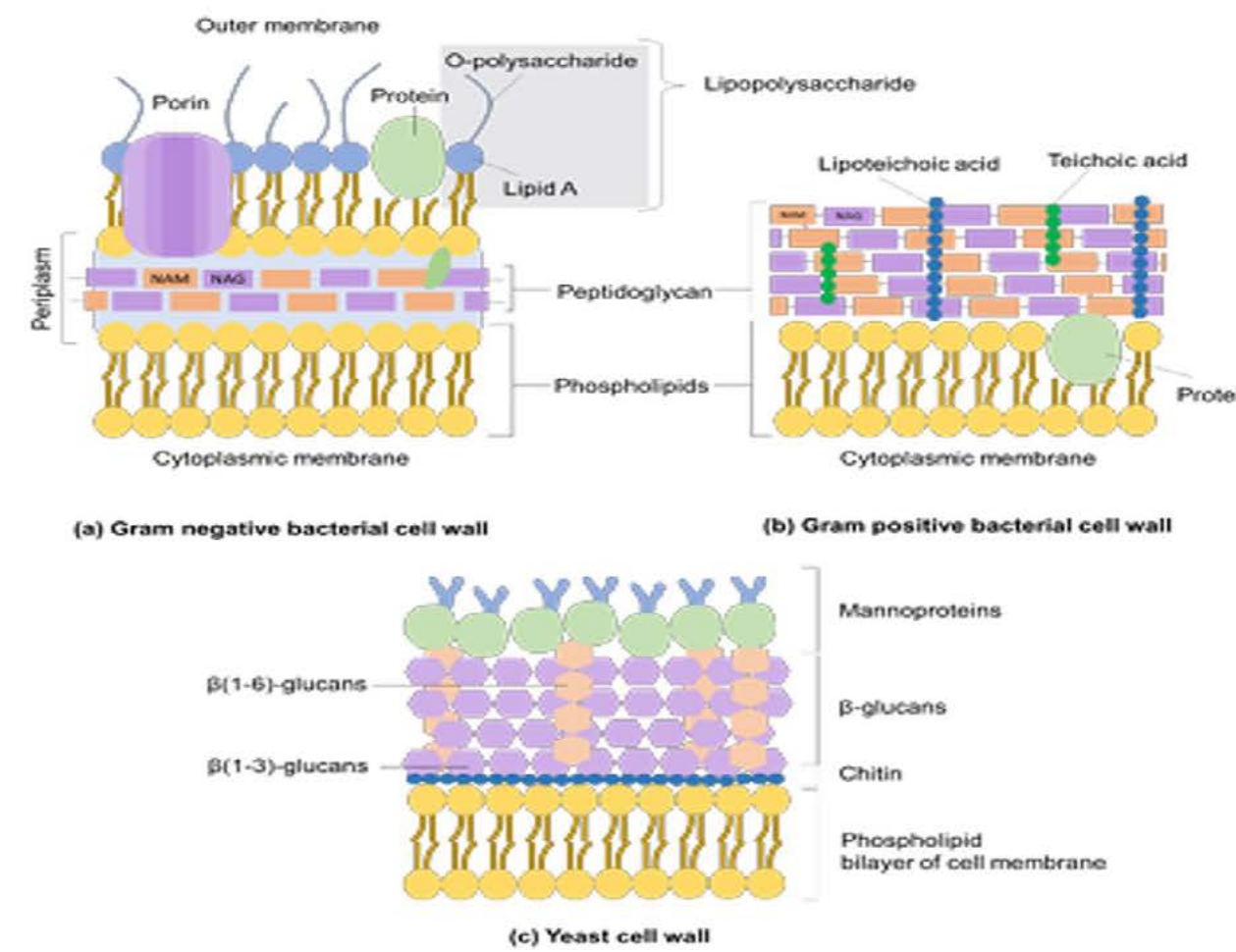
Ad esempio, gli stiramenti simmetrici e antisimmetrici dell'acqua si verificano nell'intervallo compreso tra 2700 e 3700  $\text{cm}^{-1}$ , mentre la vibrazione di deformazione si verifica intorno a 1650  $\text{cm}^{-1}$ , quindi l'acqua assorbirà queste energie della luce IR.

Se facciamo passare la luce IR attraverso l'acqua, possiamo usare un rilevatore per determinare quali frequenze di luce sono state assorbite, poiché tali frequenze saranno "mancanti" dal fascio originale di luce IR.





**Membrane e componenti**







Rapid discrimination of *Pseudomonas aeruginosa* ST175 isolates involved in a nosocomial outbreak using MALDI-TOF Mass Spectrometry and FTIR Spectroscopy coupled with Machine Learning

Belén Rodríguez-Sánchez<sup>1</sup>, Ana Candela<sup>1</sup>, Manuel J. Arroyo<sup>2</sup>, María Sánchez-Cueto<sup>1</sup>, Mercedes Marín<sup>1</sup>, Emilia Cercenado<sup>1</sup>, Gema Méndez<sup>2</sup>, Patricia Muñoz<sup>1</sup>, Luis Mancera<sup>2</sup>, and David Rodríguez-Temporal<sup>1</sup>

<sup>1</sup>Hospital General Universitario Gregorio Marañon

<sup>2</sup>Clover Bioanalytical Software Av del Conocimiento 41 18016 Granada Spain

July 29, 2022

**Abstract**

**Objectives:** Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and Fourier Transform Infrared-Spectroscopy (FTIR-S) as diagnostic alternatives to DNA-based methods for the detection of *Pseudomonas aeruginosa* sequence type (ST) 175 isolates involved in a hospital outbreak. **Methods:** Twenty-seven *P. aeruginosa* isolates from a 2014 outbreak in the Hematology department of our hospital were previously characterized by PFGE and WGS. Besides, 8 *P. aeruginosa* isolates were analyzed as unrelated controls. MALDI-TOF MS spectra were acquired by applying the colony on the MALDI target plate followed by 1 µl of formic acid 100% and 1 µl of HCCA matrix. For the analysis with FTIR-S, colonies were resuspended in 70% ethanol and sterile water according to the manufacturer instructions. Spectra from both methodologies were analyzed using Clover Biosoft® software, that allowed data modelling using different algorithms and validation of the classifying models. **Results:** Three outbreak-specific biomarkers were found at 5169, 6915 and 7236 m/z in MALDI-TOF MS spectra. Classification models based on these three biomarkers showed the same discrimination power displayed by PFGE. Besides, K-Nearest Neighbor algorithm allowed the discrimination of the same clusters provided by whole-genome sequencing and the validation of this model achieved 97.0% correct classification. On the other hand, FTIR-S showed a discrimination power similar to PFGE and reached correct discrimination of the different STs analyzed. **Conclusions:** The combination of both technologies evaluated, paired with Machine Learning tools, may represent a powerful tool for real-time monitoring of high-risk clones and isolates involved in nosocomial outbreaks.



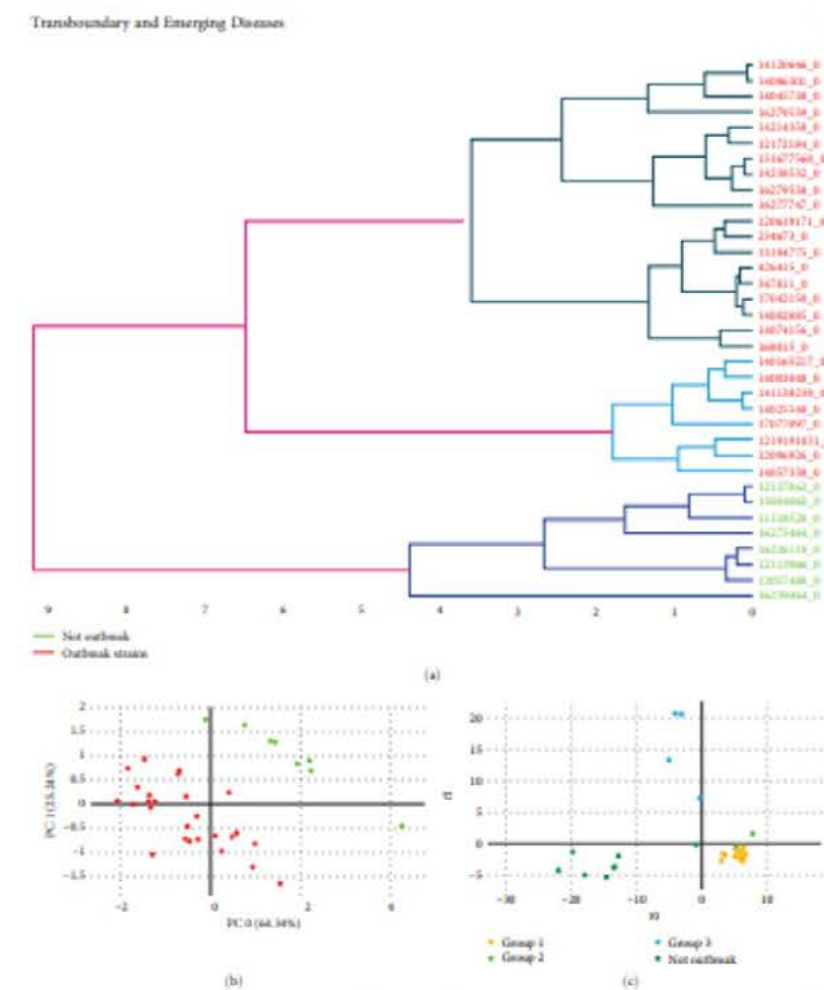
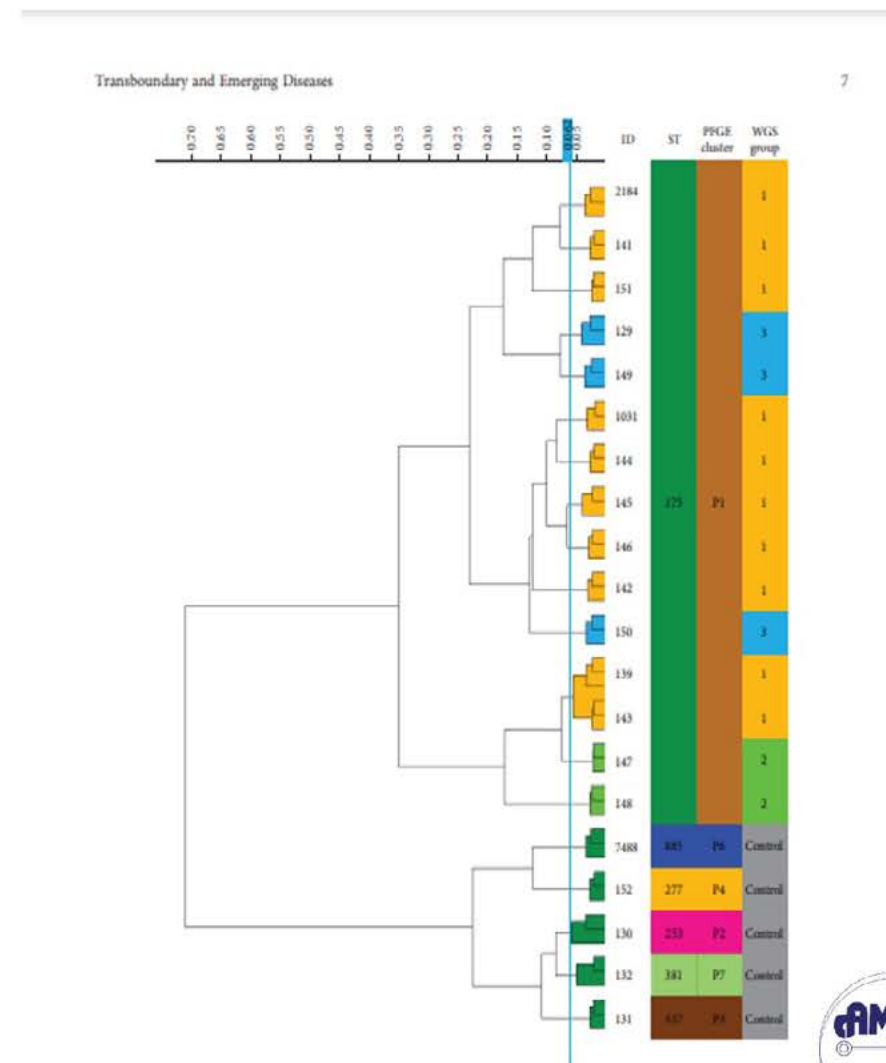


Figure 2. Classification of MALDI-TOF MS spectra between "outbreak" and "not outbreak" isolates. (a) Hierarchical clustering analysis with Euclidean distance and ward metric; (b) principal component analysis; and (c) differentiation of WGS-groups by PLS-DA (partial least square discriminant analysis) algorithm. MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; WGS, whole-genome sequencing.



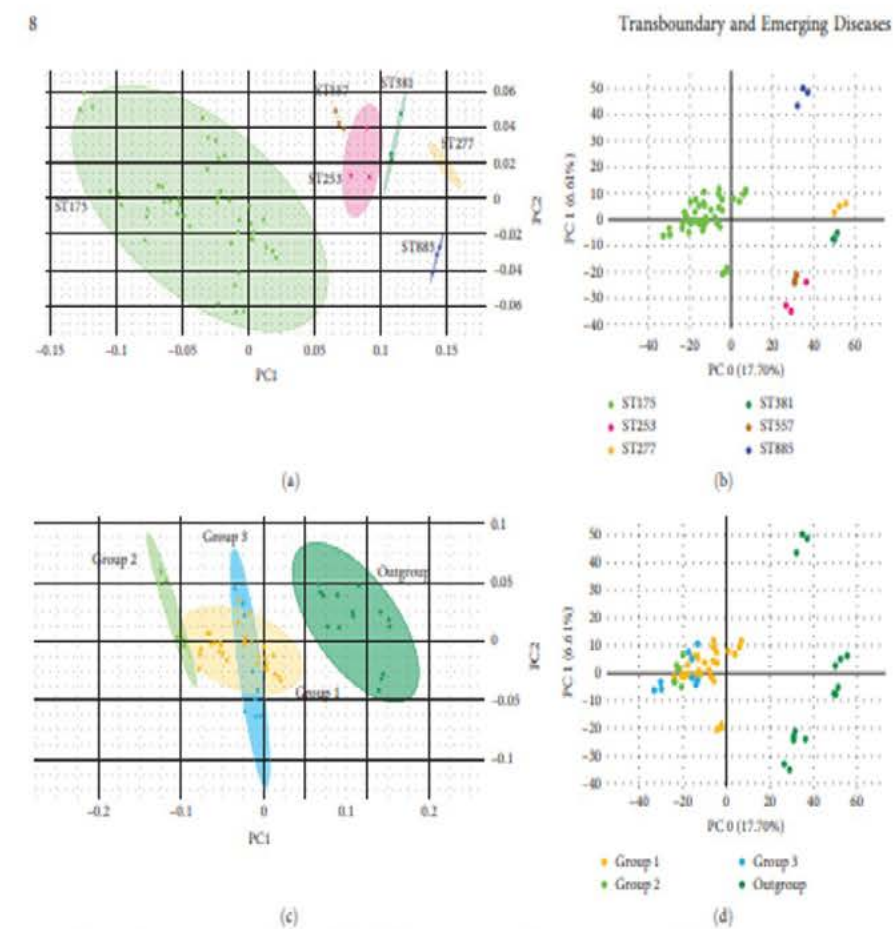


FIGURE 4: Principal component analysis scatter plot obtained by Fourier-transform infrared spectroscopy. (a) Plot according to sequence type (ST) of isolates obtained by IR Biotyper Software. (b) Plot according to ST obtained by Clover MS Data Analysis Software. (c) Plot according to whole genome sequencing (WGS) outbreak groups obtained by IR Biotyper Software. (d) Plot according to WGS groups obtained by Clover MS Data Analysis Software.

Transboundary and Emerging Diseases

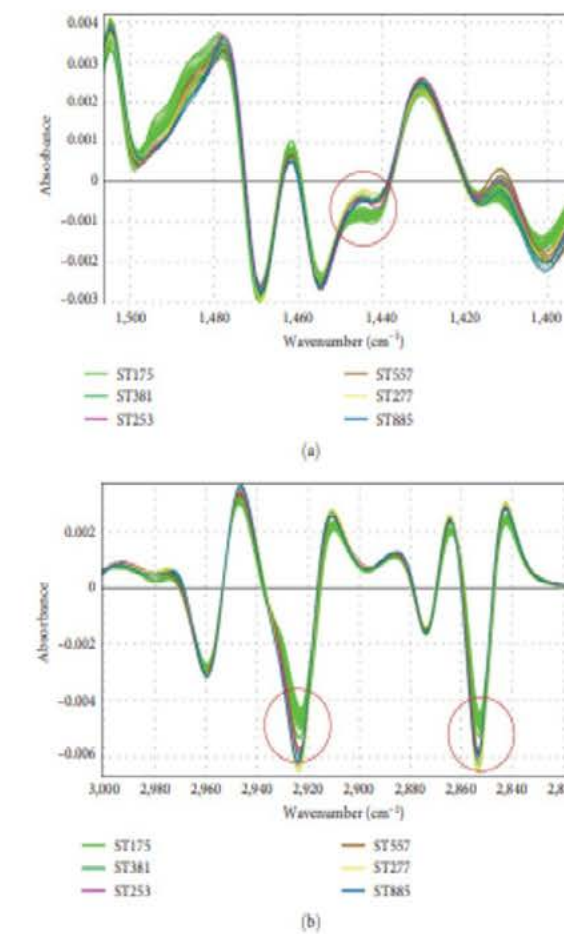


FIGURE 5: Fourier-transform infrared spectroscopy spectra of *Pseudomonas aeruginosa* isolates in the lipids region after apply derivative. (a) 1,400-1,500 cm<sup>-1</sup> region with different absorbance among outbreak and not-outbreak isolates (1,445; 2,800-3,000 cm<sup>-1</sup> region with different absorbance among outbreak and not-outbreak isolates (2,925 and 2,955 cm<sup>-1</sup>).



**RINGRAZIAMENTI al Laboratorio di Microbiologia**



**Prof. Carlo Federico Perno**  
**Dott.ssa Paola Bernaschi**



**Prof. Maurizio Sanguinetti**  
**Dott.ssa Elena De Carolis**

**Marilena Agosta**  
**Marta Argentieri**  
**Venere Cortazzo**  
**Valentino Costabile**  
**Nour Essa**  
**Barbara Lucignano**  
**Livia Mancinelli**  
**Manuela Onori**  
**Laura Pansani**  
**Martina Rossitto**  
**Anna Maria Sisto**  
**Vanessa Tuccio Guarna Assanti**  
**Gianluca Vrenna**  
**Venere**

**Anna Angelaccio**  
**Vittoria Cetra**  
**Maria Luisa De Santis**  
**Francesca Di Leva**  
**Teresa D'urbano**  
**Giulia Ferri**  
**Carmen Parlavecchio**  
**Silvia Tredici**  
**Ilaria Zullino**



**Gianluca Foglietta**  
U.O. Microbiologia e Diagnostica di Immunologia Ospedale Pediatrico Bambino Gesù



### **Delitti in materia di violazione del diritto d'autore (Art. 25-novies, D.Lgs. n. 231/2001) [articolo aggiunto dalla L. n. 99/2009]**

- Messa a disposizione del pubblico, in un sistema di reti telematiche, mediante connessioni di qualsiasi genere, di un'opera dell'ingegno protetta, o di parte di essa (art. 171, legge n.633/1941 comma 1 lett. a) bis)
- Reati di cui al punto precedente commessi su opere altrui non destinate alla pubblicazione qualora ne risulti offeso l'onore o la reputazione (art. 171, legge n.633/1941 comma 3)
- Abusiva duplicazione, per trarne profitto, di programmi per elaboratore; importazione, distribuzione, vendita o detenzione a scopo commerciale o imprenditoriale o concessione in locazione di programmi contenuti in supporti non contrassegnati dalla SIAE; predisposizione di mezzi per rimuovere o eludere i dispositivi di protezione di programmi per elaboratori (art. 171-bis legge n.633/1941 comma 1)
- Riproduzione, trasferimento su altro supporto, distribuzione, comunicazione, presentazione o dimostrazione in pubblico, del contenuto di una banca dati; estrazione o reimpiego della banca dati; distribuzione, vendita o concessione in locazione di banche di dati (art. 171-bis legge n.633/1941 comma 2)
- Abusiva duplicazione, riproduzione, trasmissione o diffusione in pubblico con qualsiasi procedimento, in tutto o in parte, di opere dell'ingegno destinate al circuito televisivo, cinematografico, della vendita o del noleggio di dischi, nastri o supporti analoghi o ogni altro supporto contenente fonogrammi o videogrammi di opere musicali, cinematografiche o audiovisive assimilate o sequenze di immagini in movimento; opere letterarie, drammatiche, scientifiche o didattiche, musicali o drammatico musicali, multimediali, anche se inserite in opere collettive o composite o banche dati; riproduzione, duplicazione, trasmissione o diffusione abusiva, vendita o commercio, cessione a qualsiasi titolo o importazione abusiva di oltre cinquanta copie o esemplari di opere tutelate dal diritto d'autore e da diritti connessi; immissione in un sistema di reti telematiche, mediante connessioni di qualsiasi genere, di un'opera dell'ingegno protetta dal diritto d'autore, o parte di essa (art. 171-ter legge n.633/1941)
- Mancata comunicazione alla SIAE dei dati di identificazione dei supporti non soggetti al contrassegno o falsa dichiarazione (art. 171-septies legge n.633/1941)
- Fraudolenta produzione, vendita, importazione, promozione, installazione, modifica, utilizzo per uso pubblico e privato di apparati o parti di apparati atti alla decodificazione di trasmissioni audiovisive ad accesso condizionato effettuate via etere, via satellite, via cavo, in forma sia analogica sia digitale (art. 171-octies legge n.633/1941).

**[Torna all'inizio](#)**