CASO

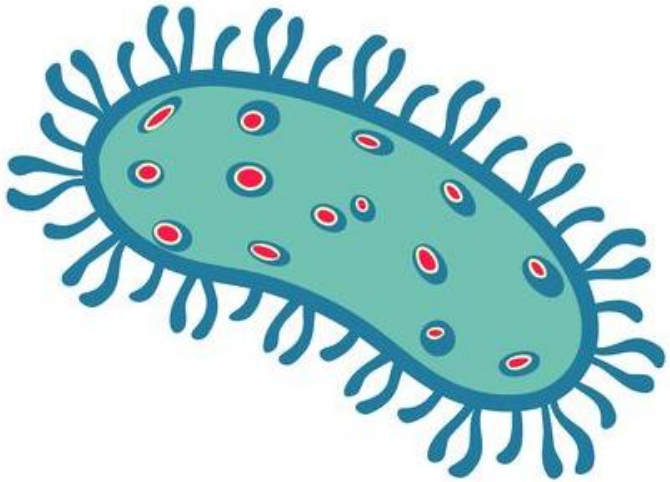
ACINETOBACTER? EPPUR SI MUOVE!

Dott.ssa Cesira Giordano – *SD Microbiologia Batteriologica*
Dott. Alessandro Leonildi – *SD Microbiologia Batteriologica*
Dott.ssa Giulia Gemignani – *Coordinatore Team AID*

Azienda Ospedaliero Universitaria Pisana

CASO:

ACINETOBACTER? EPPUR SI MUOVE!



Sommario

- *Acinetobacter*: chi è?
- Epidemiologia isolamenti *A. baumannii* in AOUP
- Il «caso pisano»
- Indagine fenotipica
- Indagine molecolare
- Conclusioni

Acinetobacter baumannii

• *Acinetobacter* è un genere di batteri ubiquitari Gram negativi, aerobi, asporigeni, catalasi positivi ed ossidasi negativi.

• Immobile 

- ✓Motilità per contrazione "*twitching*" (pili di tipo IV) → biofilm
- ✓Motilità associata alla superficie "*swarming*" (molecole polimeriche extracellulari)

• E' considerato uno dei più temibili patogeni opportunisti nosocomiali*

- ✓Persistenza e adattamento ambientale
- ✓ESKAPE
- ✓Antibiotico resistenza

• Tasso di mortalità

- ✓Polmoniti associate a ventilazione: 40-70%[1]
- ✓Infezioni del torrente circolatorio 28-58% [2,3]

•European Centre for Disease Prevention and Control (ECDC), Infectious Diseases Society of America (IDSA), WHO and Center for Disease Control and Prevention of America (CDC)

•[1]Garnacho-Montero J, Ortiz-Leyba C, Fernández-Hinojosa E, Aldabó-Pallás T, Cayuela A, Marquez-Vácaro JA, Garcia-Curiel A, Jiménez-Jiménez FJ. *Acinetobacter baumannii* ventilator-associated pneumonia: epidemiological and clinical findings. *Intensive Care Med.* 2005;31(5):649–655.

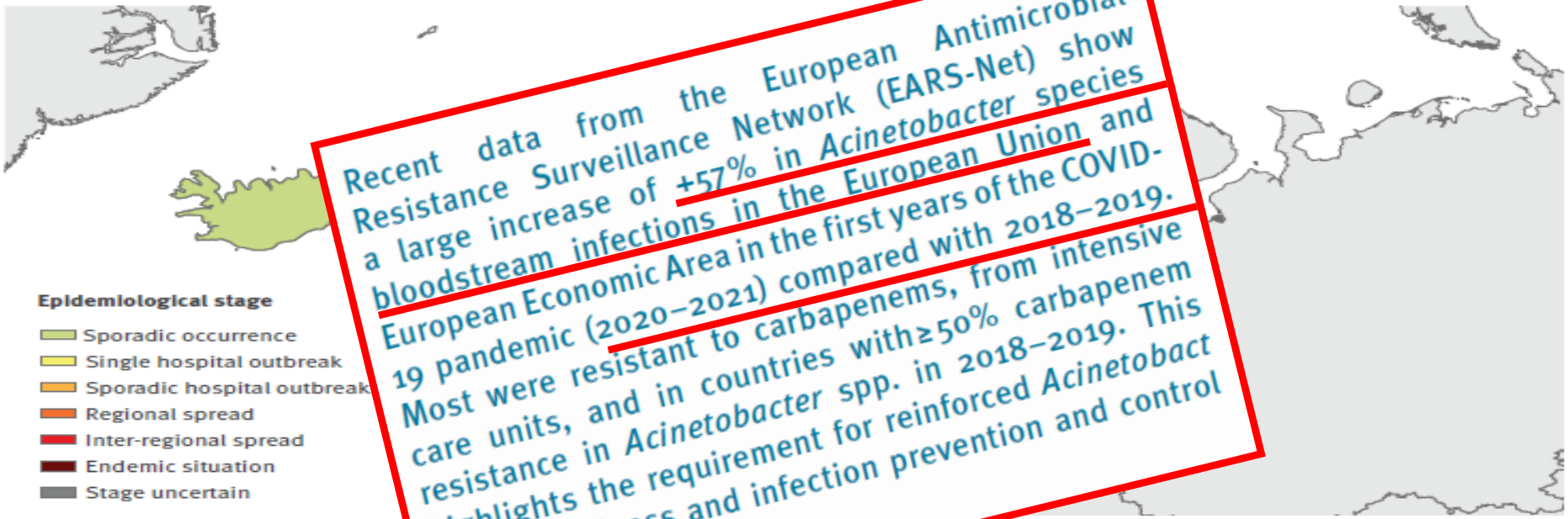
[2]Chopra T, Marchaim D, Awali RA, Krishna A, Johnson P, Tansek R, Chaudary K, Lephart P, Slim J, Hothi J, Ahmed H, Pogue JM, Zhao JJ, Kaye KS. Epidemiology of bloodstream infections caused by *Acinetobacter baumannii* and impact of drug resistance to both carbapenems and ampicillin-sulbactam on clinical outcomes. *Antimicrob Agents Chemother.* 2013;57(12):6270–6275.

[3]Yuan P-B, Dai L-T, Zhang Q-K, Zhong Y-X, Liu W-T, Yang L, Chen D-Q. Global emergence of double and multi-carbapenemase producing organisms: epidemiology, clinical significance, and evolutionary benefits on antimicrobial resistance and virulence. Microbiol Spectr. 2024 Jun 11:e0000824.

ECDC – Cosa ci dice di A. baumannii?

FIGURE

Epidemiological situation of carbapenem-resistant Acinetobacter baumannii, assessment by national experts in European countries, 2019 (n = 37)

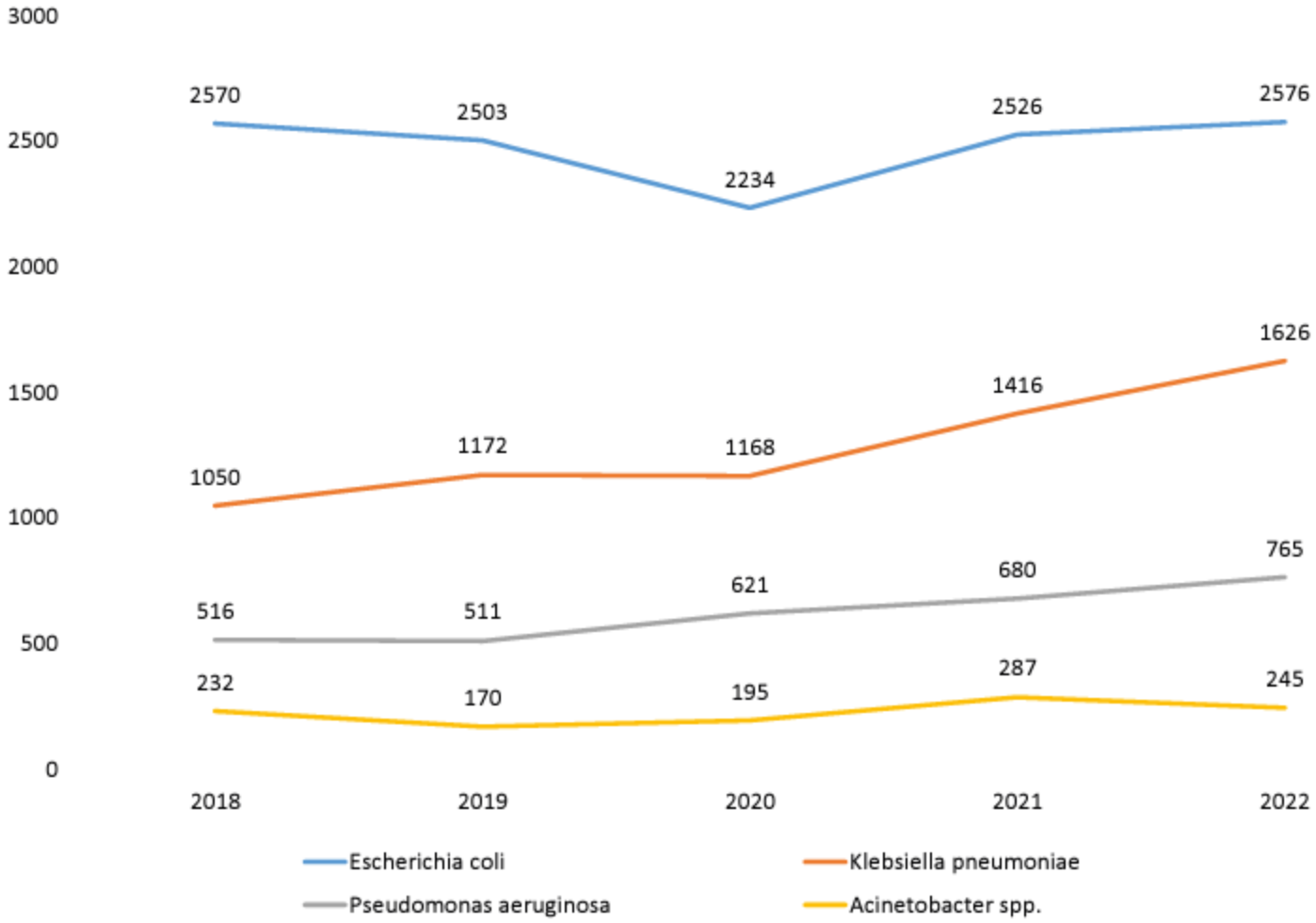


Recent data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) show a large increase of +57% in *Acinetobacter* species bloodstream infections in the European Union and European Economic Area in the first years of the COVID-19 pandemic (2020–2021) compared with 2018–2019. Most were resistant to carbapenems, from intensive care units, and in countries with ≥ 50% carbapenem resistance in *Acinetobacter* spp. in 2018–2019. This highlights the requirement for reinforced *Acinetobacter* preparedness and infection prevention and control in Europe.

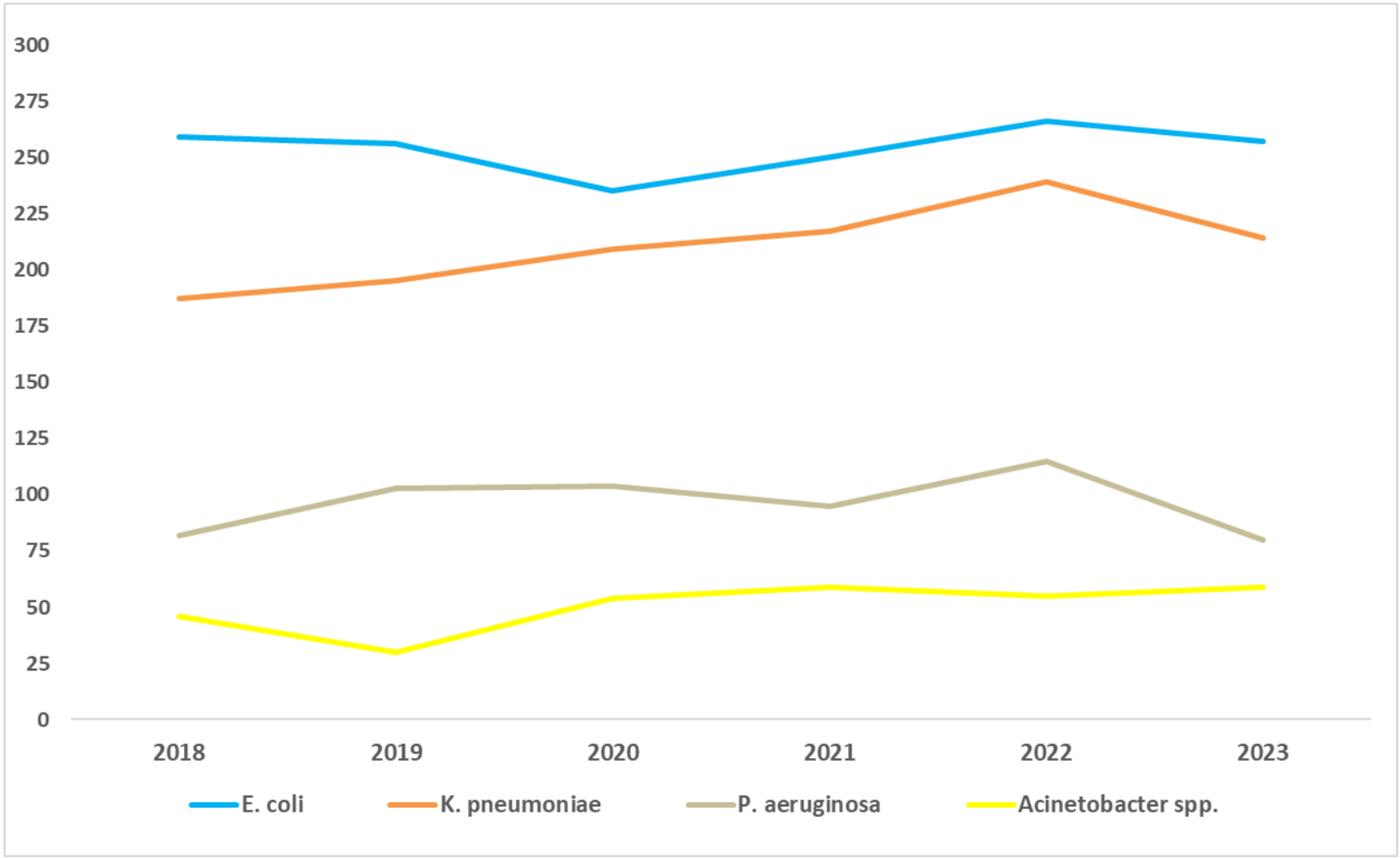
Increase in bloodstream infections with carbapenem-resistant Acinetobacter species during the first 2 years of the COVID-19 pandemic, EU/EEA, 2020 and 2021

Nov 2022 | Eurosurveillance | 27

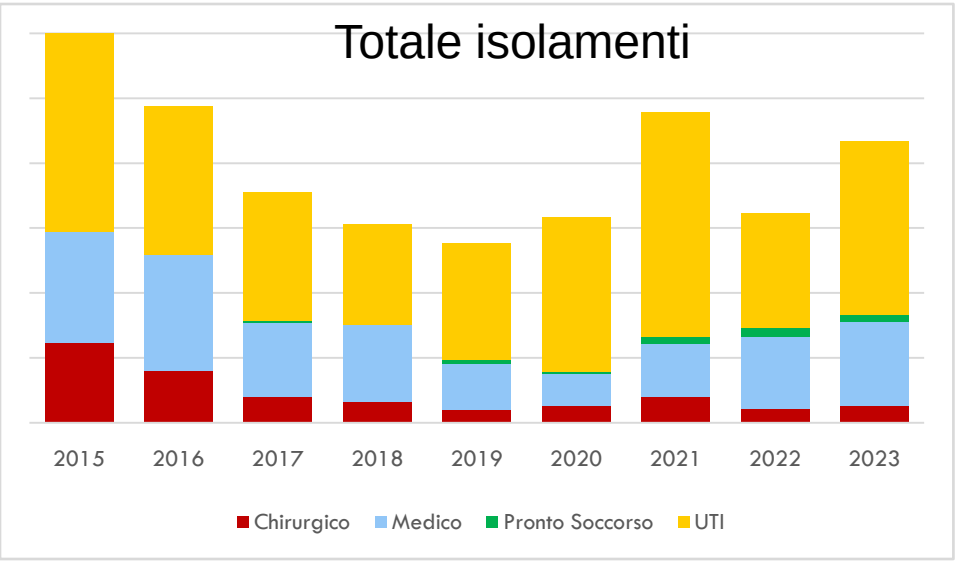
Toscana 2018-2022 - Emocolture
Andamento degli isolati Gram negativi

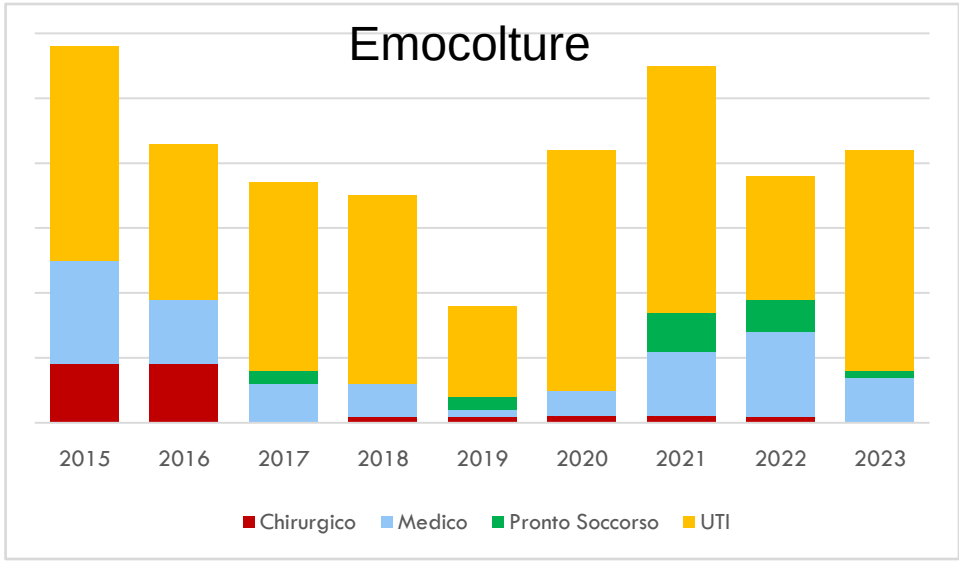


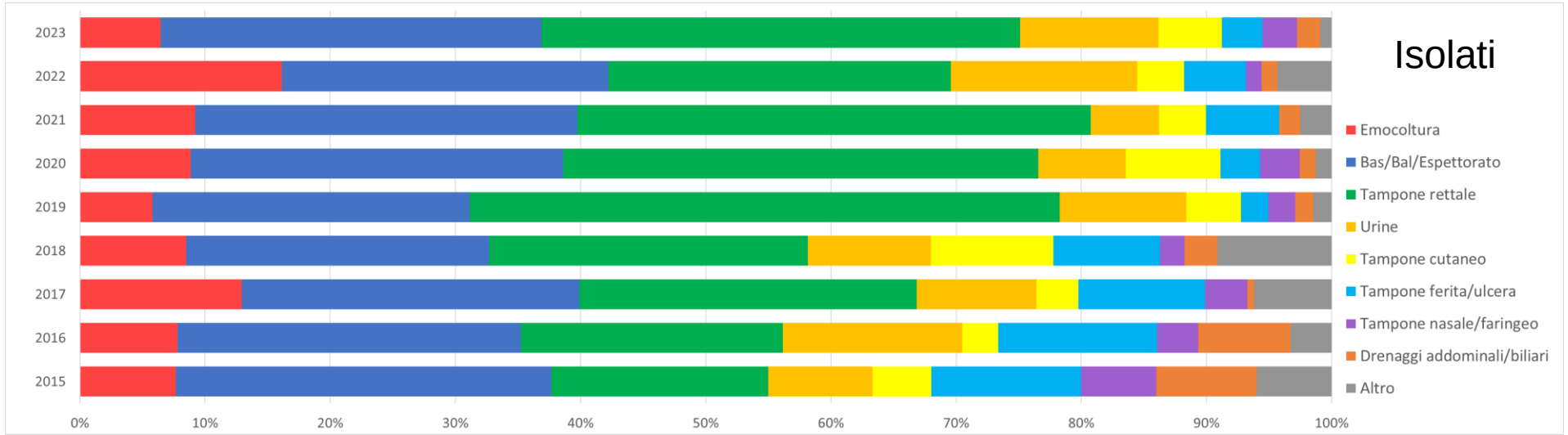
AOUP 2018-2023 - Emocolture
Andamento degli isolati Gram negativi



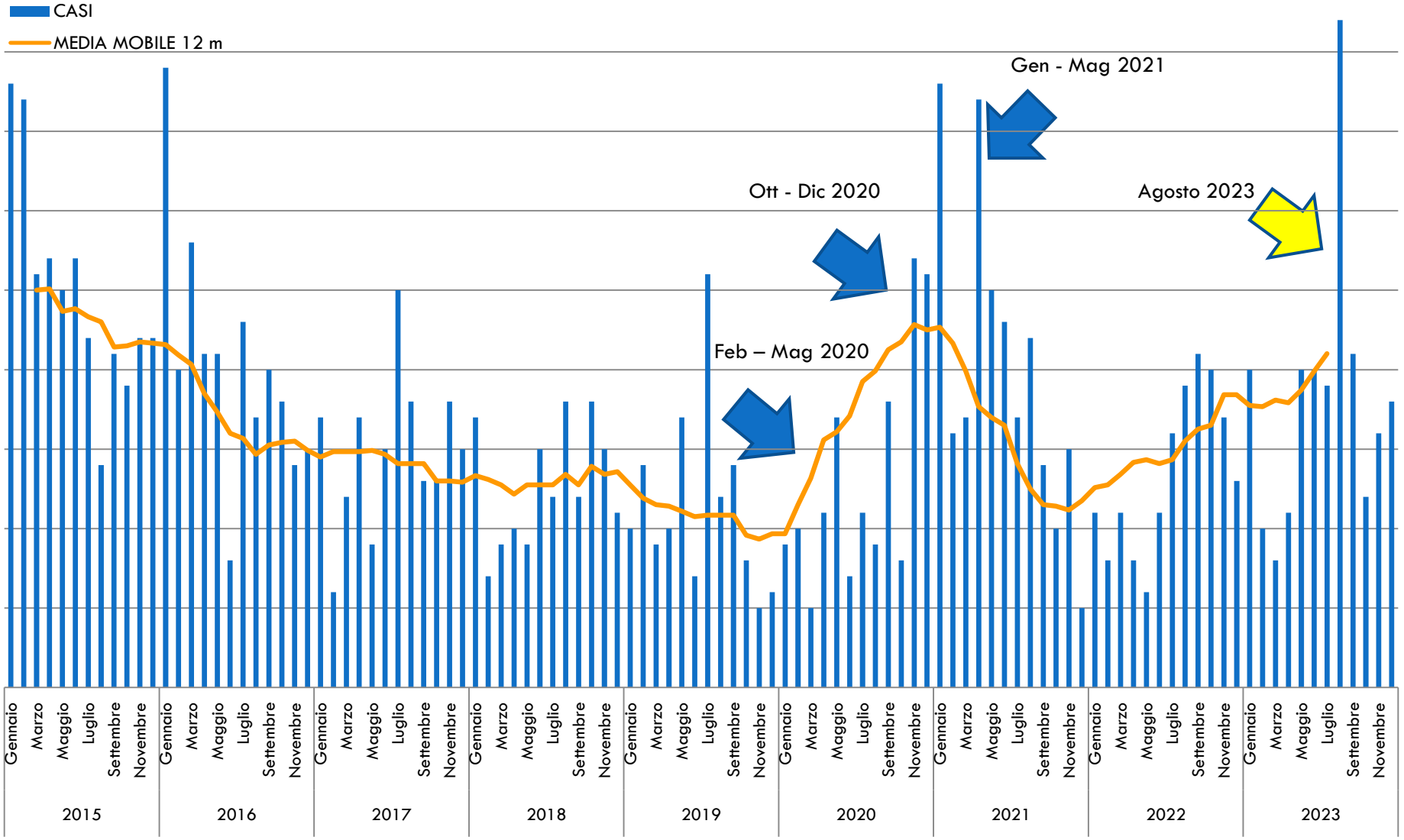
AOUP 2015-2023 – *A. baumannii* Andamento degli isolati







AOUP 2015-2023 – *A. baumannii*
Andamento degli isolati



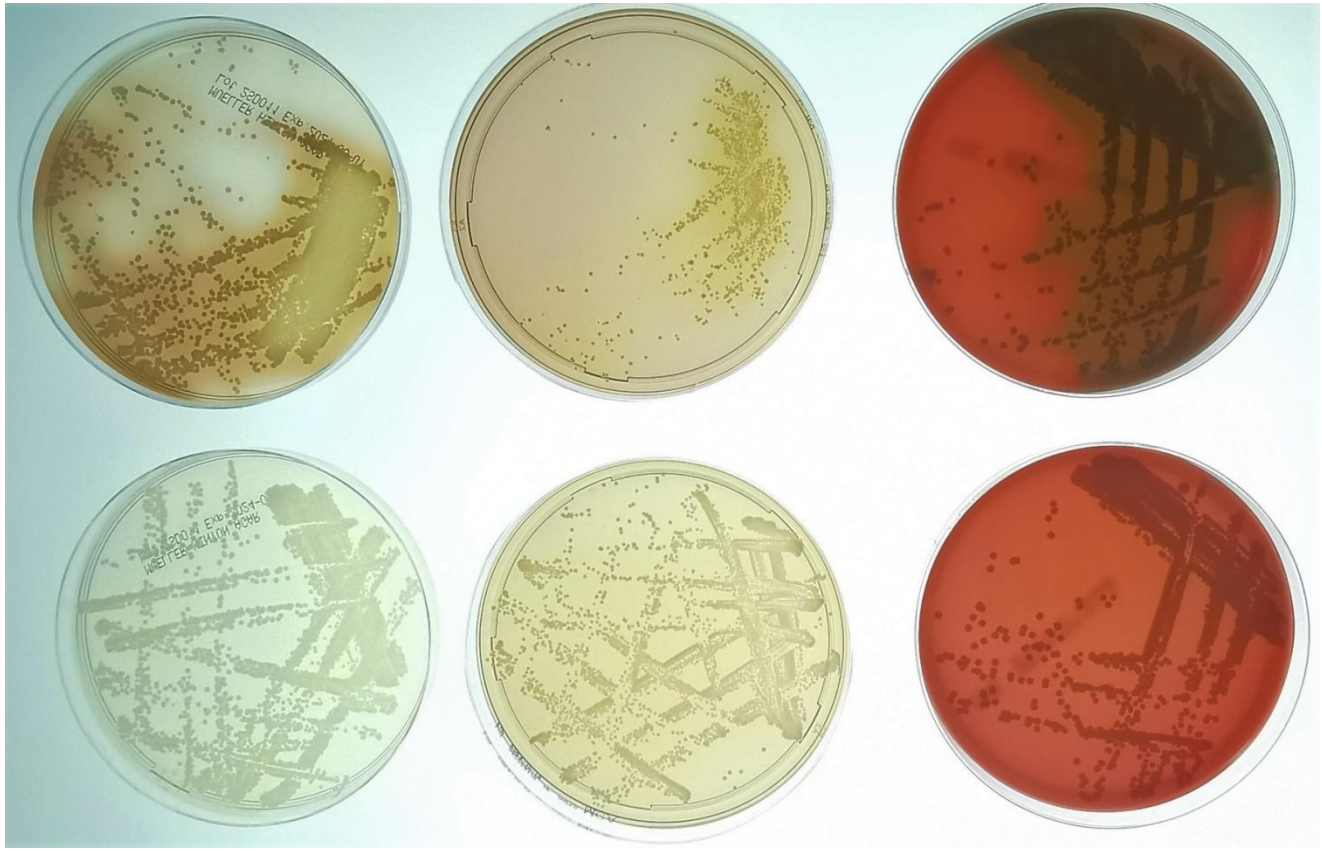
Screening per resistenza a cefiderocol in *A. baumannii*

Fenotipo pigmentato e non pigmentato in *A. baumannii*

Fenotipo pigmentato e non pigmentato in *A. baumannii*

PIGMENTATO

NON PIGMENTATO



Mueller-Hinton | Mc Conkey | Columbia sangue

JMM Correspondence

Pyomelanin production: a rare phenotype in *Acinetobacter baumannii*

Talita Coelho-Souza,[1] Natacha Martins,[1] Fernanda Maia,[2] Susana Frases,[3] Raquel Regina Bonelli,[1] Lee W. Riley[4] and Beatriz Meurer Moreira[1]

DOI 10.1099/jmm.0.064089-0

ORIGINAL ARTICLE

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *115*: e200371, 2020

Pyomelanin biosynthetic pathway in pigment-producer strains from the pandemic *Acinetobacter baumannii* IC-5

Érica Fonseca[1]/+, Fernanda Freitas[1], Raquel Caldart[2], Sérgio Morgado[1], Ana Carolina Vicente[1]

[1]Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Genética Molecular de Microrganismos, Rio de Janeiro, RJ, Brasil
[2]Universidade Federal de Roraima, Boa Vista, RR, Brasil

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE

Check for updates

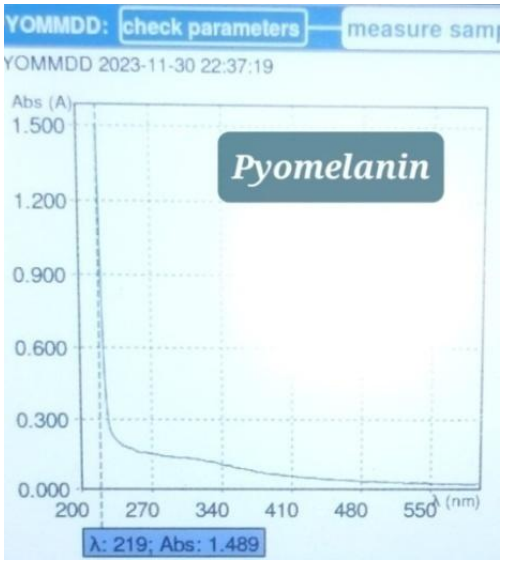
Description of a Rare Pyomelanin-Producing Carbapenem-Resistant *Acinetobacter baumannii* Strain Coharboring Chromosomal OXA-23 and NDM-1

Feng Zhao,[a,b] Haiyang Liu,[c,d,e] Yue Yao,[c,d,e] Linghong Zhang,[c,d,e] Zhihui Zhou,[c,d,e] Sebastian Leptihn,[c,f,g] Yunsong Yu,[c,d,e] Xiaoting Hua,[c,d,e] Ying Fu[a,b]

Estrazione del pigmento

- Escissione di agar pigmentato
- Precipitazione in HCl 6 M
- Risospensione in NaOH 0.5 M

- Scansione spettrofotometrica a 200-600 nm



Original Research Article https://doi.org/10.20546/ijcmas.2020.906.275

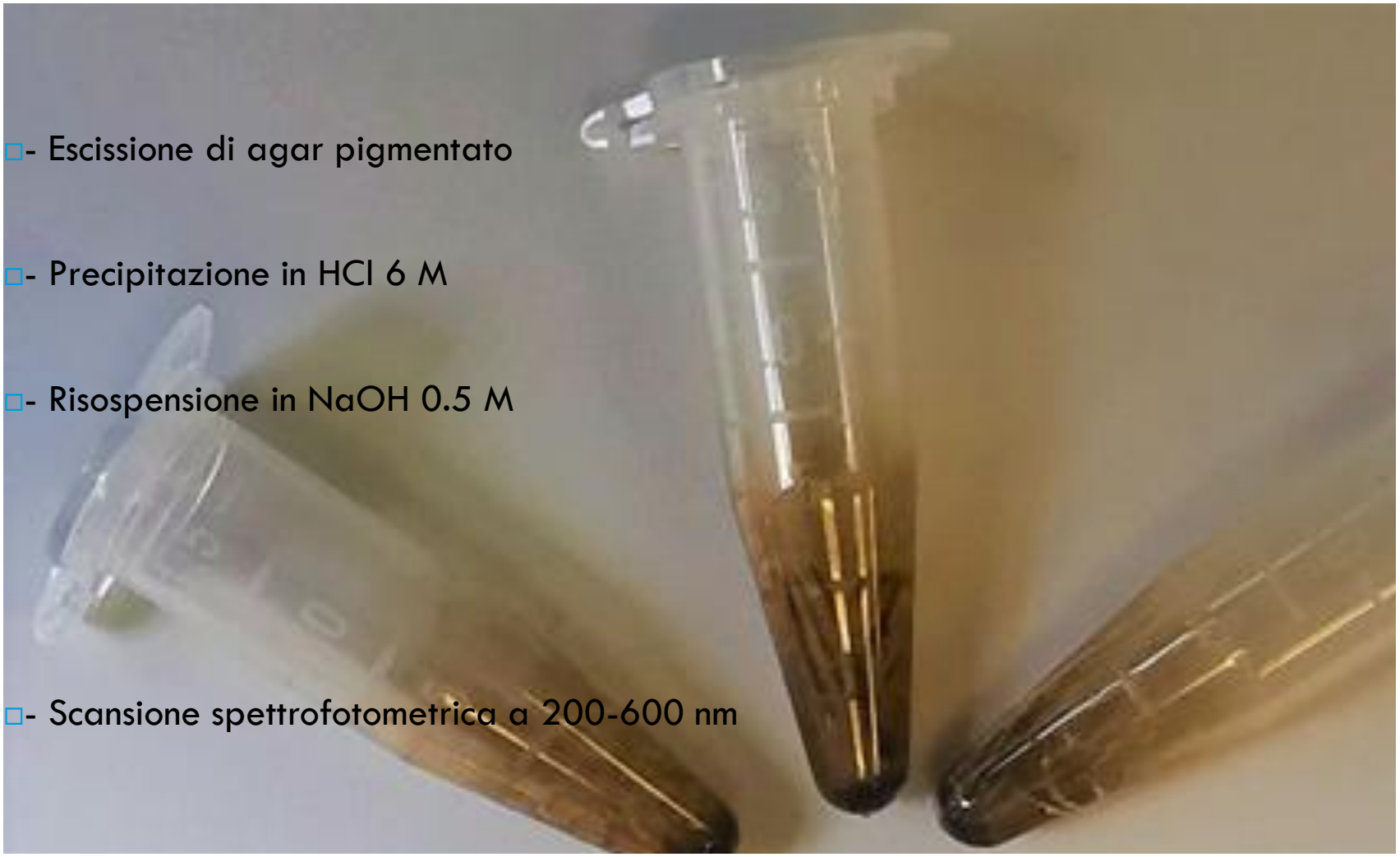
Pyomelanin Production From a Marine Isolate of *Acinetobacter Spp.*

Jean Loi[1], Tan Shi Yi[1], Attapol Pinsa[1], Sakinah Mulyana[1], Lloyd George Singaretnam[2], Meliana Riwanto[1] and Jason Chang[1]*

[1]*School of Applied Science, Temasek Polytechnic, 21 Tampines Avenue 1, Singapore 529757*
[2]*Present address: School of Applied Science, Republic Polytechnic, 9 Woodlands Avenue 9, Singapore 738964*

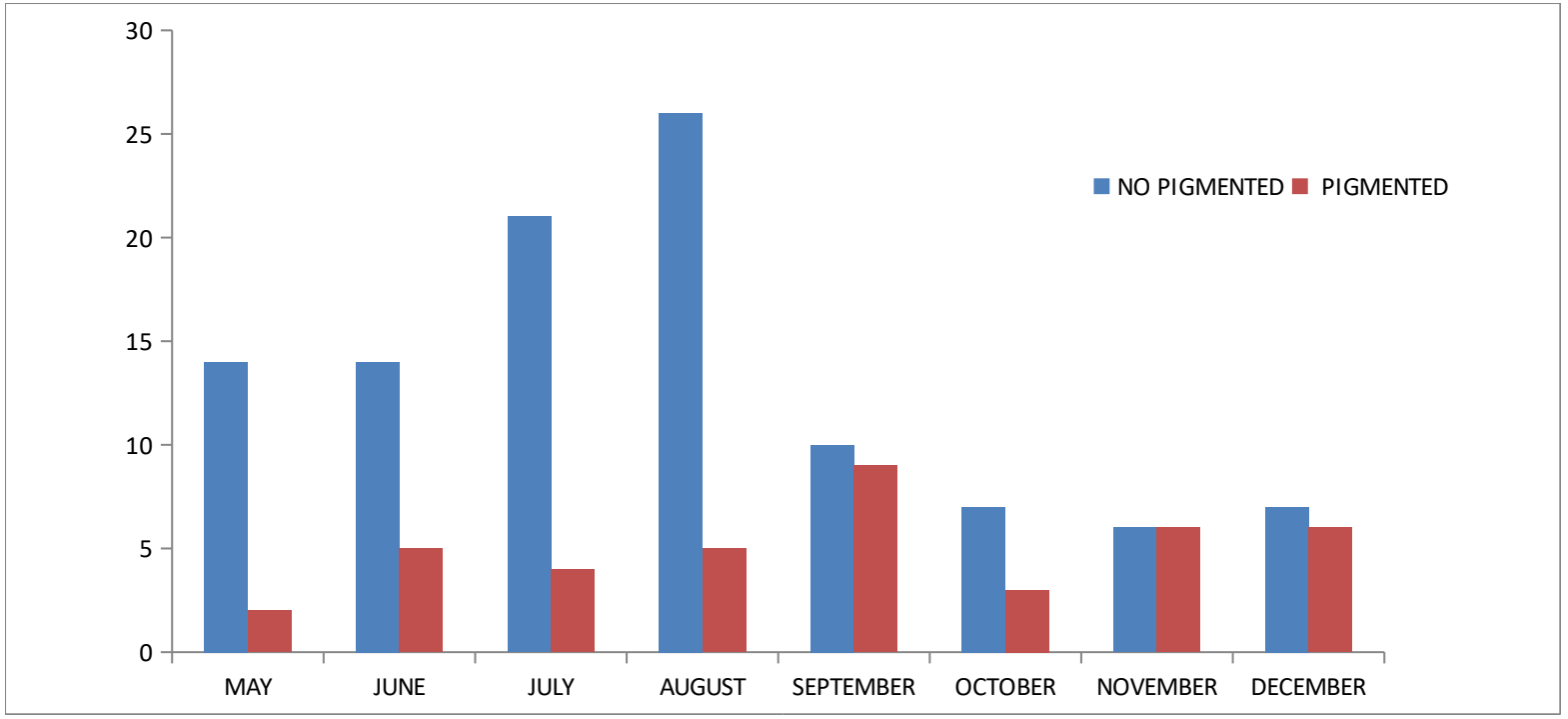
**Corresponding author*

Estrazione del pigmento

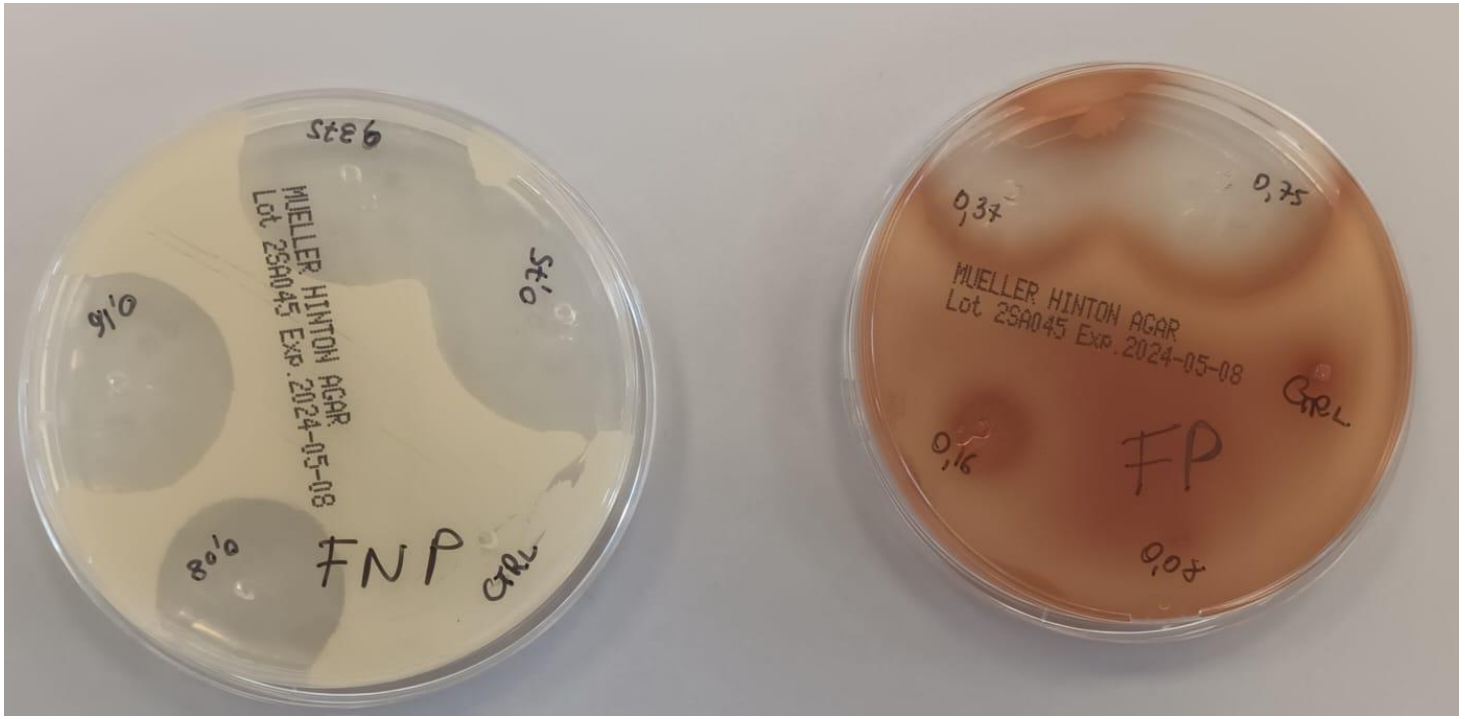


- Escissione di agar pigmentato
- Precipitazione in HCl 6 M
- Risospensione in NaOH 0.5 M
- Scansione spettrofotometrica a 200-600 nm

Isolamento nel periodo di studio maggio-dicembre 2023

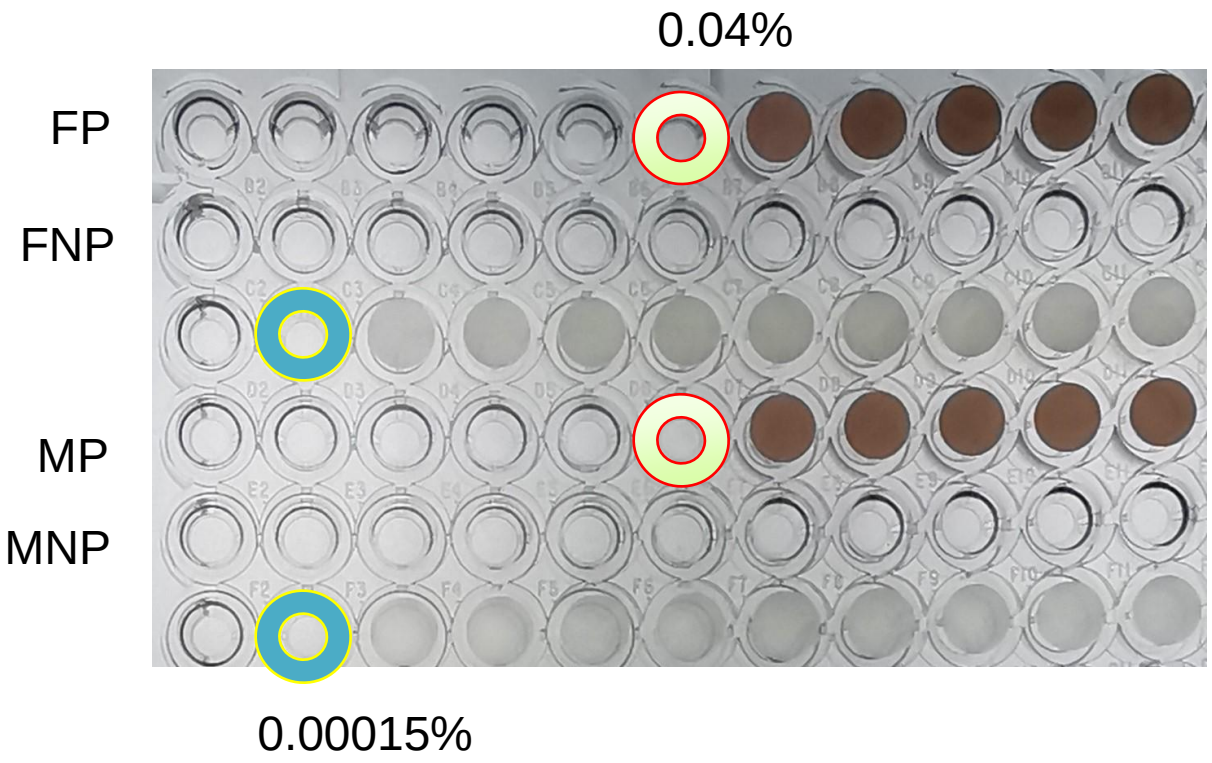


Test di **agar-diffusione** di perossido di idrogeno



	HYDROGEN PEROXIDE %				SALINE	
	0,75%	0,37%	0,16%	0,08%	CTRL	inoculum
MP	34	24	10	0	0	1,3x10E5
MNP	40	32	30	24	0	4,6x10E5
FP	36	25	10	0	0	1,1x10E5
FNP	36	32	30	24	0	1,3x10E5

Test di **brodo-diluizione** di perossido di idrogeno



PIGMENTI MICROBICI: PROPRIETA'

- ANTIMICROBICHE
- ANTIOSSIDANTI
- ANTIVIRALI
- ANTITUMORALI
- ANTIPARASSITICHE
- UV RESISTENZA
- ROS RESISTENZA
- IMMUNOMODULATORIE
- SIDEROFORI
- EPATOPROTETTIVE

TAVOLOZZA DEI PIGMENTI MICROBICI

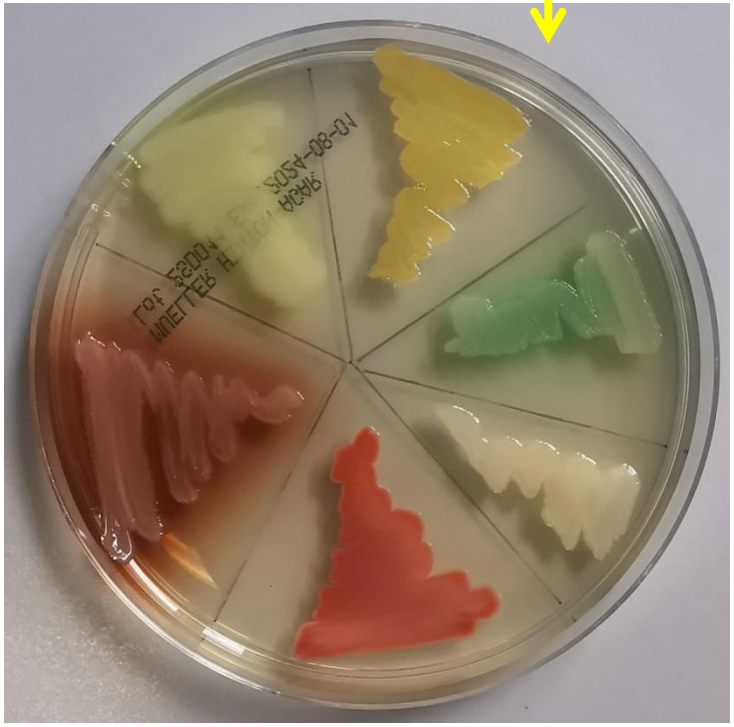
NO PIGMENT

TAVOLOZZA DEI PIGMENTI MICROBICI

PYOCIANIN

NO PIGMENT

TAVOLOZZA DEI PIGMENTI MICROBICI

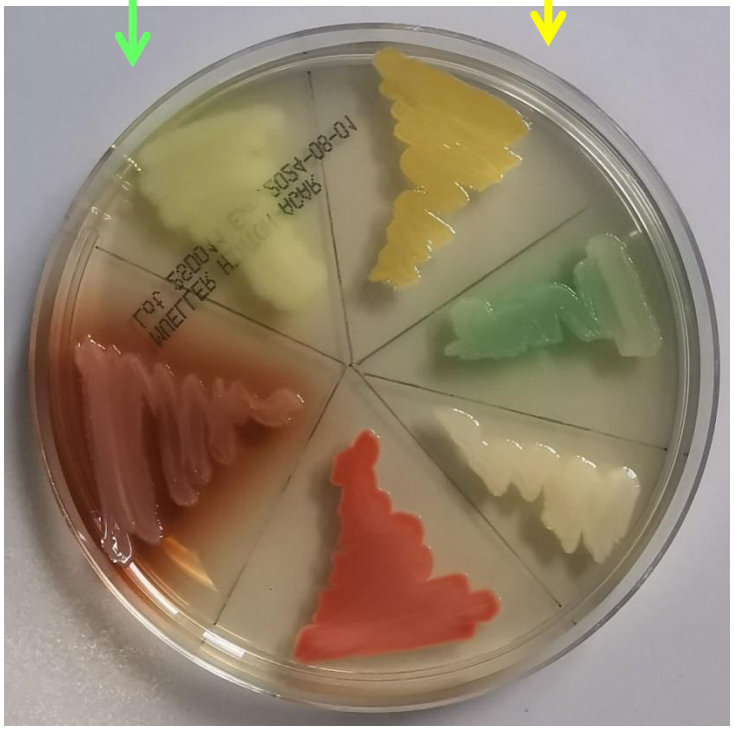


FLEXIRUBIN

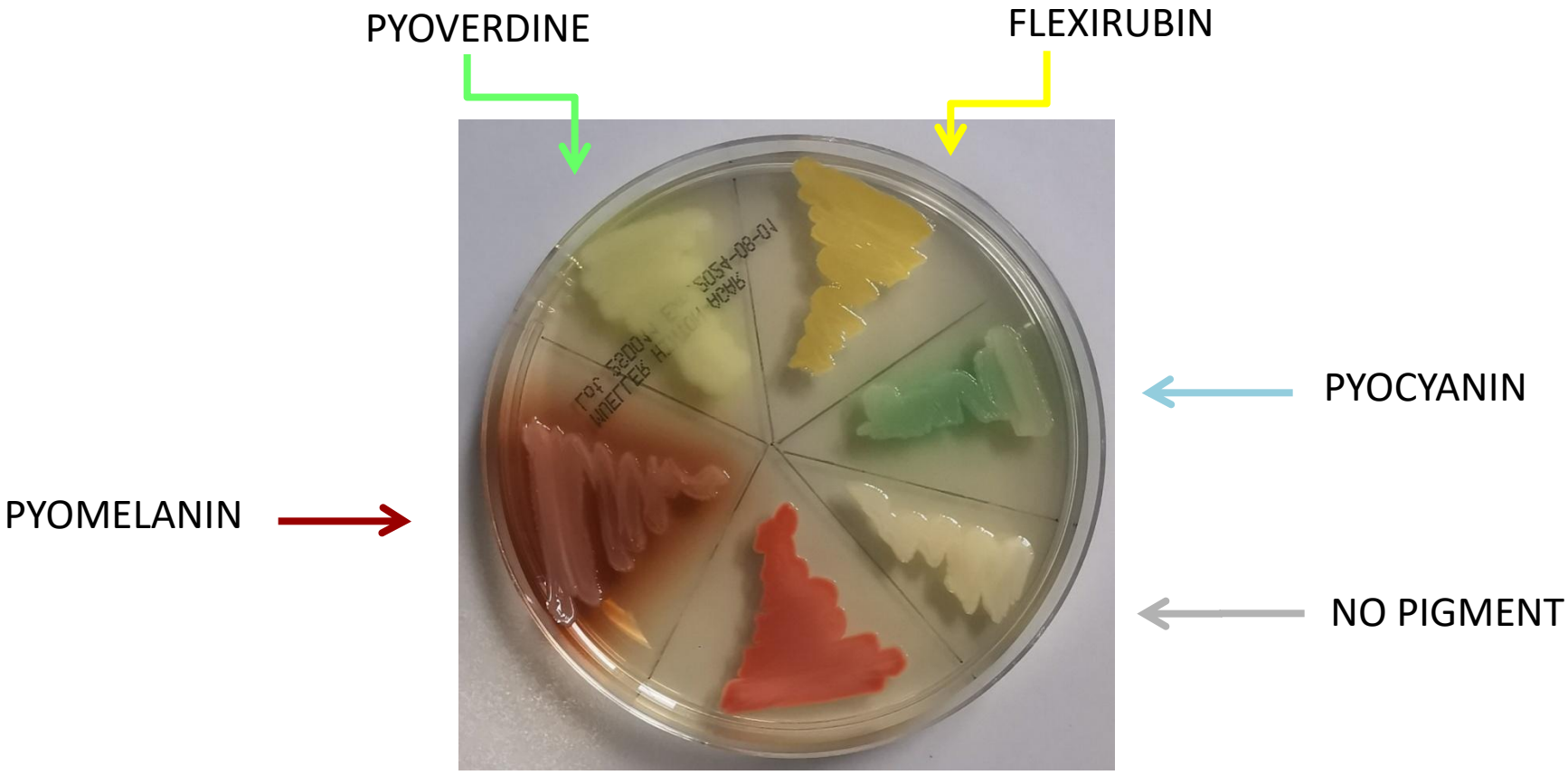
PYOCIANIN

NO PIGMENT

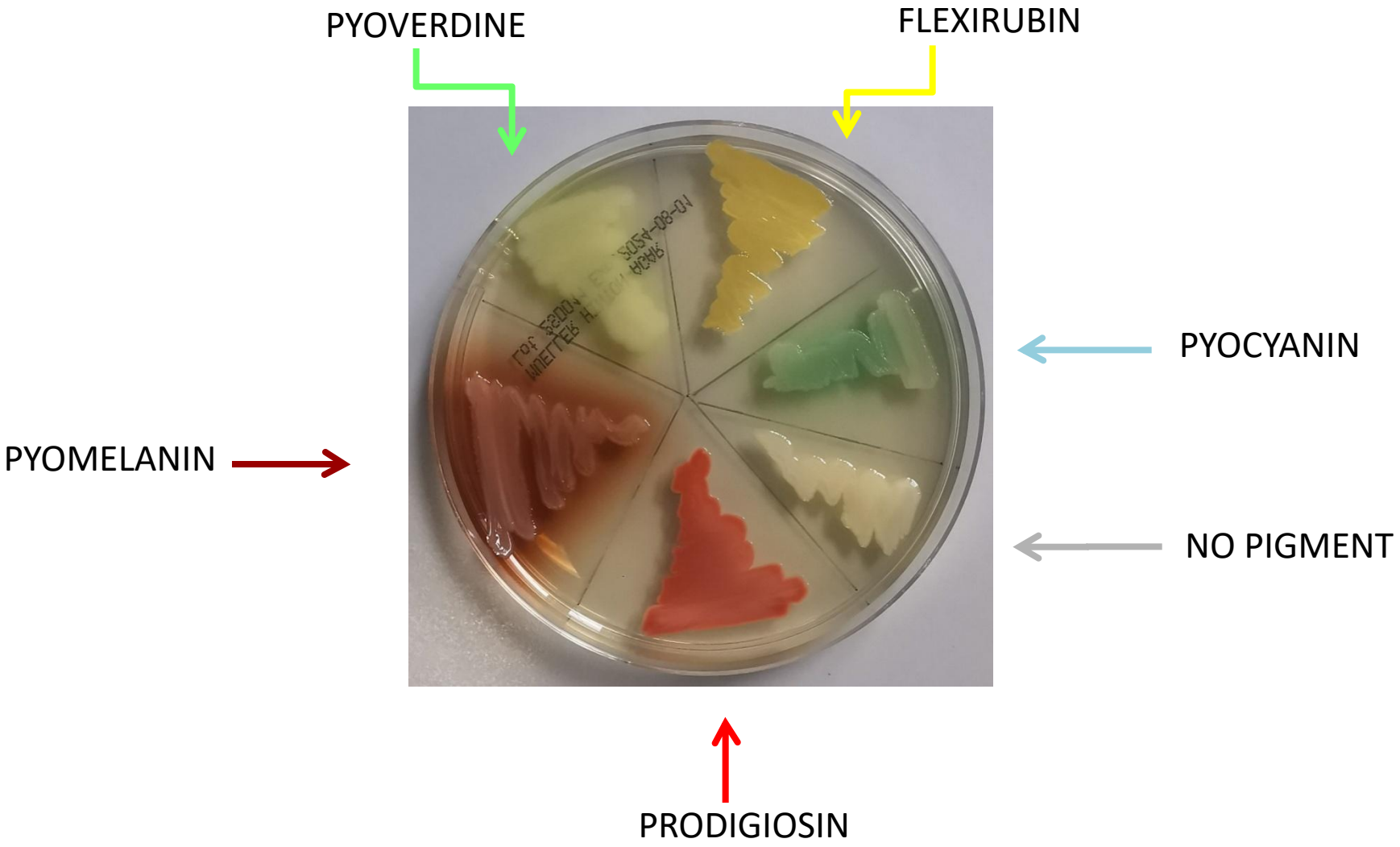
TAVOLOZZA DEI PIGMENTI MICROBICI



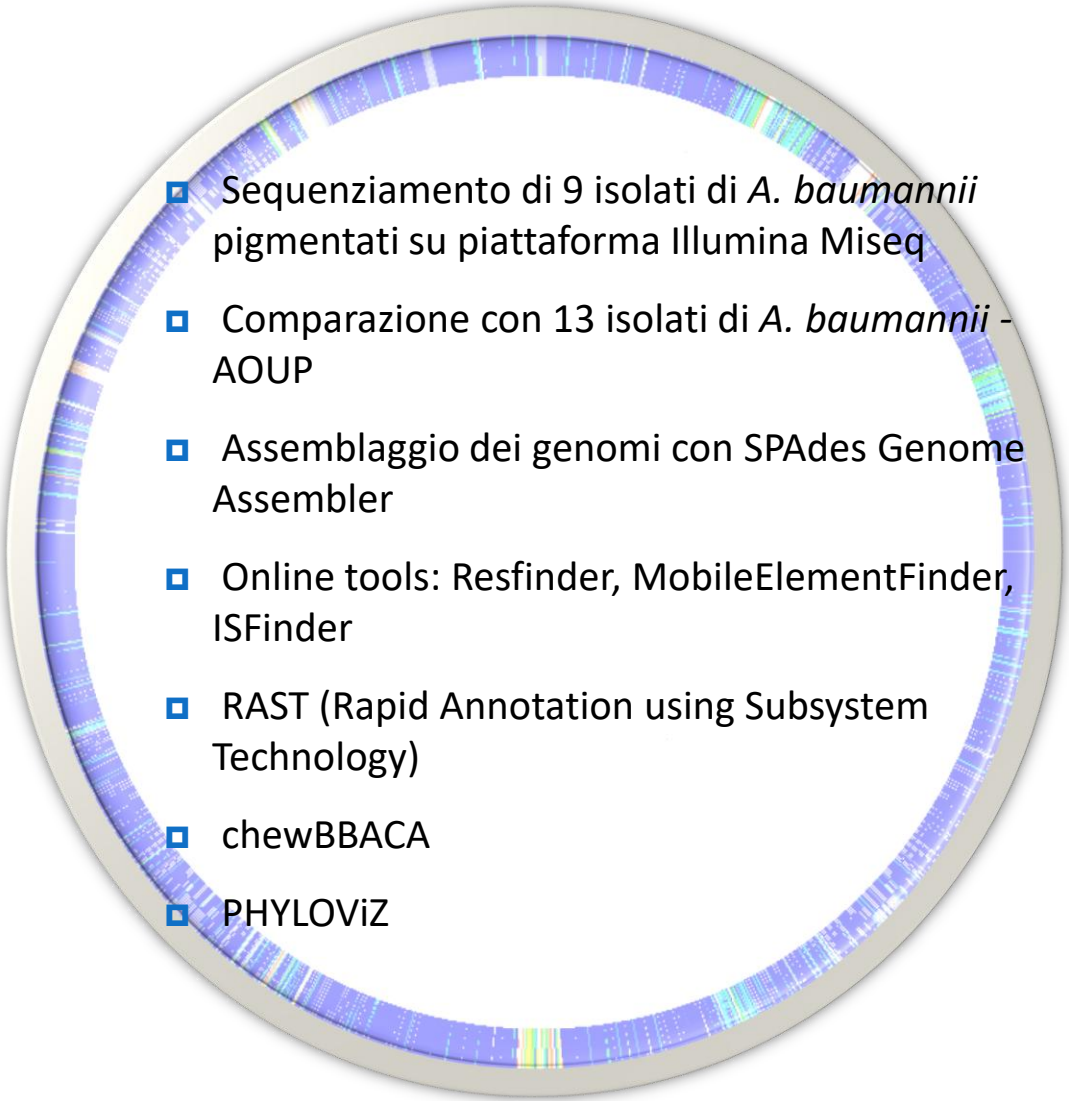
TAVOLOZZA DEI PIGMENTI MICROBICI



TAVOLOZZA DEI PIGMENTI MICROBICI



Indagine molecolare



- Sequenziamento di 9 isolati di *A. baumannii* pigmentati su piattaforma Illumina Miseq
- Comparazione con 13 isolati di *A. baumannii* - AOUP
- Assemblaggio dei genomi con SPAdes Genome Assembler
- Online tools: Resfinder, MobileElementFinder, ISFinder
- RAST (Rapid Annotation using Subsystem Technology)
- chewBBACA
- PHYLOViZ

Caratteristiche degli isolati di *A. baumannii* in AOUP

- **13 ceppi non produttori di piomelanina**

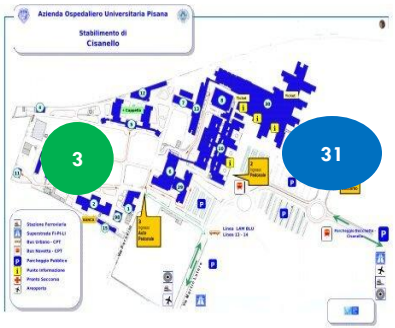
  - Resistenti a beta-lattamici (OXA -72, OXA-66, TEM-1D, and ADC-25)
  - Resistenti ad amminoglicosidi (aph(6)-Id, aph(3'')-Ib, armA)
  - Resistenti a cefiderocol (piuA K384fs, fepA P635-ISAba125; PBP3 K235N)*
  - Suscettibili a colistina
  - $ST2_{Pas}$; $ST1839/ST281_{Oxf}$
  - Pili type: I/IV
  - Porine: OmpA, OprD, OprB, Aquaporin Z

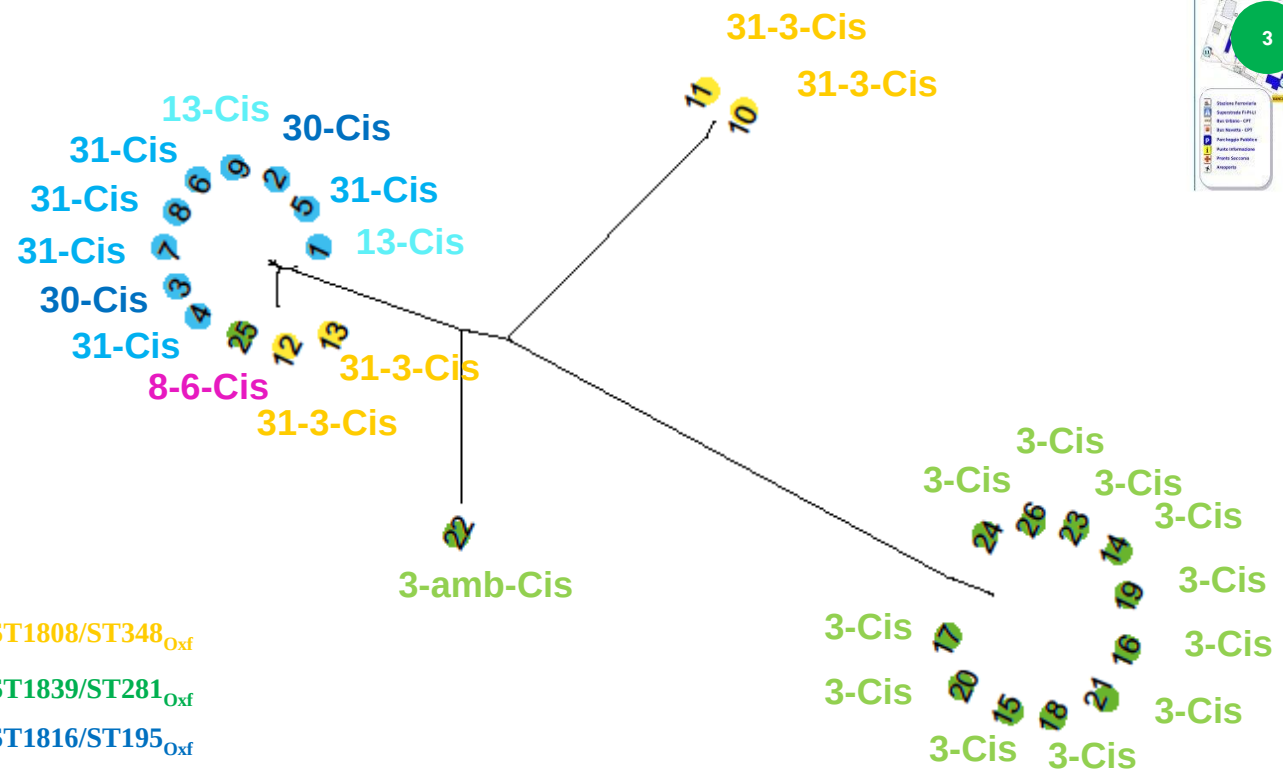
- **9 ceppi produttori di piomelanina**

  - Resistenti a beta-lattamici (OXA-23, OXA-66, TEM-1D, and ADC-25)
  - Resistenti ad amminoglicosidi (aph(6)-Id, aph(3')-Ia, aph(3'')-Ib, armA)
  - Suscettibili a colistina e cefiderocol
  - ST2Pas; ST1816/ST195Oxf
  - Pili type: I/II/IV
  - Porine: OprB, Aquaporin Z

*C.Giordano, S.Barnini. Glycine restores the sensitivity to antibiotics in multidrug resistant bacteria. 2024. Microbiology Spectrum. 18/06/24 https://journals.asm.org/doi/10.1128/spectrum.00164-24.

Outbreaks di *A. baumannii* in AOUP





2020 $ST2_{Pas}$; $ST1808/ST348_{Oxf}$

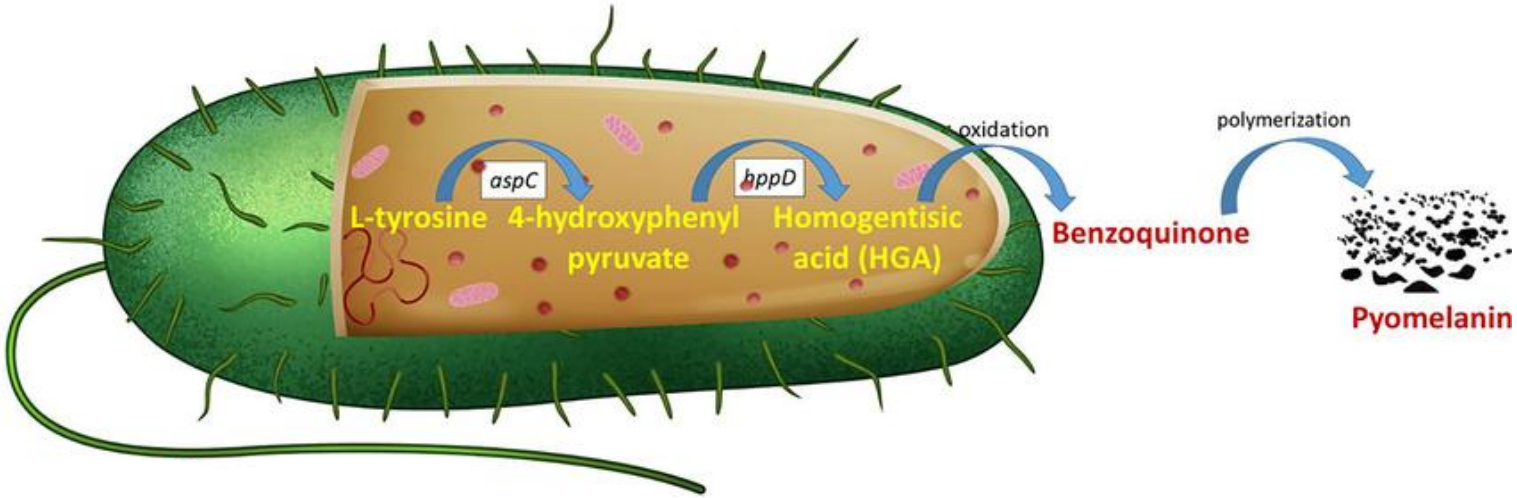
2022 $ST2_{Pas}$; $ST1839/ST281_{Oxf}$

2023 $ST2_{Pas}$; $ST1816/ST195_{Oxf}$

50

The chewBBACA software was run in Conda environment for creation, evaluation and use of core genome (cg) multilocus sequence typing (MLST) schemas, based on an *ad hoc* structure including 2390 alleles (Silva). ChewTree tool was used to calculate a phylogenetic tree from chewBBACA alleles on the web tool ARIES (Advanced Research Infrastructure for Experimentation in Genomics - Galaxy Instance at ISS) (Knijn). Tree was

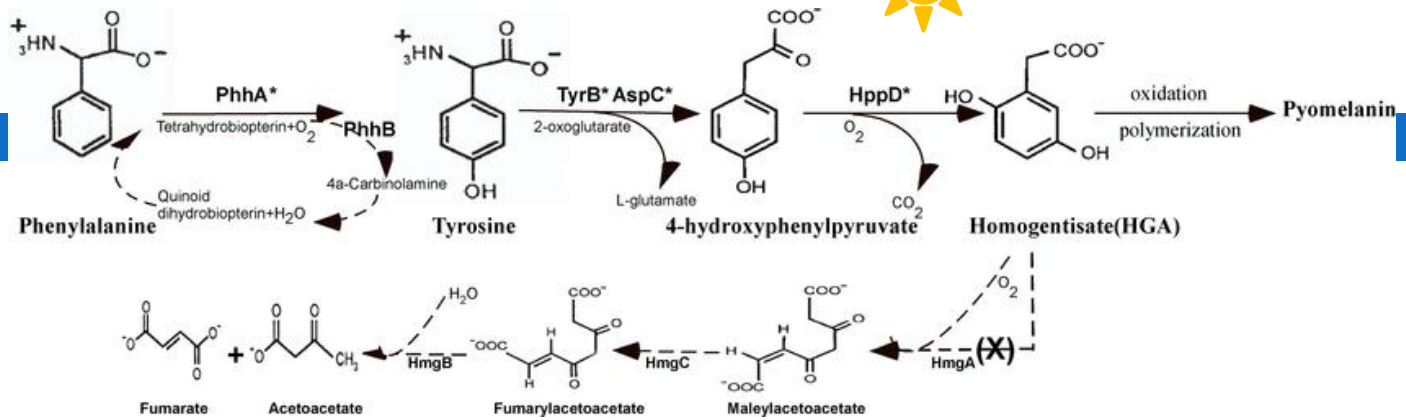
Pathway biochimico della produzione di piomelanina

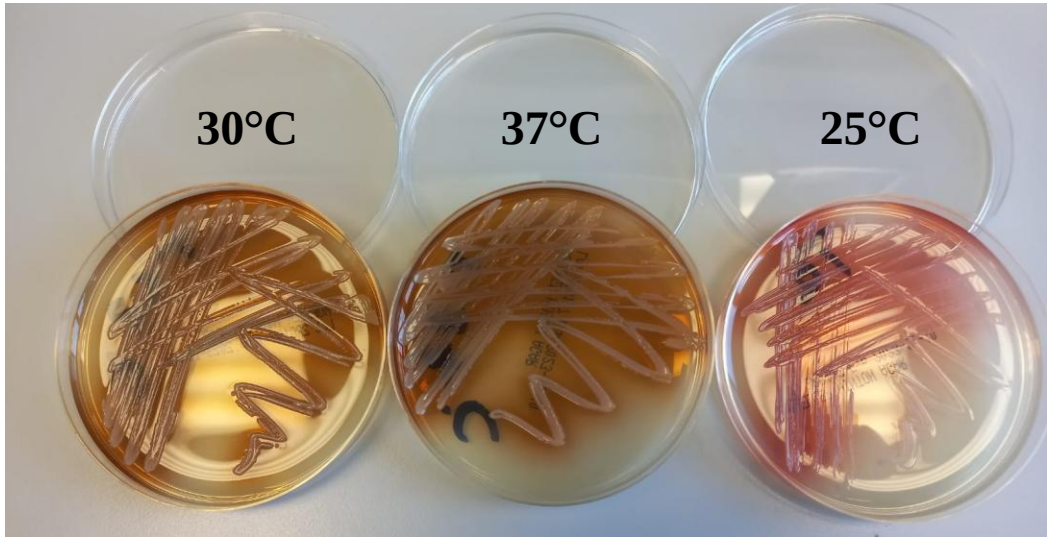


Arias-Barrau E, Olivera ER, Luengo JM, Fernández C, Galán B, García JL, Díaz E, Miñambres B. The homogentisate pathway: a central catabolic pathway involved in the degradation of L-phenylalanine, L-tyrosine, and 3-hydroxyphenylacetate in Pseudomonas putida. J Bacteriol. 2004 Aug;186(15):5062-77.

*4-hydroxyphenylpyruvate dioxygenase

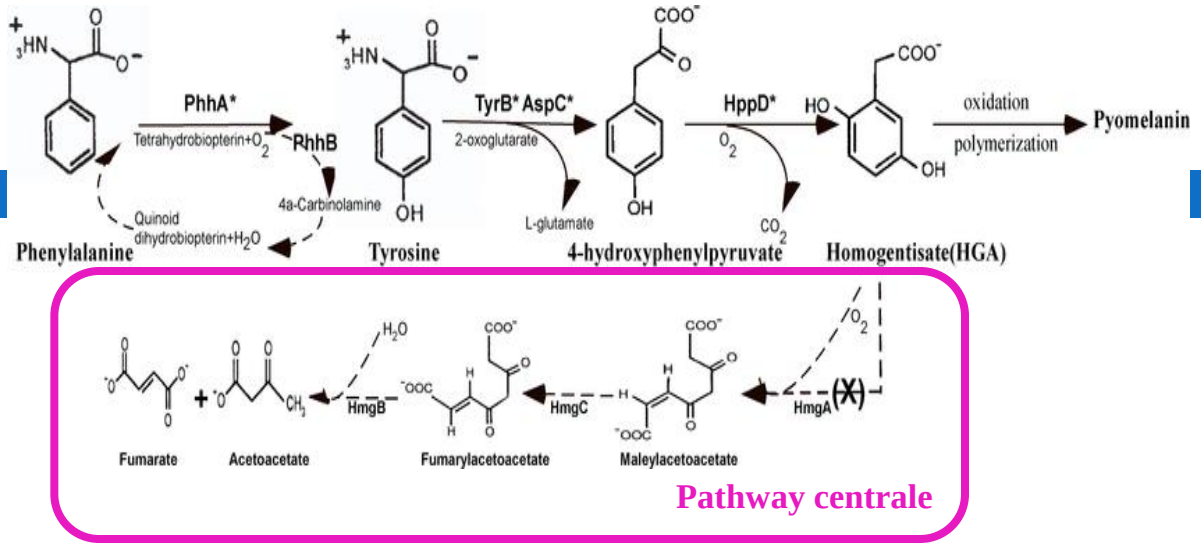
DOI:10.1371/journal.pone.0120923





Fonseca É, Freitas F, Caldart R, Morgado S, Vicente AC. Pyomelanin biosynthetic pathway in pigment-producer strains from the pandemic Acinetobacter baumannii IC-5. Mem Inst Oswaldo Cruz. 2020 Nov 6;115:e200371.

Singh D, Kumar J, Kumar A. Isolation of pyomelanin from bacteria and evidences showing its synthesis by 4-hydroxyphenylpyruvate dioxygenase enzyme encoded by hppD gene. Int J Biol Macromol. 2018 Nov;119:864-873.



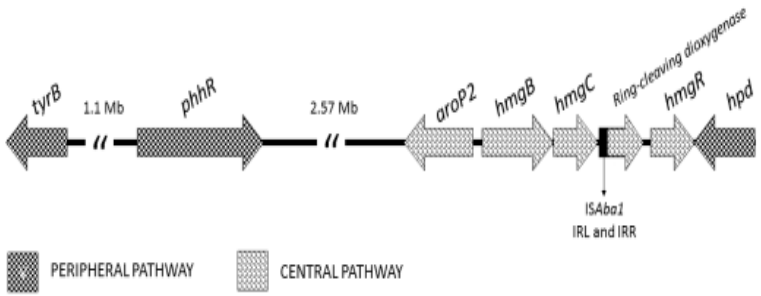


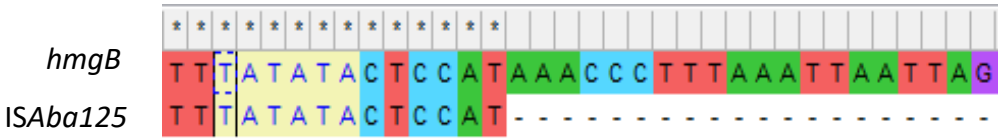
Fig. 2: gene organisation of the pyomelanin biosynthetic pathway in *Acinetobacter baumannii* AB4353. Genes are represented by arrows and the central and peripheral pathways genes are highlighted with different patterns.

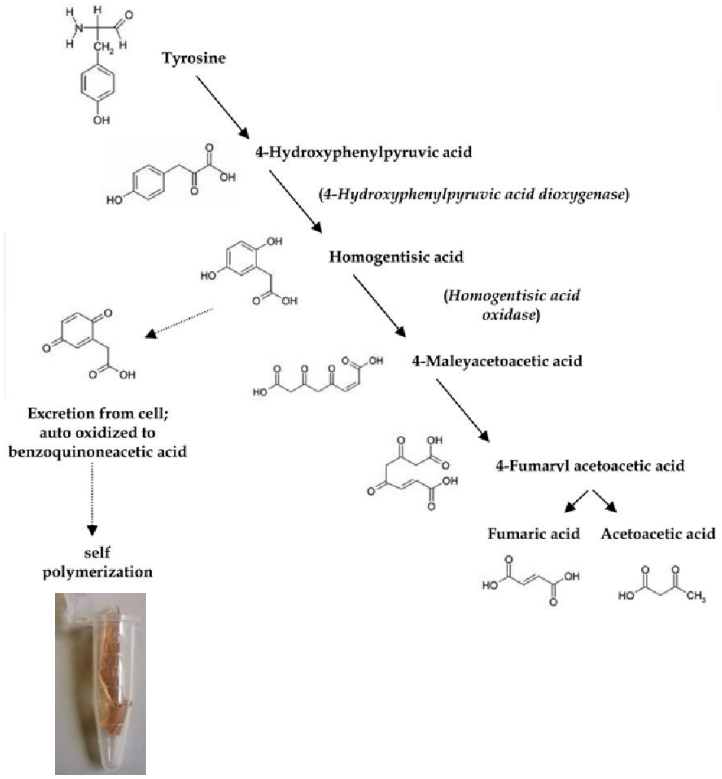
hmgA: homogentisate dioxygenase **hmgB**: fumarylacetoacetase hydrolase **hmgC**: maleylacetoacetate isomerase

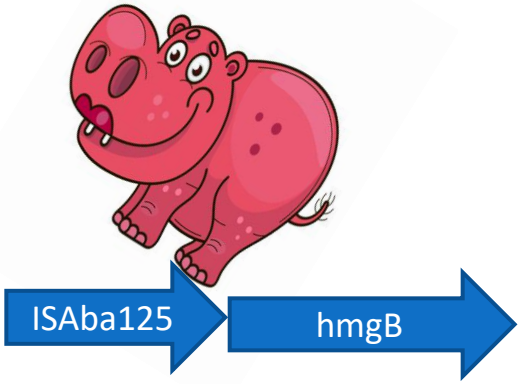
ISAba125-IS30 family

Analisi preliminare della comparazione *in silico* dei principali geni coinvolti

MEGA (molecular evolutionary genetics analysis) software







Lopes BS, Amyes SGB. Role of ISAba1 and ISAba125 in governing the expression of blaADC in clinically relevant Acinetobacter baumannii strains resistant to cephalosporins. J Med Microbiol. 2012 Aug;61(Pt 8):1103-1108.

Conclusioni

- *A. baumannii* è un microrganismo dotato di notevole plasticità genomica che si adatta a qualsiasi ambiente
- La produzione di pigmento è sempre associata ad un aumento di virulenza del ceppo che lo produce.
- I ceppi produttori di piomelanina non sembrano mostrare ad oggi un aumento significativo di mortalità né un aumento nel numero di sepsi rispetto ai ceppi non pigmentati
- L'outbreak del 2023 di *A. baumannii* pigmentato costituisce un tracciante del movimento dei ceppi all'interno dell'ospedale

Grazie per l’attenzione

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