Carbapenemases other than MBL in *Pseudomonas aeruginosa*

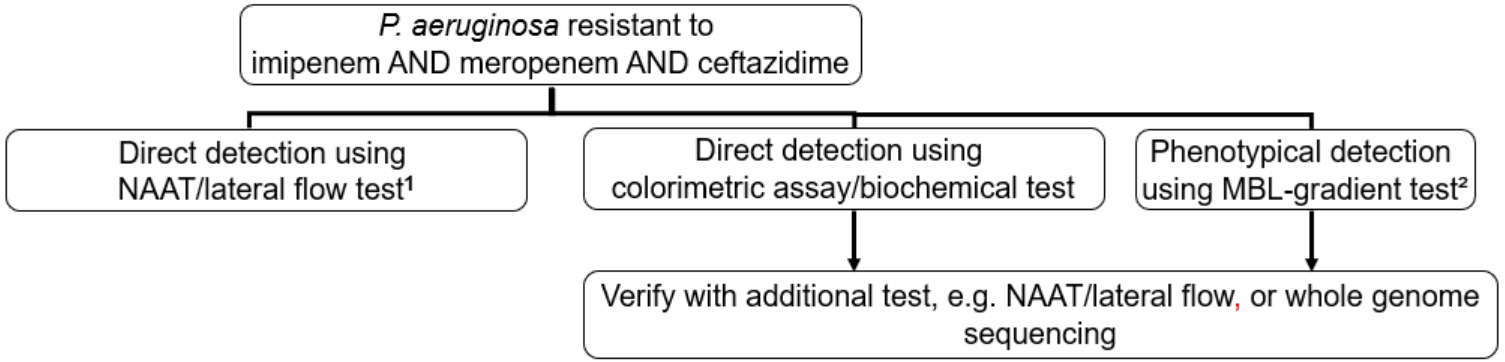
-insights from an outbreak. Is our screening algorithm good enough?

Anna-Karin Smekal, Karolinska University Hospital

NordicAST workshop 12-13 May 2026

NordicAST algorithm for carbapenemase detection in *P. aeruginosa*

ALGORITHM FOR CARBAPENEMASE DETECTION



[1]NAAT (nucleic acid amplification test, e.g. PCR, LAMP)/lateral flow test should include the most common carbapenemases (NDM, VIM and IMP). If the test is negative, but the isolate is still suspected to be carbapenemase producing (e.g. due to laboratory or epidemiological reasons), consider additional testing for rarer carbapenemases or whole genome sequencing.
[2] Disk diffusion with ceftolozane-tazobactam can be used as an additional test to rule out MBL. MBL-producing isolates are always R.

The most common carbapenemases in *P. aeruginosa* are metallo-beta-lactamases (MBL; class B) (3). MBLs can hydrolyze almost all beta-lactams, with the exception of aztreonam and to a large extent the novel siderophore cephalosporin cefiderocol (4). The main MBL types in *P. aeruginosa* are VIM and NDM, but IMP and some others may occur. Class A carbapenemases, including KPC, can also be found, particularly in South America and China (5). Class D carbapenemases have occasionally been described in Europe (6).

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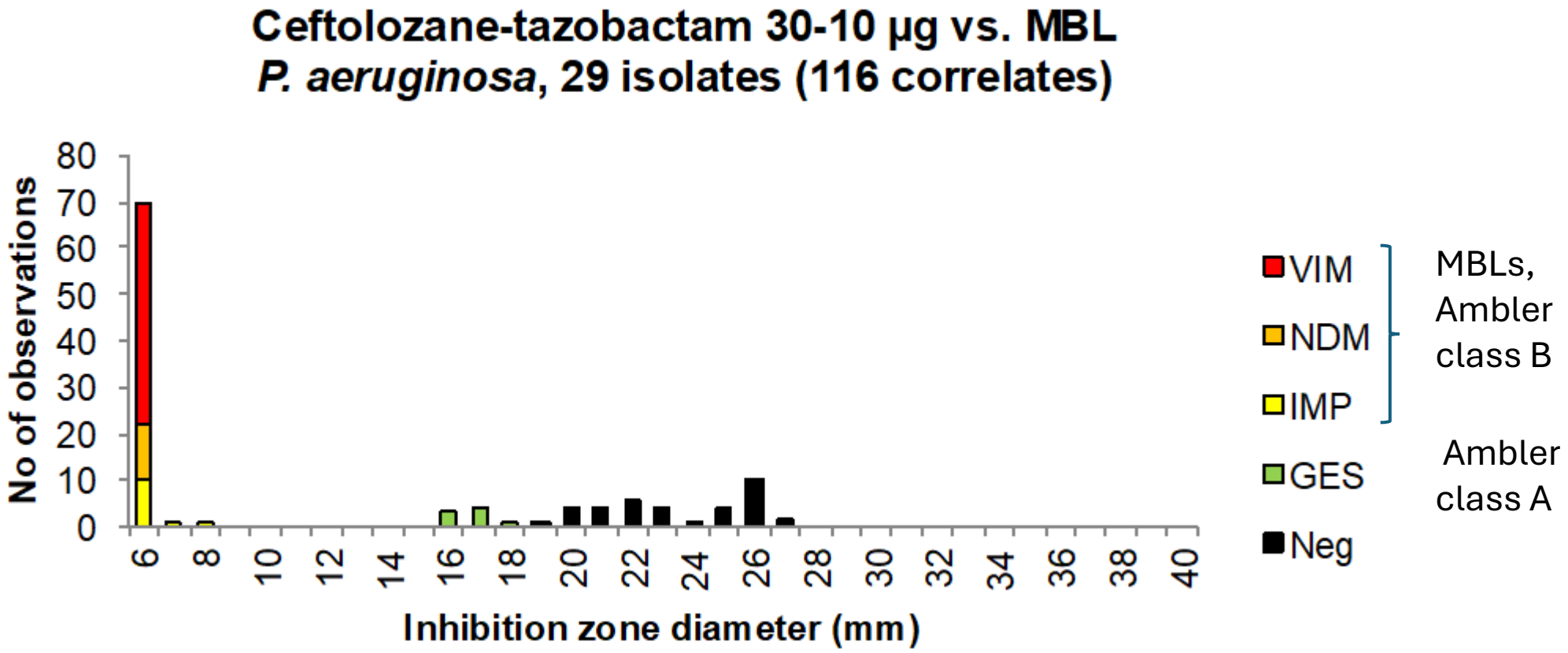
Ceftolozane-tazobactam as a marker for carbapenemases in *P. aeruginosa*



S≥ 23 mm R< 23 mm

Data from EDL and C. Giske-group

Published sequenced cases of KPC in P. aeruginosa in the world

Conclusion: The great diversity of STs identified reveals an alarming increase of unrelated clones that have acquired the *bla*$_{KPC}$ gene; in addition, there is a predominance of specific STs among populations with different geographical locations, which may have significant public healthcare implications.

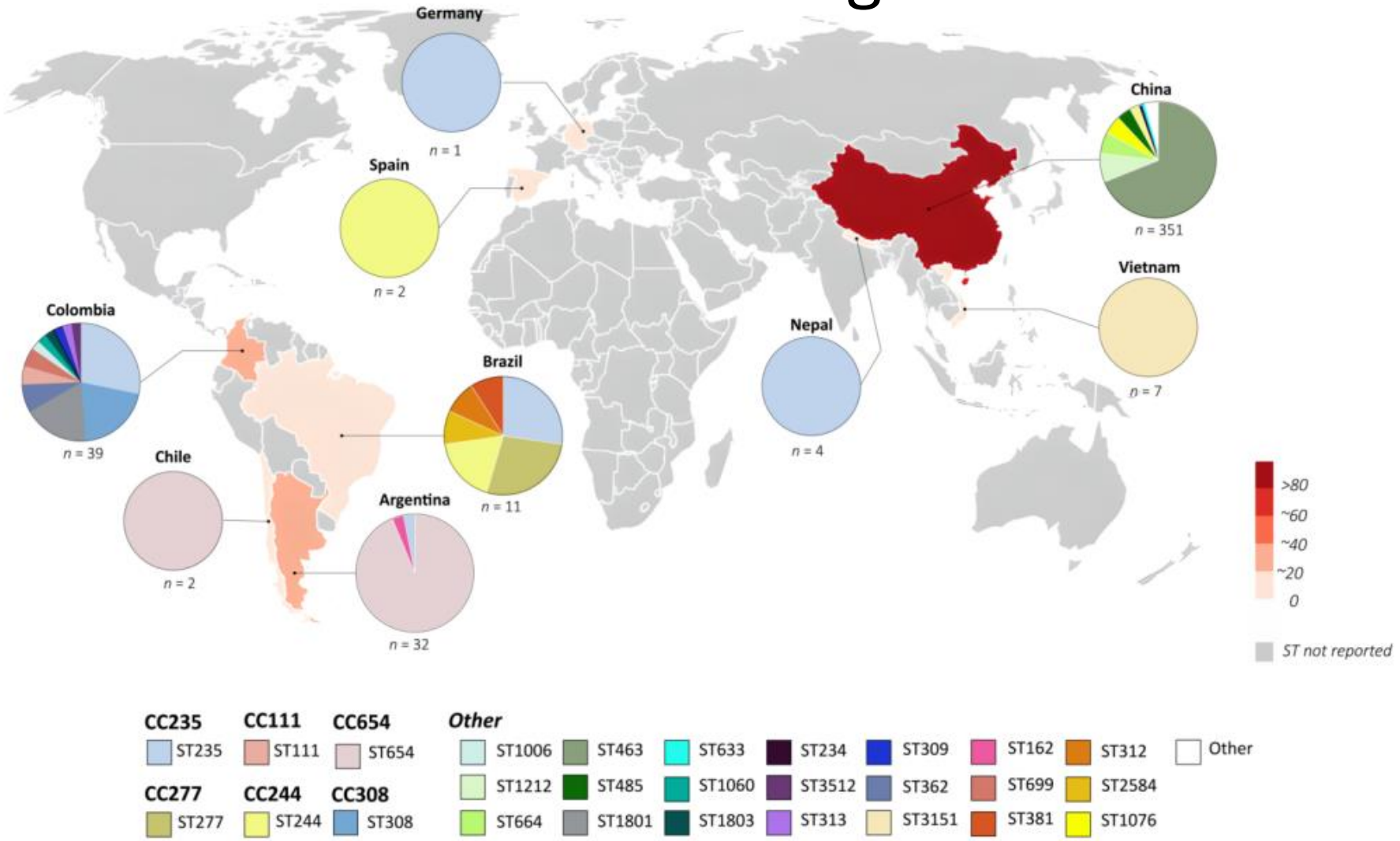
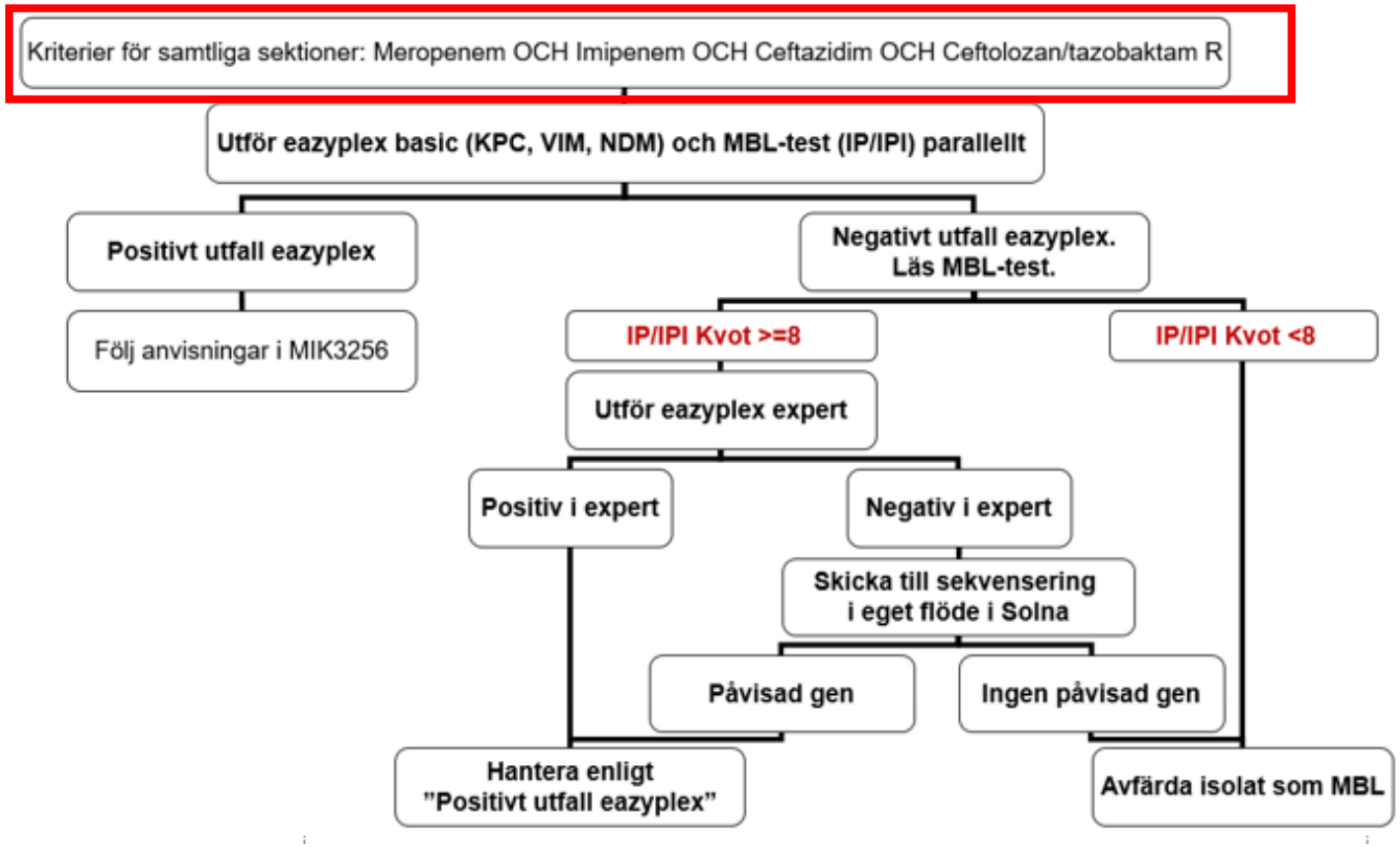


Figure 2. Geographic distribution of the *bla*$_{KPC}$-harboring *Pseudomonas aeruginosa* isolates with known sequence types. The color shading represents the total number of isolates with known sequence types. Pie charts refer to the proportion of representative sequence *bla*$_{KPC}$-harboring types found per country. Clonal complexes (CC) were assigned according to Del Barrio-Tofiño et al. in 2020 [73].

Forero-Hurtado *et al.Review 2023 in Antibiotics*

New algorithm at Karolinska UH from December 2023

Flödesschema 4: Algoritm för detektion av karbapenemaser ($ESBL_{CARBA}$) hos *P. aeruginosa*



- Only investigates isolates that are: Meropenem + imipenem+ ceftazidime +ceftolozane-tazobactam R
- Direct detection using Eazyplex Basic kit (NDM, VIM, KPC, OXA-48)
- Always MBL-test in parallel
- If positive MBL-test but negative för **NDM/VIM**→Eazyplex expert (**IMP**, **GIM** GES, IMI), if neg→ WGS

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Carbapenemases in *Pseudomonas aeruginosa*- highlighted in yellow

Vilka karbapenemaser (ESBL-carba) kan *P. aeruginosa* ha?

	Ambler klass A	Ambler klass B (Metallo-betalaktamaserna)	Ambler klass C	Ambler klass D
Viktigaste karbapenemas-grupperna och typiska karaktäristika	**KPC** (inaktiverar de flesta betalaktamer inkl. karbapenemer) **GES:** ovanlig men har gett sjh-utbrott i bla Brasilien och Japan **IMI:** kromosomal karbapenemas hos *E. cloacae*. Klonala utbrott beskrivna. Inaktiverar imipenem mest uttalat **Ovanliga: NMC** och **SME**	**NDM, VIM, IMP, GIM** (inaktiverar de flesta betalaktamer förutom aztreonam) Ovanligt: **SPM**, förekommer i Sydamerika	Inga karbapenemaser i denna Ambler grupp men AmpC + porinförlust kan ge reducerad känslighet för karbapenemer.	A) **OXA-48-gruppen** (inkl OXA-181, -244 mfl undergrupper) Ofta endast lätt nedsatt känslighet mot karbapenemer. Känslig för cefalosporiner. B) **OXA-23, 24/40,58** (inaktiverar de flesta betalaktamer inkl karbapenemer)
Förekommer hos dessa arter/familjer (Inom parentes: ovanliga arter med enzymet)	*Enterobacterales* (*P. aeruginosa*: KPC i Sydamerika mfl områden, GES)	*Enterobacterales* (NDM, VIM, IMP, GIM) *P. aeruginosa* (alla) *A. baumannii*-gruppen: (NDM)	*Enterobacterales*	A) *Enterobacterales* B) *A. baumannii*-gruppen

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And then on a January morning in 2024 someone picked the wrong Eazyplex kit ...

The case

- Male 55 years old, liver transplanted in November , complications with abdominal abscesses around the liver.

- Known ESBLcarba/CPE carrier : *K variicola* KPC
- Since November repeated findings of *P aeruginosa* in tracheal secretion cultures, abdominal abscesses, urine cultures.
- All tested negative with MBL-test. Same antibiogram
- End of Jan, culture from abscess in the abdomen: *E. faecium* + *P. aeruginosa*

PTZ: 11:R CZA: 20:S CXA: 17:R CID: 26:S IMI: 6:R IMR: 13:R MER: 6:R AZT: 21:I AMI: 10:R CFZ: 12:R CIP: 6:R
OBS! Klassificering I betyder KÄNSLIG vid ökad exponering (högdos).

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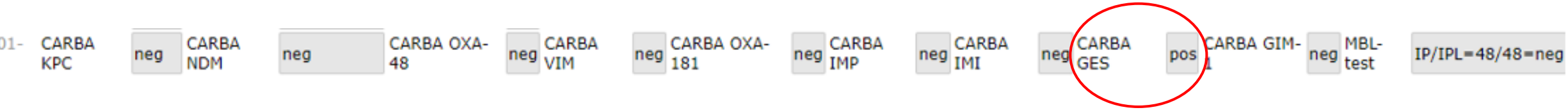
The case

- By mistake the staff uses the Eazyplex Expert kit instead of Basic kit…
- Basic kit: NDM, VIM, KPC, OXA-48
- Expert kit: IMP, IMI, GES, GIM



- The isolate sent to Solna site, the same results with the Expert kit
- Recommendation to treat the patient with ceftazidime-avibactam (CAZ-AVI) in combination with cefiderocol
- Sent for WGS

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The case

- Eazyplex expert:
  - Detects GES-1 to GES-20
  - Not all are classified as carbapenemases
  - WGS needed

Results:

- Sequence type 235 (CC235)
- GES-5 detected, classified as a carbapenemase

GES			Se
A	GES-1	2f	ESBL view
A	GES-2	2f	Carbapenemase
A	GES-3	2f	ESBL
A	GES-4	2f	Carbapenemase
A	GES-5	2f	Carbapenemase view
A	GES-6	2f	Carbapenemase
A	GES-7	2f	ESBL
A	GES-8	2f	ESBL
A	GES-9	2f	ESBL
A	GES-10	2f	ESBL
A	GES-11	2f	ESBL view
A	GES-12	2f	ESBL view
A	GES-13	2f	ESBL
A	GES-14	2f	Carbapenemase view
A	GES-15	2f	Carbapenemase
A	GES-16	2f	Carbapenemase
A	GES-17	2f	ESBL
A	GES-18	2f	Carbapenemase view
A	GES-19	2f	ESBL
A	GES-20	2f	Carbapenemase

Naas, T., Oueslati, S., Bonnin, R. A., Dabos, M. L., Zavala, A., Dortet, L., ... Iorga, B. I. (2017). Beta-lactamase database (BLDB) – structure and function. Journal of Enzyme Inhibition and Medicinal Chemistry, 32(1), 917–919. https://doi.org/10.1080/14756366.2017.1344235

Source: bldb.eu

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GES-5

- GES (Guiana extended spectrum), first discovered in French Guiana in the late 1990
- The following resistance patterns are typical for GES-5
  - Ceftazidime+imipenem+meropenem+ceftolozane-tazobactam R, BUT ceftazidime-avibactam S.

- Rapid spread in hospital settings has been described from among others South Africa, Saudi Arabia, Japan in recent years.
- Associated with CC235. Might be the most common carbapenemase in *Pseudomonas aeruginosa*.
- Usually located on class 1 integron

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GES-5 some examples

Open Access Article

High Prevalence of GES-5 Variant and Co-Expression of VIM-2 and GES-45 among Clinical *Pseudomonas aeruginosa* Strains in Tunisia

by Meha Fethi [1], Beatriz Rojo-Bezares [2,†], Ameni Arfaoui [1,†], Raoudha Dziri [1], Gabriela Chichón [2], Farouk Barguellil [3,4], María López [2], Mohamed Selim El Asli [3,4], Paula Toledano [2], Hadda-Imen Ouzari [1], Yolanda Sáenz [2,*] and Naouel Klibi [1,*]

Antibiotics **2023**, *12*(9), 1394; https://doi.org/10.3390/antibiotics12091394

Abstract

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) are a global health concern. The antimicrobial resistance, virulence, and molecular typing of 57 CRPA isolated from 43 patients who attended a specific Tunisian hospital from September 2018 to July 2019 were analyzed. All but one were multidrug-resistant CRPA, and 77% were difficult-to-treat-resistant (DTR) isolates. The *bla*$_{VIM-2}$ gene was detected in four strains (6.9%), and among the 36 *bla*$_{GES}$-positive CRPA (62%), the *bla*$_{GES-5}$ gene was the predominant variant (86%). Three strains co-harbored the *bla*$_{VIM-2}$ and *bla*$_{GES-45}$ genes, and seven CRPA carried the *bla*$_{SHV-2a}$ gene (14%). OprD alterations, including truncations by insertion sequences, were observed in 18 strains. Regarding the 46 class 1 integron-positive CRPA (81%), the *bla*$_{GES-5}$ gene was located in integron In717, while the *bla*$_{GES-29}$ and *bla*$_{GES-45}$ genes were found in two new integrons (In2122 and In4879), and the *bla*$_{VIM-2}$ gene was found in In1183 and the new integron In2142. Twenty-four PFGE patterns and thirteen sequence types (three new ones) were identified. The predominant serotype O:11 and *exoU* (81%) were mostly associated with ST235 and the new ST3385 clones. The seven *bla*$_{SHV-2a}$-CRPA from different patients belonged to ST3385 and the same PFGE pattern. The *bla*$_{GES-5}$- and *bla*$_{VIM-2}$ + *bla*$_{GES-45}$-positive CRPA recovered mostly from ICU patients belonged to the high-risk clone ST235. Our results highlight the alarming prevalence of *bla*$_{GES-5}$- and ST235-CRPA, the co-existence of *bla*$_{GES-45}$ and *bla*$_{VIM-2}$, and their location within integrons favoring their dissemination.

RESEARCH ARTICLE

Spread of GES-5 carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates in Japan due to clonal expansion of ST235

Tomomi Hishinuma[1], Tatsuya Tada[1]*, Kyoko Kuwahara-Arai[1], Norio Yamamoto[1], Masahiro Shimojima[2], Teruo Kirikae[1]

artment of Microbiology, Juntendo University School of Medicine, Tokyo, Japan, **2** BML Inc., Kawagoe, na, Japan

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stract

irst outbreak in Japan of GES-5 carbapenemase-producing *Pseudomonas aeruginosa* rred in a long-term care facility in 2014. To assess the spread of GES-5 producing *P. aeruginosa* clinical isolates in medical settings in Japan, 1,476 carbapenem-resistant *P. aeruginosa* isolates obtained from 2012 to 2016 were characterized. Of these 1,476 isolates, 104 (7.0%) harbored *bla*$_{GES-5}$. Southern blotting revealed that the *bla*$_{GES-5}$ was located on the chromosome. The isolation rates of these GES-5 producers increased significantly every year, from 2.0% (6 of 295) in 2012 to 2.8% (8 of 283) in 2013 to 5.3% (16 of 303) in 2014 to 9.7% (29 of 300) in 2015 to 15.3% (45 of 295) in 2016. Of the 104 GES-5 producers, 102 belonged to clonal complex (CC) 235, including 99 belonging to ST235 and three belonging to ST2233). Whole genome sequence analysis revealed that CC235 *P. aeruginosa* harboring *bla*$_{GES-5}$ spread in a clonal manner. These results indicate that these GES-5 producing CC235 *P. aeruginosa* clinical isolates have spread in medical settings throughout Japan.

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A few months later the second and the third case came along..

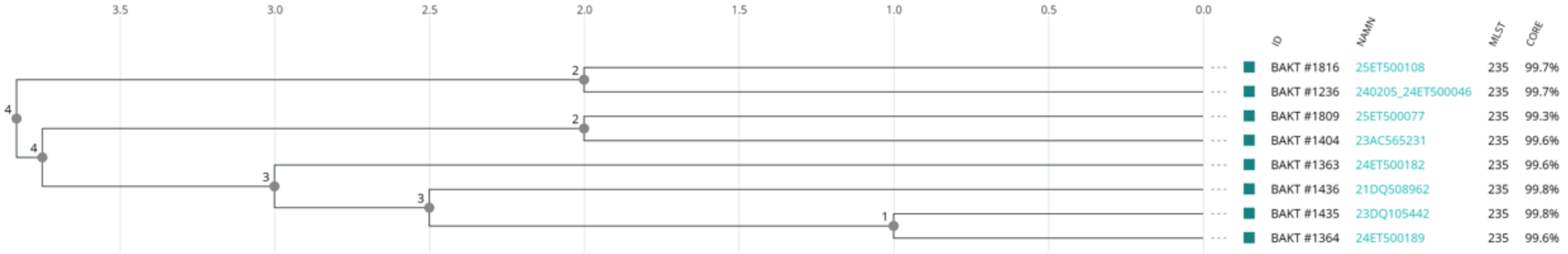
P aeruginosa GES ST235 cluster

Patient	ST	PTZ	CZA	CXA	CID	IMI	IMR	MER	AMI	CFZ	CIP
3	ST235	9	18	18	26	6	13	6	11	13	6
2	ST235	8	19	17	28	8	14	6	10	11	6
1	ST235	11	20	17	26	6	13	6	10	12	6
4x	ST235	10	19	16	26	6	12	6	12	13	6
5x	ST235	11	19	20	25	8	13	6	11	12	6
6x	ST235	11	19	18	26	6	13	6	12	12	6
7	ST235	10	20	18	28	10	15	7	10	11	8
8	ST235	10	20	18	25	7	14	6	11	11	6
9	ST235	11	21	18	26	7	12	6	10	11	6

*Aztreonam tested I for 5 patients with respiratory tract infection (DQ-numbers). The rest not tested

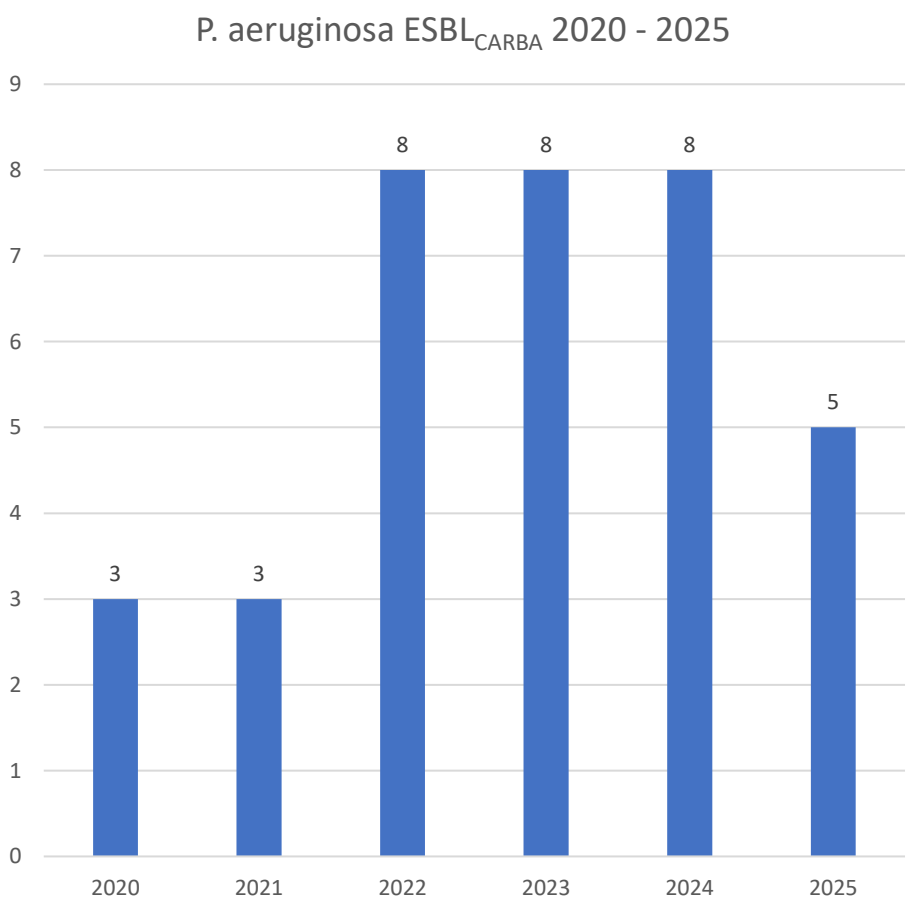
- Nine patients in total, all were isolates from clinical cultures
- Two patients died because of their untreatable infection with PA GES (in combination with heavy immunosuppression)
- Indexpatient: 2021, patient from Egypt

P. aeruginosa $ESBL_{CARBA}$ GES ST235 Cluster



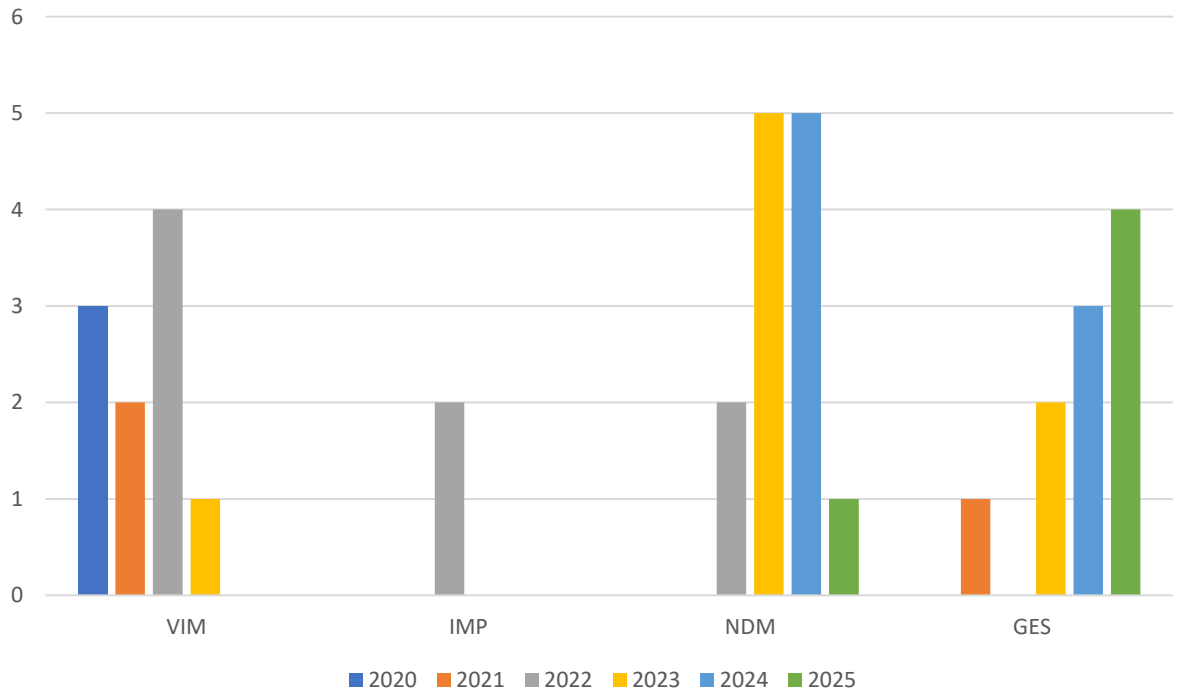
cgMLST cluster analysis

Pseudomonas aeruginosa $ESBL_{CARBA}$ in the county of Stockholm



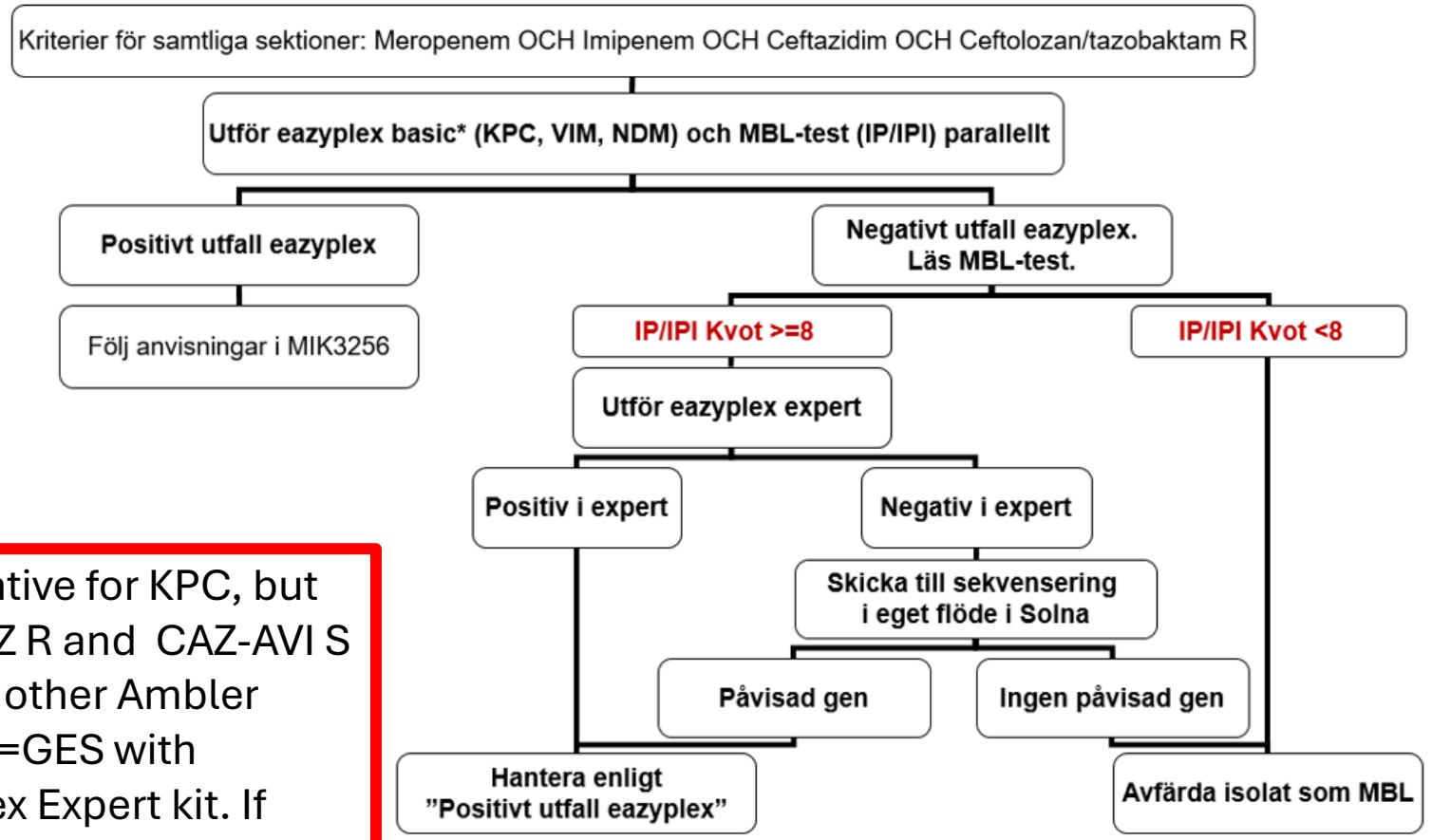
Påvisad genotyp 2025:

- NDM (n=1)
- GES (n=4)



Current algorithm for detection at Karolinska UH

Flödesschema 4: Algoritm för detektion av karbapenemaser ($ESBL_{CARBA}$) hos *P. aeruginosa*



*If negative for KPC, but TOL-TAZ R and CAZ-AVI S test för other Ambler class A=GES with Eazyplex Expert kit. If negative –> WGS

- Only investigates isolates that are: Meropenem + imipenem+ ceftazidime +ceftolozane-tazobactam R
- Direct detection using Eazyplex Basic kit (NDM, KPC, VIM, OXA-48)
- Always MBL-test in parallel
- If positive MBL-test but negative för NDM/VIM→Eazyplex expert (IMP), if neg→ WGS

Is the NordicAST screening algorithm good enough?

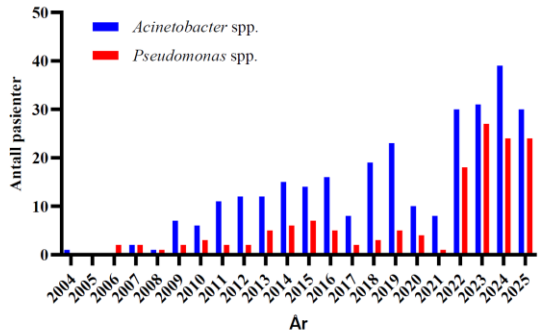
Nordic data on carbapenemases in *P. aeruginosa*

- Swedish data

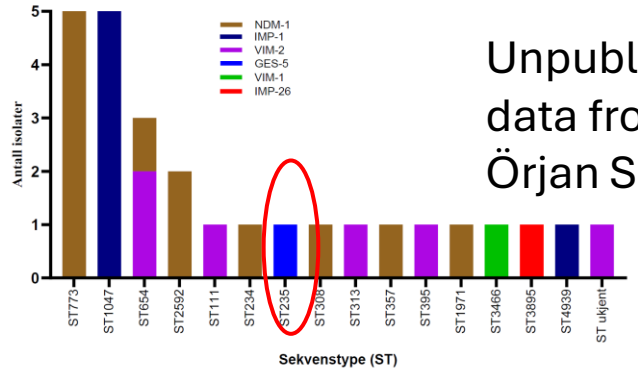
	Antal	Antal NDM	Antal VIM	Antal IMP	Antal KPC	Antal GES
2023	12	9	3	0	0	0
2024	18	10	5	1	0	2
2025	16	8	2	2	1	3
2026*	9	4	3	2	1	0

Unpublished data from Fohm/Vilhelm Müller:
three active clusters in 2025, two with NDM (4 resp 2 patients), 1 with GES (4 patients)

- Norwegian data



Figur 15. Antall personer med påvist karbapenemaseproduserende *Pseudomonas* spp. og *Acinetobacter* spp. i Norge 2004-2025.



Unpublished data from K-res, Örjan Samuelsen

Figur 16: Fordeling av ST og karbapenemasevarianter blant karbapenemaseproduserende *Pseudomonas* spp. (n=27) isolert i Norge i 2025.

- Danish data

2025 11 isolates (5 NDM, 3 IMP, 3 VIM)
2024 19 isolates (11 NDM, 5 IMP, 3 VIM)

Unpublished data from DANMAP, Henrik Hesman

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Algorithm to Guide Definitive Carbapenemase Testing to Identify Carbapenemase-Producing *Pseudomonas aeruginosa*

Table 1. Characteristics of the challenge set of 92 *P. aeruginosa* isolates utilized in algorithm development.

Susceptibility	Carbapenemase Producers, $n = 57$	Non-Carbapenemase Producers,		Test Performance	
		Cephalosporinase or Efflux/Porin Mutation, $n = 20$	Wild Type, $n = 15$	Sensitivity, % (95% CI)	Specificity, % (95% CI)
IPM + MEM- Resistant	57 (100%)	15 (75%)	1 (7%)	100% (94–100%)	54% (37–71%)
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible	57 (100%)	12 (60%)	0 (0%)	100% (94–100%)	66% (48–81%)
IPM + MEM- Resistant AND FEP + CAZ + TZP- Resistant	47 (82%)	6 (30%)	0 (0%)	83% (70–91%)	83% (66–93%)
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + CZA- Resistant	49 (86%)	8 (40%)	0 (0%)	86% (74–94%)	77% (60–90%)
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + C/T- Resistant	57 (100%)	4 (20%)	0 (0%)	100% (94–100%)	89% (73–97%)
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + C/T- Resistant + CZA- Resistant	49 (86%)	3 (15%)	0 (0%)	86% (74–94%)	91% (77–98%)

IPM = imipenem; MEM = meropenem; FEP = cefepime; CAZ = ceftazidime; TZP = piperacillin/tazobactam; CZA = ceftazidime/avibactam; C/T = ceftolozane/tazobactam; Sensitivity and Specificity = calculated based on prediction the criteria accurately identify carbapenemase production; 95%CI = 95% confidence interval.

Carbapenemase-producers: NDM (n=10), IMP (n=10), VIM (n=10), KPC (n=8), SPM (n=10), GES (n=9)

Gill CM et al. Antibiotics 2020, 9, 738; doi:10.3390/antibiotics9110738

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Algorithm for CP-PA (cont.)

Table 2. Performance of algorithm after application to 1209 clinical *P. aeruginosa* isolates from a US surveillance study.

Algorithm-Derived Screening Criteria	Number Meeting Criteria	Carbapenemase Producers Detected	Carbapenemase Producers Missed by Criteria
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible	116	7/116	0
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + CZA- Resistant	43	7/43	0
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + C/T- Resistant	21	6/21	1 *
IPM + MEM- Resistant AND FEP + CAZ + TZP- Non-Susceptible + C/T- Resistant + CZA-Resistant	19	6/19	1 *

IPM = imipenem; MEM = meropenem; FEP = cefepime; CAZ = ceftazidime; TZP = piperacillin/tazobactam; CZA = ceftazidime/avibactam; C/T = ceftolozane/tazobactam. * Genotype: bla_{OXA-2}, bla_{OXA-50}, and PAO.

The isolate that was ”missed” had positive mCIM-test, but genotypically no carbapenemases

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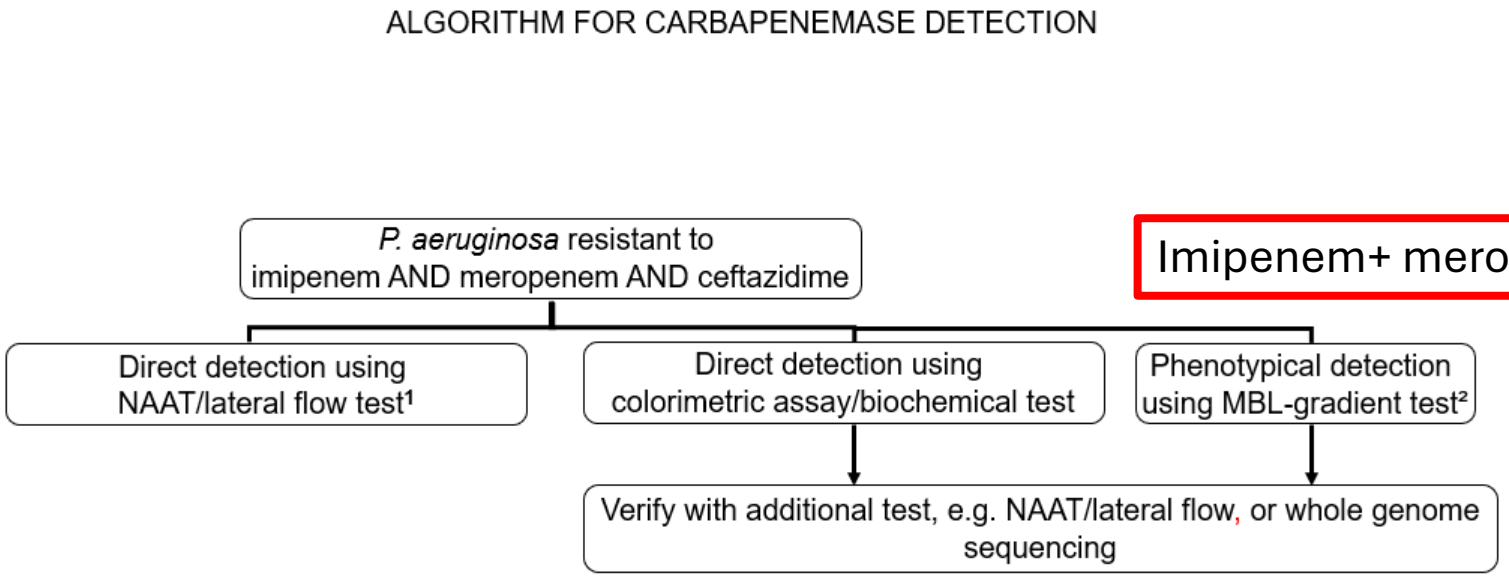
Up-coming discussion regarding update of the NordicAST algorithm



[1]NAAT (nucleic acid amplification test, e.g. PCR, LAMP)/lateral flow test should include the most common carbapenemases (NDM, VIM and IMP). If the test is negative, but the isolate is still suspected to be carbapenemase producing (e.g. due to laboratory or epidemiological reasons), consider additional testing for rarer carbapenemases or whole genome sequencing.
[2] Disk diffusion with ceftolozane-tazobactam can be used as an additional test to rule out MBL. MBL-producing isolates are always R.

Imipenem+ meropenem+ceftazidime+ceftolozane-tazobactam

Update common carbapenemases with Ambler class A (KPC, GES)
Add that isolates CAZ-AVI S should be examined for KPC/GES,
Aztreonam I, could possible be used to examine KPC negative isolates further (for GES)

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Possible antibiogram guidance to use in a detection algorithm for Pseudomonas aeruginosa

Mechanism	TOL-TAZ	CAZ-AVI	AZT[1]
Class A KPC	R	S	R
Class A GES	R	S	I
Class B MBL	R	R	I

[1]Aztreonam resistance can also arise through other mechanisms (PDC, efflux)

Montero MM et al. Eur J of Clin Micro Inf Dis 2025; 44:1077-1087

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Question 1 (PA)

An isolate of PA features resistance to imipenem, and low-level resistance to meropenem. What is the most likely resistance mechanism?

A) AmpC hyperproduction plus efflux
B) Only efflux
C) Class A carbapenemase
D) Porin loss

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Question 2 (PA)

A strain of PA features resistance to PTZ, CAZ, IMI, MER. Extended testing shows resistance to TOL-TAZ and susceptibility to CAZ-AVI. What is the most likely mechanism?

A) AmpC+porin loss
B) Class B metallo-beta-lactamase
C) Porin loss+efflux
D) Class A carbapenemase

Question 2 (PA)

A strain of PA features resistance to PTZ, CAZ, IMI, MER. Extended testing shows resistance to TOL-TAZ and susceptibility to CAZ-AVI. What is the most likely mechanism?

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