1er CONGRÈS DE LA RÉGION MIDDLE EAST AND NORTH AFRICA DE MICROBIOLOGIE CLINIQUE ET DE PATHOLOGIE INFECTIEUSE

34ème CONGRÈS NATIONAL DE LA SOCIÉTÉ TUNISIENNE DE PATHOLOGIE INFECTIEUSE

Antibiotic Resistance profiles and WGS of XDR *A.baumannii* strains circulating in Tunisia

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LR99-ES09.

Medicine Faculty of Tunis

World Health Organization

Antimicrobial Resistance (AMR): A global threat facing Humanity!

- AMR is one of the top global public health threats in the 21st century
- Inappropiate use of antimicrobials in humans, animals and plants: Genetic changes
- New resistance mechanisms are emerging and spreading
- Development of resistance: “superbugs”
- HAI in the ICU+++

AMR: Prioritisation of MDR Pathogens

	2017	2024
Critical priority	*Acinetobacter baumannii*, carbapenem-resistant, *Pseudomonas aeruginosa*, carbapenem-resistant; Enterobacteriaceae, carbapenem-resistant, third-generation cephalosporin-resistant	*A baumannii*, carbapenem-resistant; Enterobacterales, third-generation cephalosporin-resistant; Enterobacterales, carbapenem-resistant; *Mycobacterium tuberculosis*, rifampicin-resistant*
High priority	*Enterococcus faecium*, vancomycin-resistant; *Staphylococcus aureus*, meticillin-resistant, vancomycin intermediate and-resistant; *Helicobacter pylori*, clarithromycin-resistant; *Campylobacter* spp, fluoroquinolone-resistant; salmonellae, fluoroquinolone-resistant; *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant	*Salmonella enterica* serotype Typhi, fluoroquinolone-resistant; *Shigella* spp, fluoroquinolone-resistant; *E faecium*, vancomycin-resistant; *P aeruginosa*, carbapenem-resistant; non-typhoidal *Salmonella*, fluoroquinolone-resistant; *N gonorrhoeae*, third-generation cephalosporin-resistant, fluoroquinolone-resistant; *S aureus*, meticillin-resistant

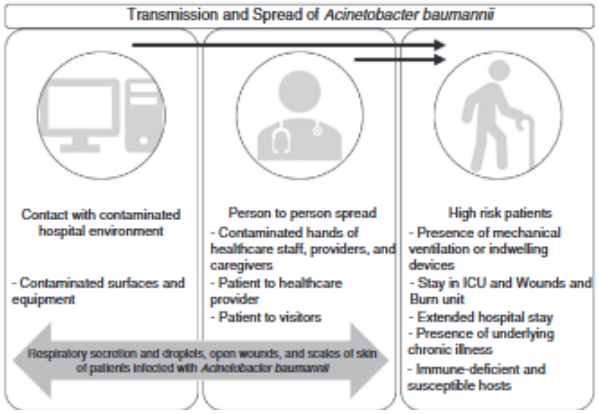
Final ranking of antibiotic-resistant bacteria in the 2024 WHO BPPL

The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance

Hatim Sati, Elena Carrara*, Alessia Savoldi, Paul Hansen, Jacopo Garlasco, Enrica Campagnaro, Simone Boccia, Juan Antonio Castillo-Polo, Eugenia Magrini, Pilar Garcia-Vello, Eve Wool, Valeria Gigante, Erin Duffy, Alessandro Cassini, Benedikt Huttner, Pilar Ramon Pardo, Mohsen Naghavi, Fuad Mirzayev, Matteo Zignol, Alexandra Cameron, Evelina Tacconelli, and the WHO Bacterial Priority Pathogens List Advisory Group†*

www.thelancet.com/infection **Published online April 14, 2025** **https://doi.org/10.1016/S1473-3099(25)00118-5**

Acinetobacter baumannii



- ➢Important nosocomial pathogen
- ➢Severe infections/ critically ill patients +++
- ➢Healthcare challenge:
  - Intrinsic and acquired antimicrobial resistance++: Severe threat to a successful treatment
  - Prospensity for clonal spread

- ➢Survive on inanimate surfaces for extended periods of time++

➡ Outbreaks and endemic persistence

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Acinetobacter baumannii

- Family: *Moraxellaxeae (GNB)*
- Genus: *Acinetobacter*
- ***Acinetobacter* complexe *baumannii***

03 Major species :

- ***A.baumannii***
- ***A. nosocomialis***
- ***A. pittii***

Expert consensus (CDC –ECDC)

A. baumannii XDR

- **MDR** (Multi-Drug Resistance) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

- **XDR** (e**X**tensive **D**rug **R**esistance) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., **bacterial isolates remain susceptible to only one or two categories).**

- **PDR** (Pan Drug Resistance) was defined as non-susceptibility to all agents in all antimicrobial categories.

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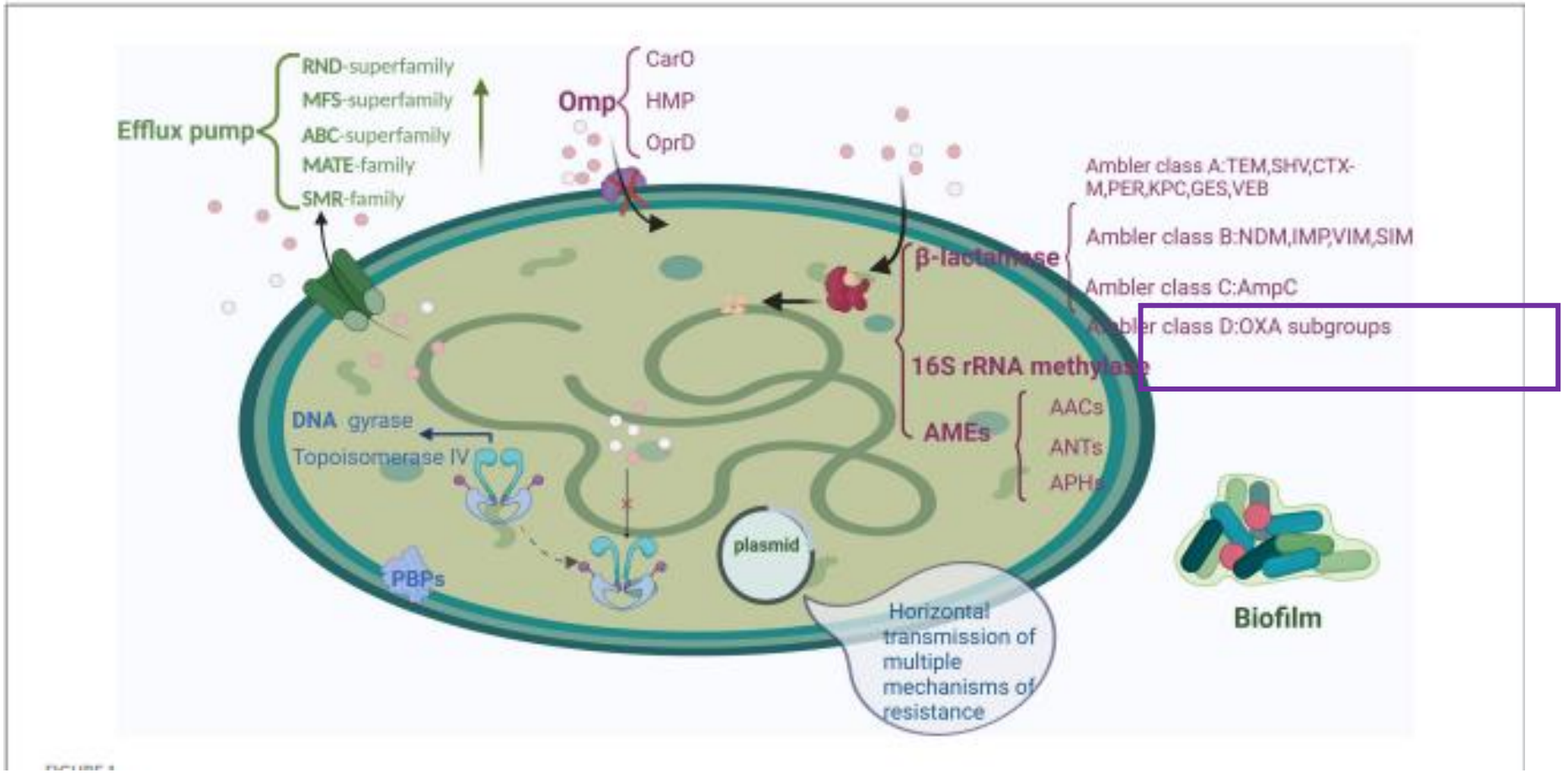
TYPE Review
PUBLISHED 22 January 2024
DOI 10.3389/fmicb.2024.1332108

Resistance to carbapenems



Mechanisms underlying drug resistance *in A. baumannii*: β-lactamase production, AMEs, and 16S rRNA methylase, efflux pump overexpression, antibiotic target location alterations, Omp permeability alterations, and biofilm formation

Carbapenemases production

Resistant mechanism	Class or subgroup	Gene	References
β-Lactamases	Ambler class A	TEM, SHV, PER, VEB, KPC	Černiauskienė et al. (2023)
	Ambler class B	IMP, VIM, SIM, NDM	Chen et al. (2017), López et al. (2019), Ayibieke et al. (2018)
	Ambler class C	AmpC	Liu and Liu (2015)
	Ambler class D	OXA23 family OXA24 family OXA51 family OXA58 family OXA143 family OXA235 family	Lin and Lan (2014), June et al. (2016), Huang et al. (2015), Donald et al. (2000), Avci et al. (2023), Zhao et al. (2019), Oliveira et al. (2019), Liao et al. (2015)

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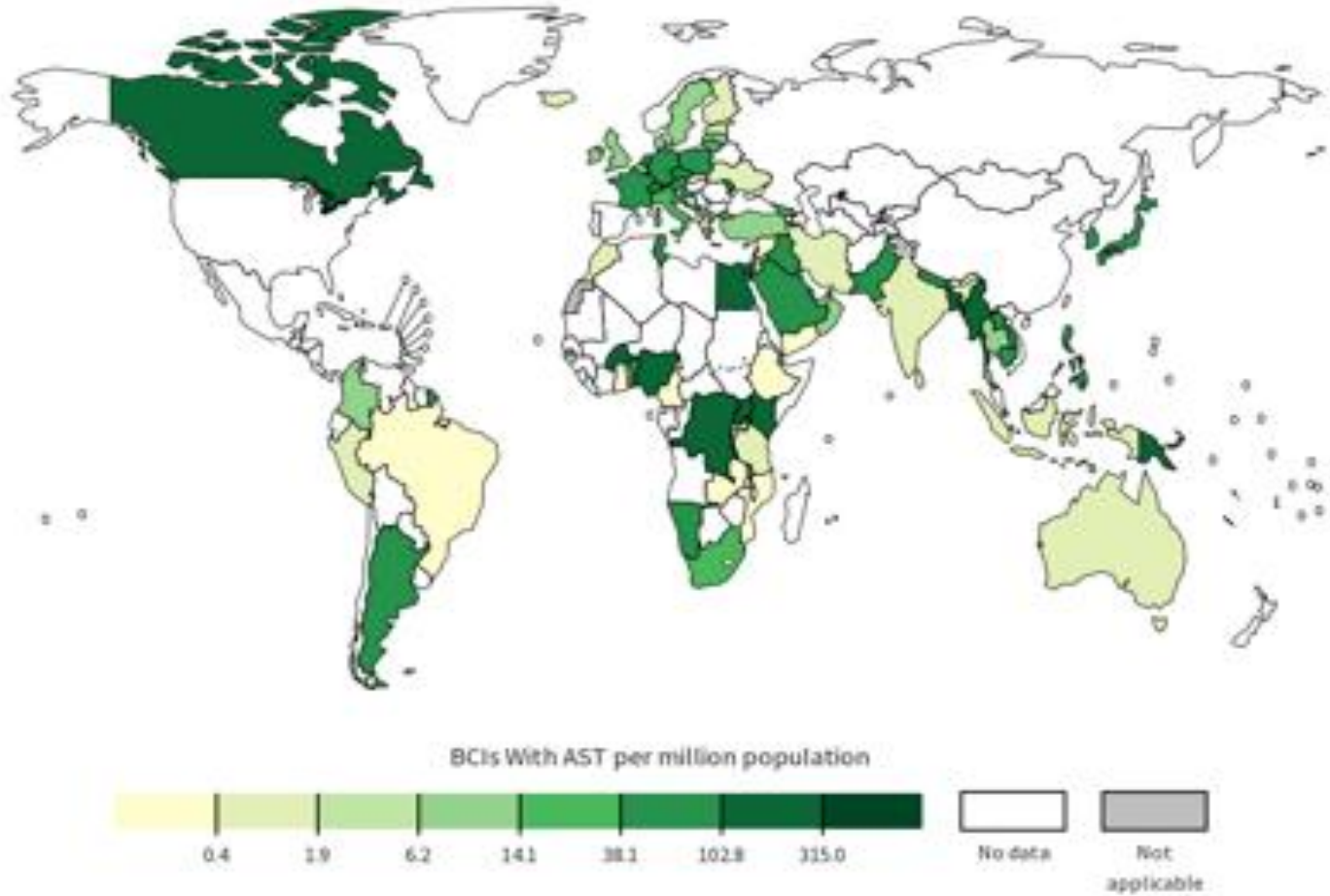
Global maps of testing coverage by bacterial pathogen and antibiotic group

Year: 2022 **Region:** All **Infection syndrome:** Bloodstream **Bacterial pathogen:** Acinetobacter spp. **Antibiotic group:** Carbapenems

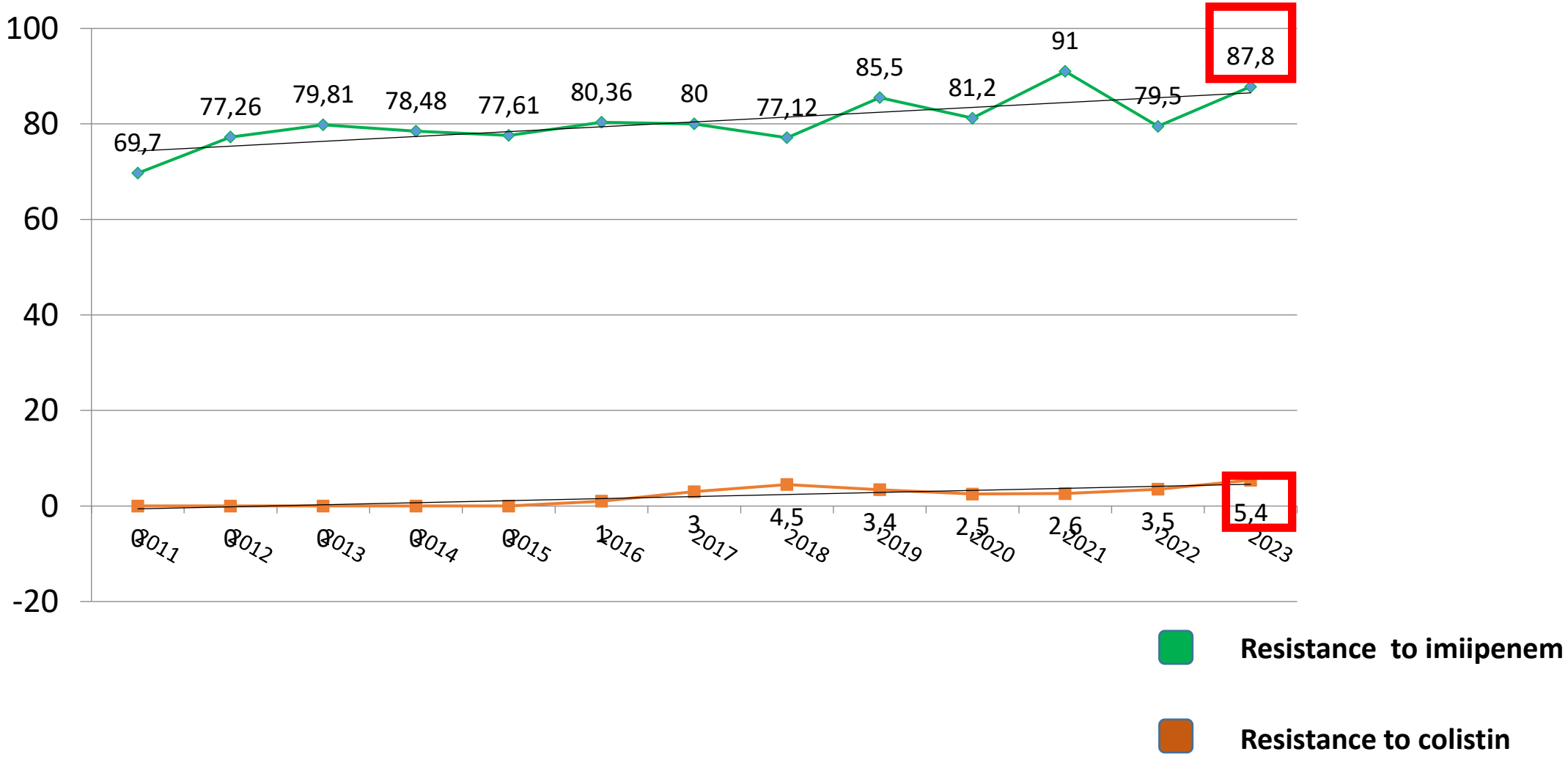


Global Antimicrobial Resistance and Use Surveillance System (GLASS) data reported by December 2023

Downloaded on 20 May 2025 from worldhealthorg.shinyapps.io/glass-dashboard/

World Health Organization

Annual evolution of imipenem and colistin resistance rates in *A.baumannii* Tunisian strains



LART 2023 Data

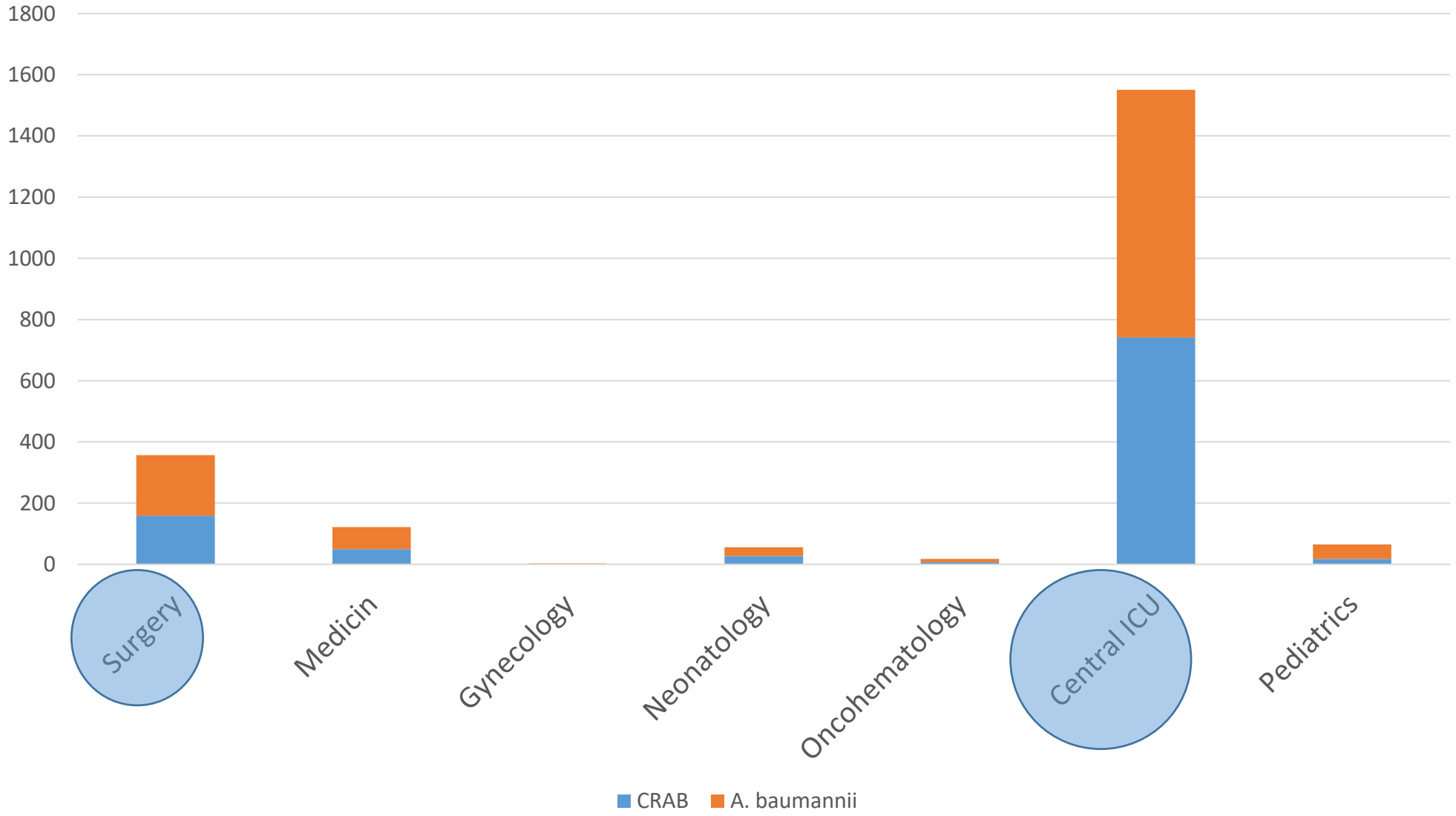
CRAB: Resistance levels to other antibiotics

Antibiotiques	Fréquence de résistance	
	Nombre	Pourcentage
Pipéracilline	926	100
Pipéracilline+Tazobactam	988	100
Céftazidime	996	100
Céfepime	894	100
Gentamicine	987	97,4
Amikacine	964	90,4
Ciprofloxacine	964	99,6
Cotrimoxazole	769	83,1
Colistine*	409	5,4

LART 2023 Data

*: MICs were determined by microdilution methods

Prevalence of CRAB Tunisian strains in ICUs



LART 2023 Data

Tunisia

To study the phenotypic and genotypic profiles of *A. baumanii* strains circulating in Tunisia through a multicenter study

Objectifs

- To give an update on the current antimicrobial resistance profiles of *A. baumannii* Tunisian strains.

- To identify the antibiotic resistance mechanisms in CRAB strains (carbapenems , colistin, aminoglycosides, fluroquinolones)

- To describe the molecular epidemiology and the global distribution of CRAB strains circulating in Tunisia

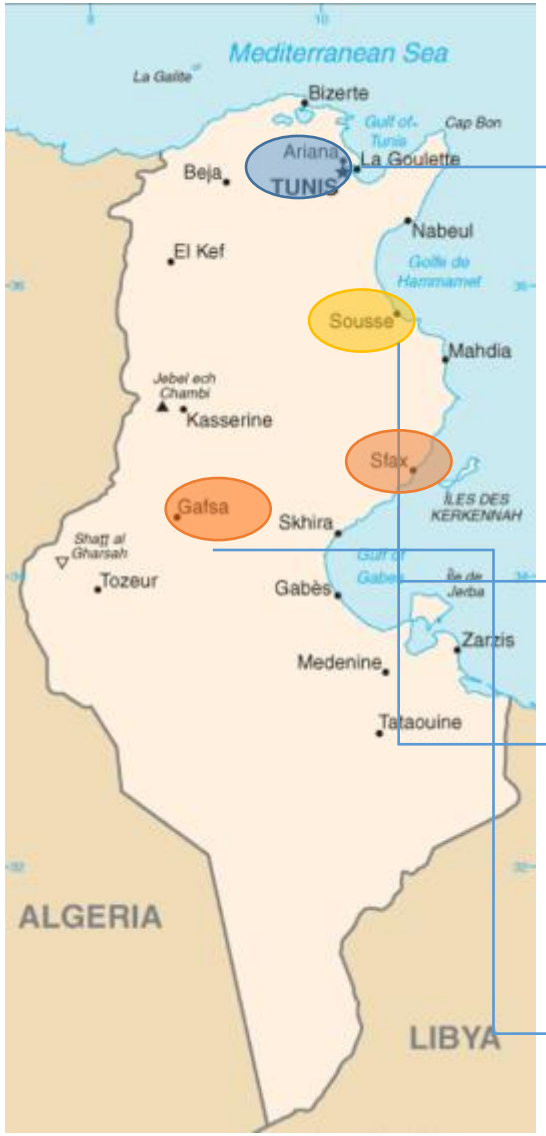
Materiel and Methods

Type of Study

- Prospective multicenter study
- 06 Months (01 September 2024-31 March 2025)
- Participation of 16 centers

Total nb= 142

Materiel



1. The North of Tunisia

2. The center of Tunisia: Sousse / Monastir

3, The south of Tunisia: Sfax. Gafsa

1. HCN (n=6)
2. Hôpital La Rabta (n=4)
3. HEBHT(n=5)
4. HMPIT(n=11)
5. HAO (n=0)
6. CNGMO(n=0)
7. CMNT(n=7)
8. Clinique Carthagène (n=5)
9. Clinique Hannabal (n=0)
10. H. Abderrahmen Mami (n=4)
11. CTGB(n=55)
12. IMKO(n=0)

13. H Farhat Hached Sousse (n=4)
14. H Sahloul (n=35)
15. H. Fattouma Bourguiba Monastir (n=0)

15. H. Habib Bourguiba Sfax(n=11)
16. H R de Gafsa(n=0)

Materiel

Study population

Included strains	Non included strains	Excluded strains
All non duplicated *A. baumannii* strains causing bacteremia ICUs	*A. baumannii* stains isolated in other types of specimens	*Acinobacter* strains not belonging to *A baumannii* complex

Phenotypic Methods

Bacterial Identification (Origin Lab)

Conventional or automated methods

Antimicrobial susceptibility testing: CA-SFM recommandations

For XDR *A. baumannii* strains ⇒ MICs:

✓Imipenem/meropenem: E-tests

✓Colistin: Broth microdilution methods

All strains were sent to the NRL(HCN) with all correspond demographic and phenotypic profiles Data

Genotypic Methods

Whole genome sequencing:

- 74 strains retained (Budget limitation)

Centers that where sent < 12 strains: All were retained

Centers that where sent > 12 strains: we retained one strain out of 02 or 03 isolated on the same day or the next day.

- DNA extraction: Qiagen DNeasy Blood and Tissue Kit (Qiagen, 168 Hilden, Germany), adhering to the manufacturer's instructions

Genotypic Methods

Whole-Genome Sequencing and Read Quality Control

- Paired-end (2 × 150 bp) libraries were prepared using **the Illumina DNA prep Kit** and sequenced on an **Illumina iSeq100 system** according to the manufacturer's instructions.

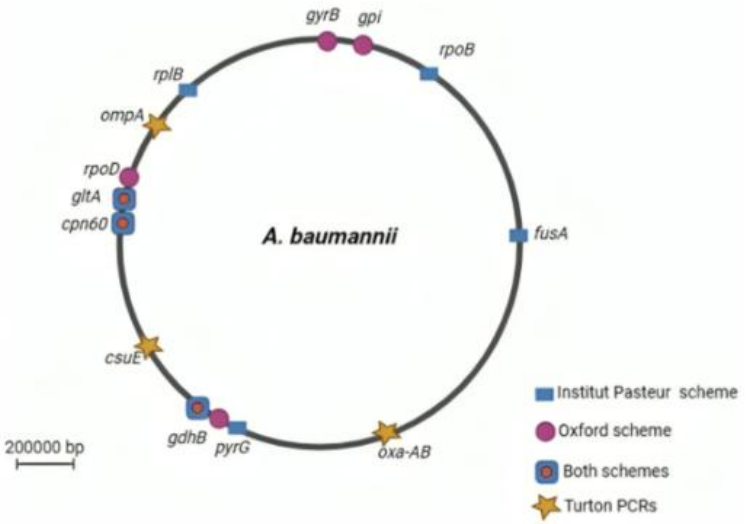
- Raw sequencing reads were quality-checked with **FastQC v0.11.9** to assess per-base quality scores, GC content, adapter contamination, and sequence duplication levels.

Genotypic Methods

Species Identification and Multilocus Sequence Typing (MLST)

- Species assignment and sequence typing were performed via the **PubMLS**T database.

- Three MLST schemes: the Institut Pasteur scheme, the Oxford scheme, and **core-genome MLST (cgMLST**)++



Genotypic Methods

De Novo Genome Assembly and Genome Annotation

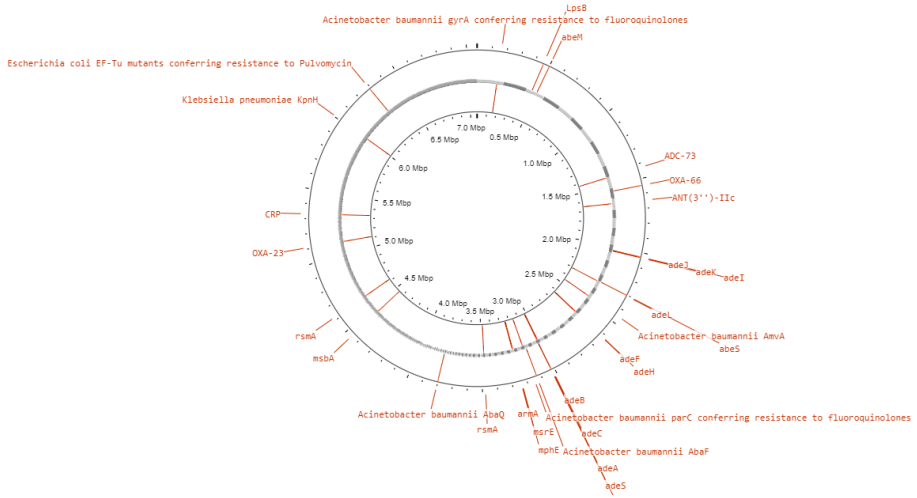
- High-quality reads were assembled de novo using the **Proksee online platform** (web version accessed May 2025), producing draft genome assemblies for each isolate.

- **Prokka v1.14 beta** was used for automatic de novo assembly annotation

Genotypic Methods

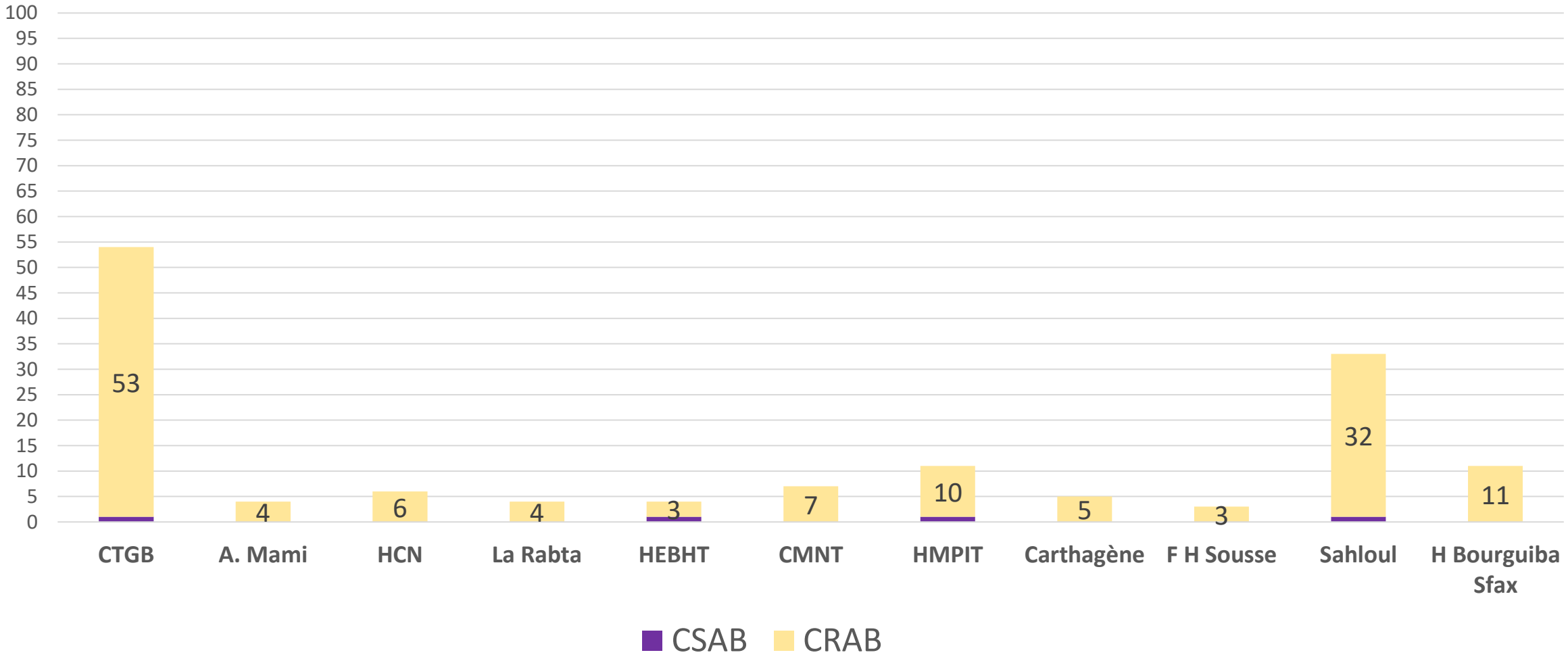
Antibiotic Resistance Gene Analysis:

Antibiotic resistance genes were identified using the **CARD database and ResFinder**, applying identity thresholds of 100 % for β-lactamase variants and 98 % for all other resistance genes.

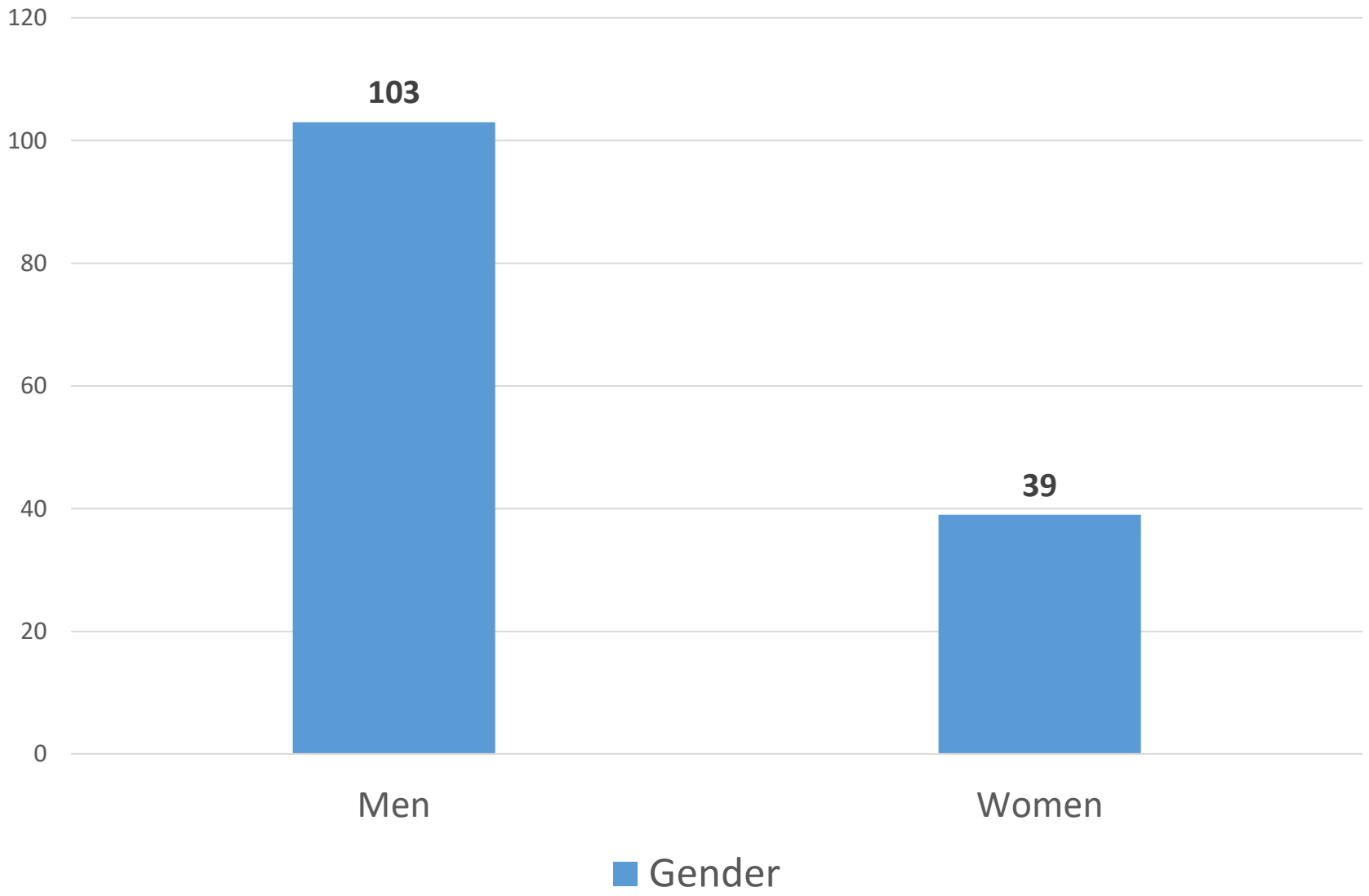


PRELIMINARY RESULTS

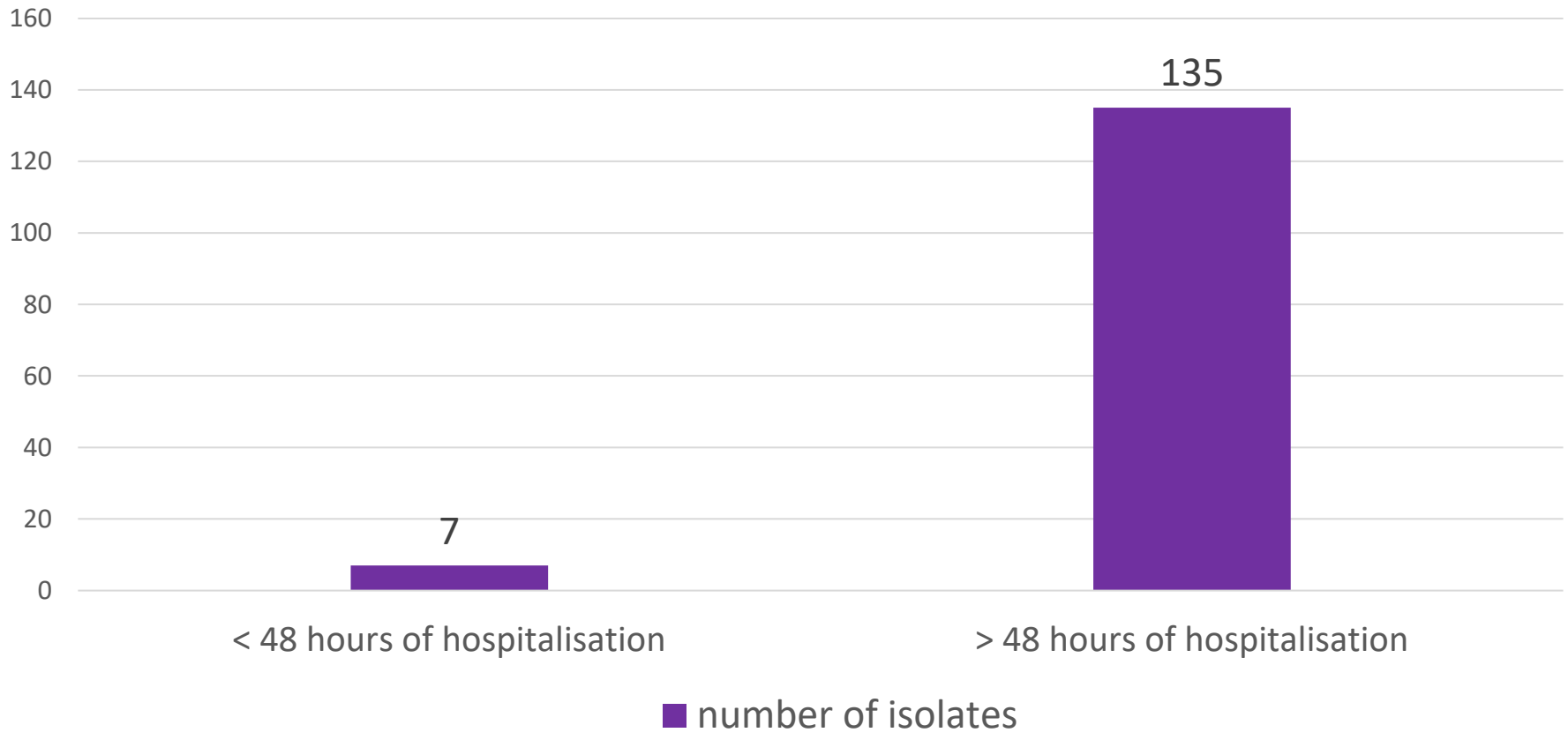
Distribution of strains by region and by hospital



Demographic Results



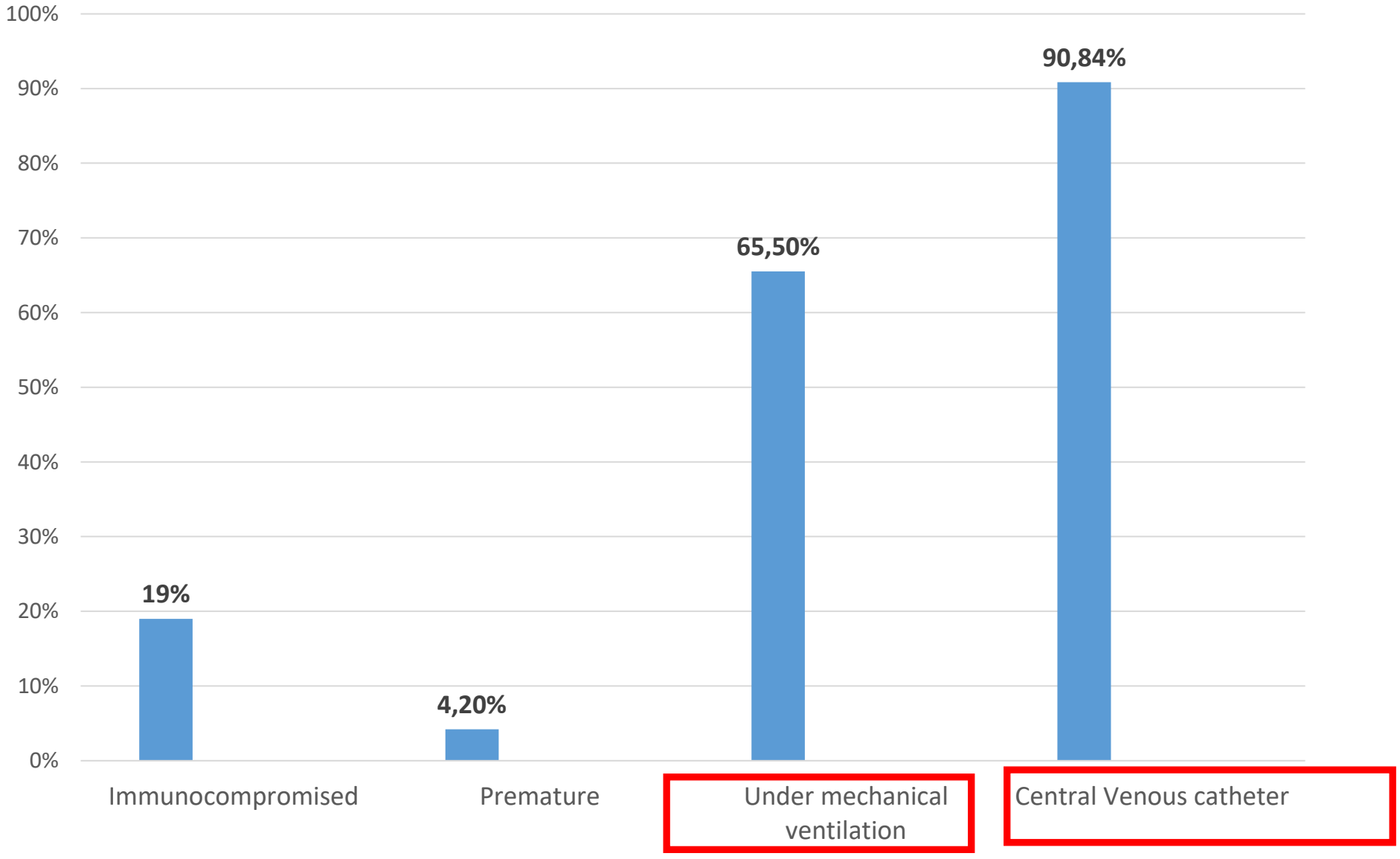
Time from admission to positive culture



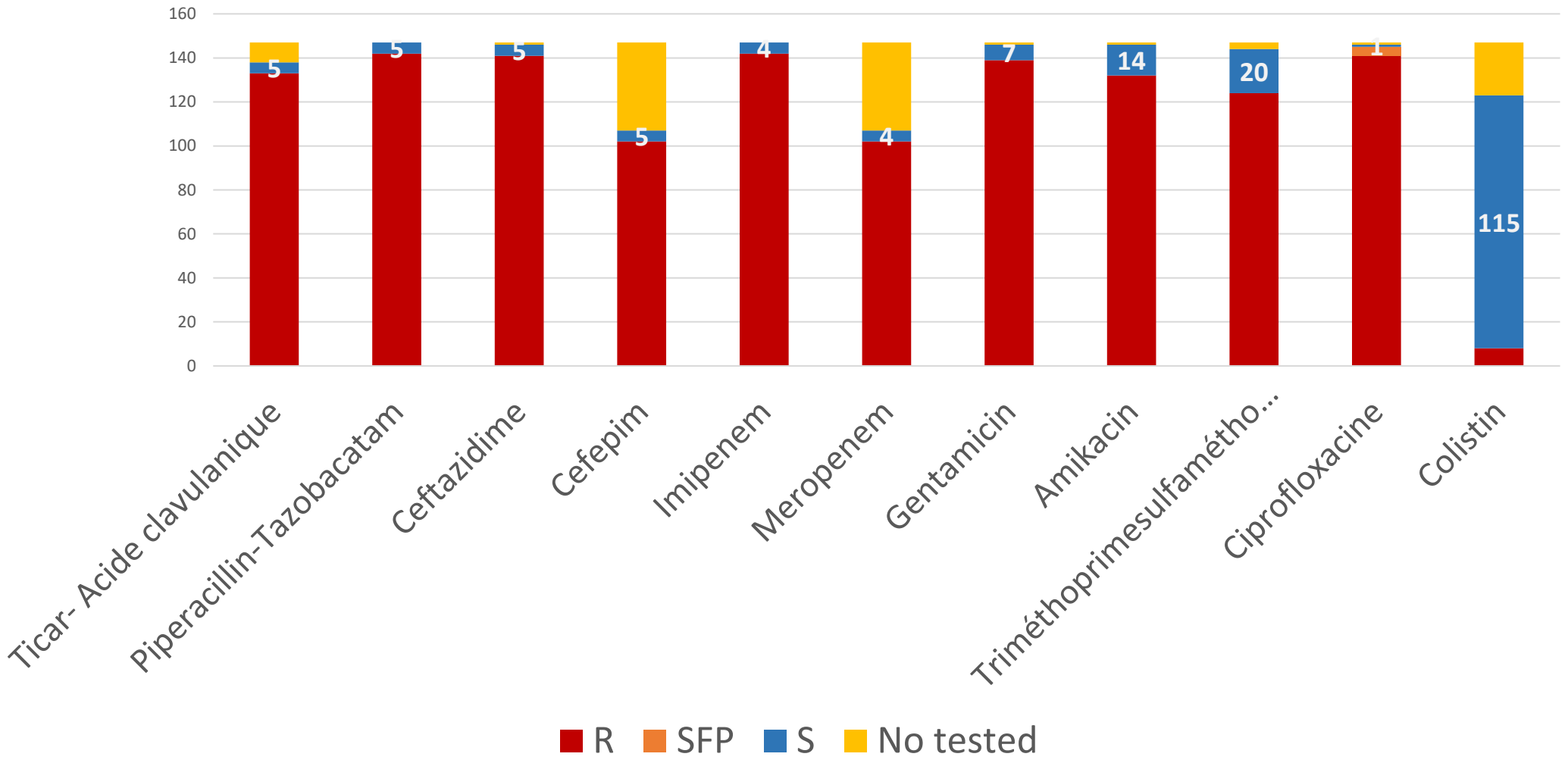
Antimicrobial treatment in patients with at least one antimicrobial administered

Antibiotics	N.	%
Colistin	66	30.7
Imipenem	40	18.6
Glycopeptides	23	10.7
Third –generation cephalosporins	15	7
Tigecyclin	14	6.51
Piperacillin-Tazo	09	4.18
Ertapenem	07	3.25
Amoxicillin+ clavulanic Acid	01	0.46
Other antibiotics	40	18.6
Total	215	100

Risk factors of CRAB infections

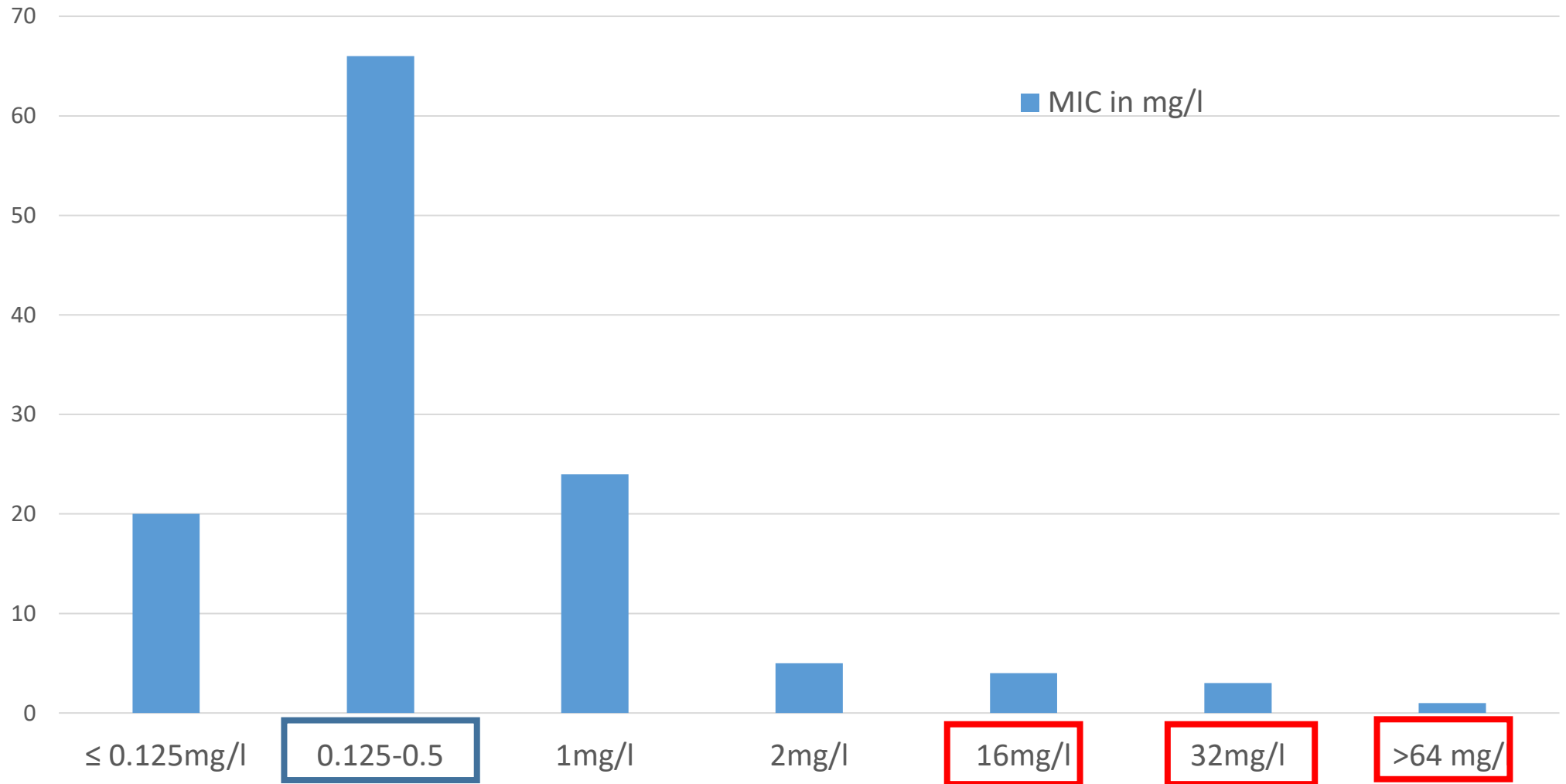


Resistance Profiles



Distibutions of Colistin MICs

5,63% of resistance (n=8)

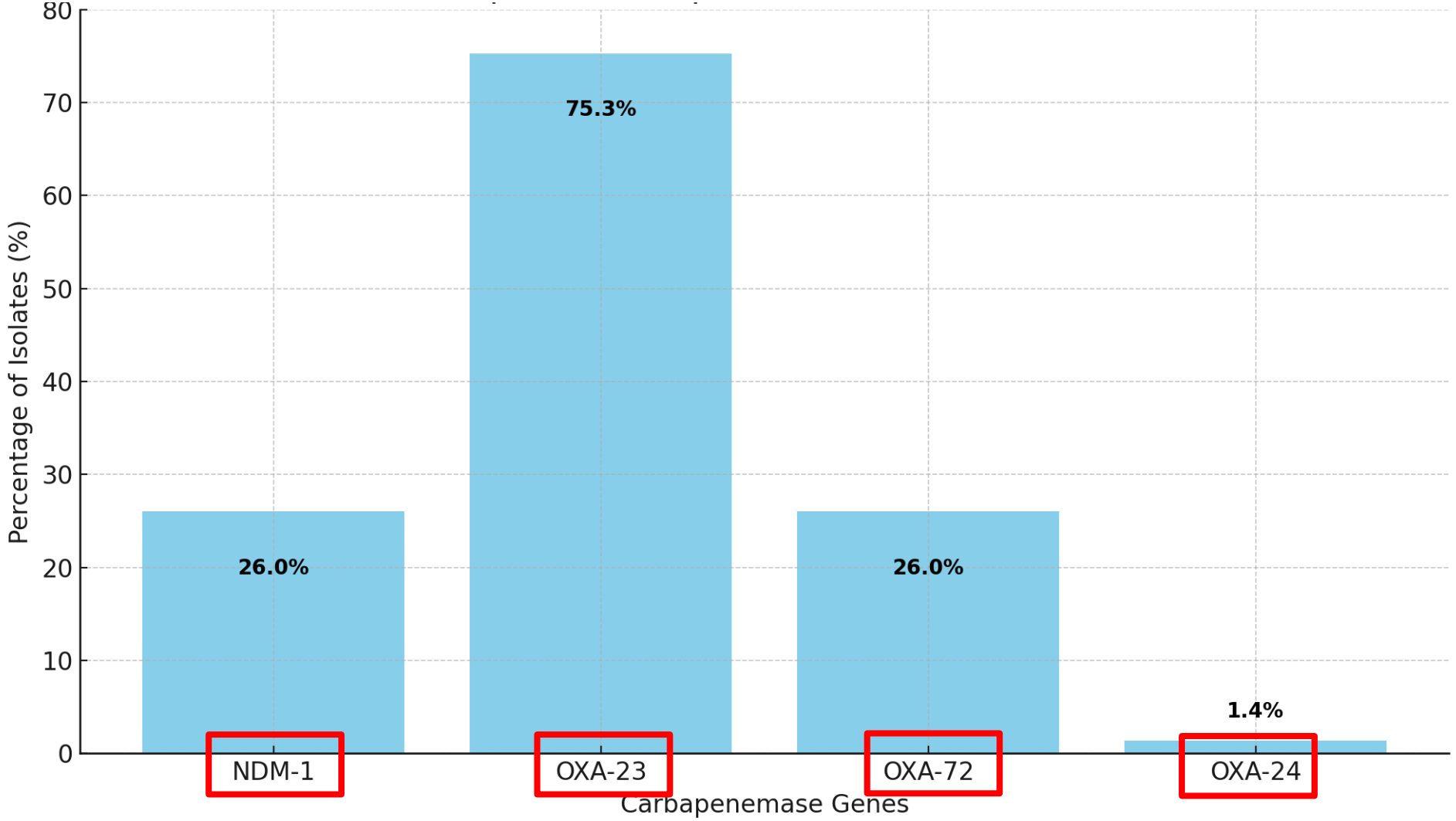


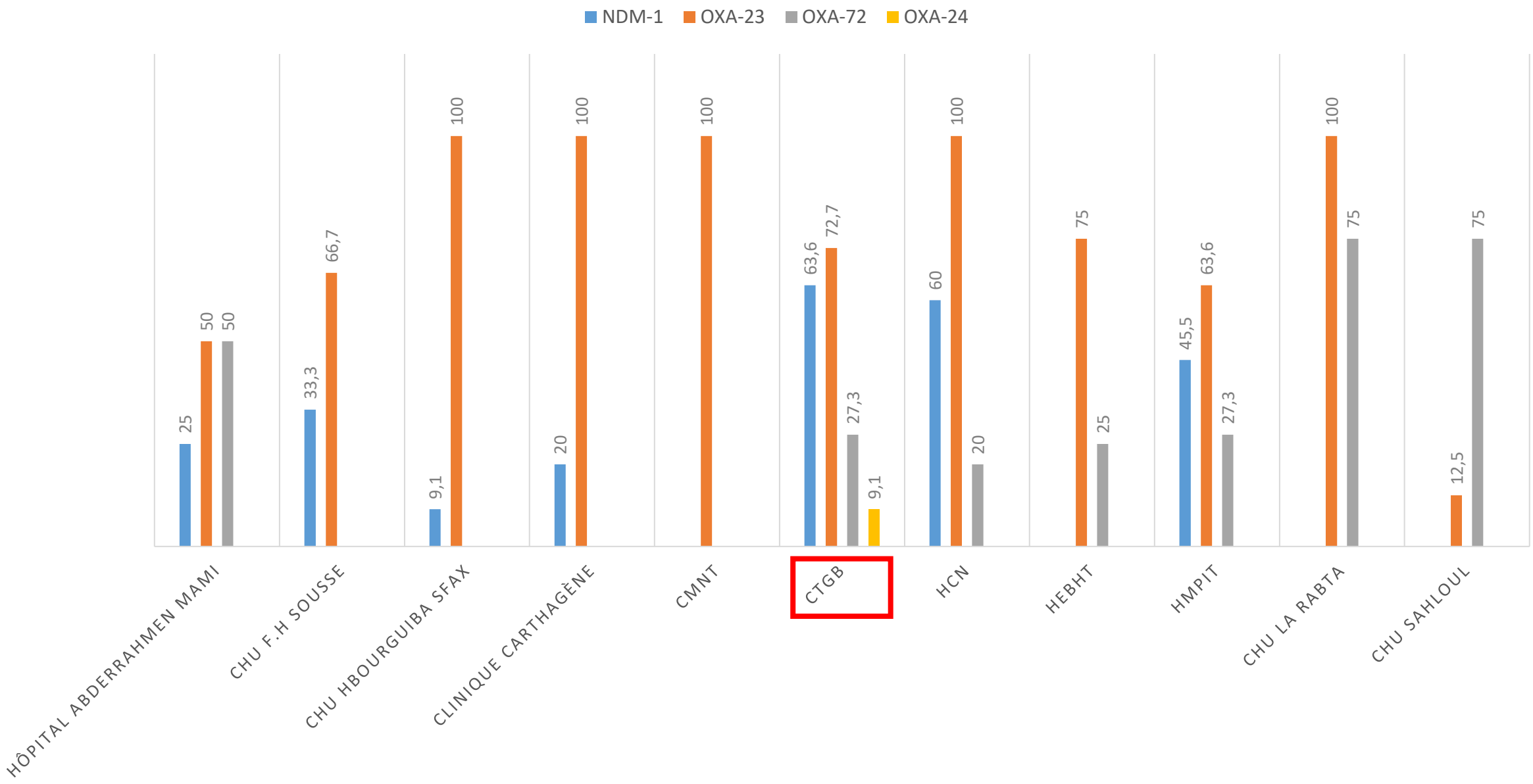
Genome Analysis (n= 74)

Species identification:

- ***A. baumannii (n=72)***
- ***A pittii (n=2)***

Distribution of acquired Carbapenemase




NDM-1
OXA-23
OXA-72
OXA-24
25
50
50
33,3
66,7
9,1
100
20
100
100
63,6
72,7
27,3
9,1
60
100
20
75
25
45,5
63,6
27,3
100
75
12,5
75
HÔPITAL ABDERRAHMEN MAMI
CHU F.H SOUSSE
CHU HBOURGUIBA SFAX
CLINIQUE CARTHAGÈNE
CMNT
CTGB
HCN
HEBHT
HMPIT
CHU LA RABTA
CHU SAHLOUL

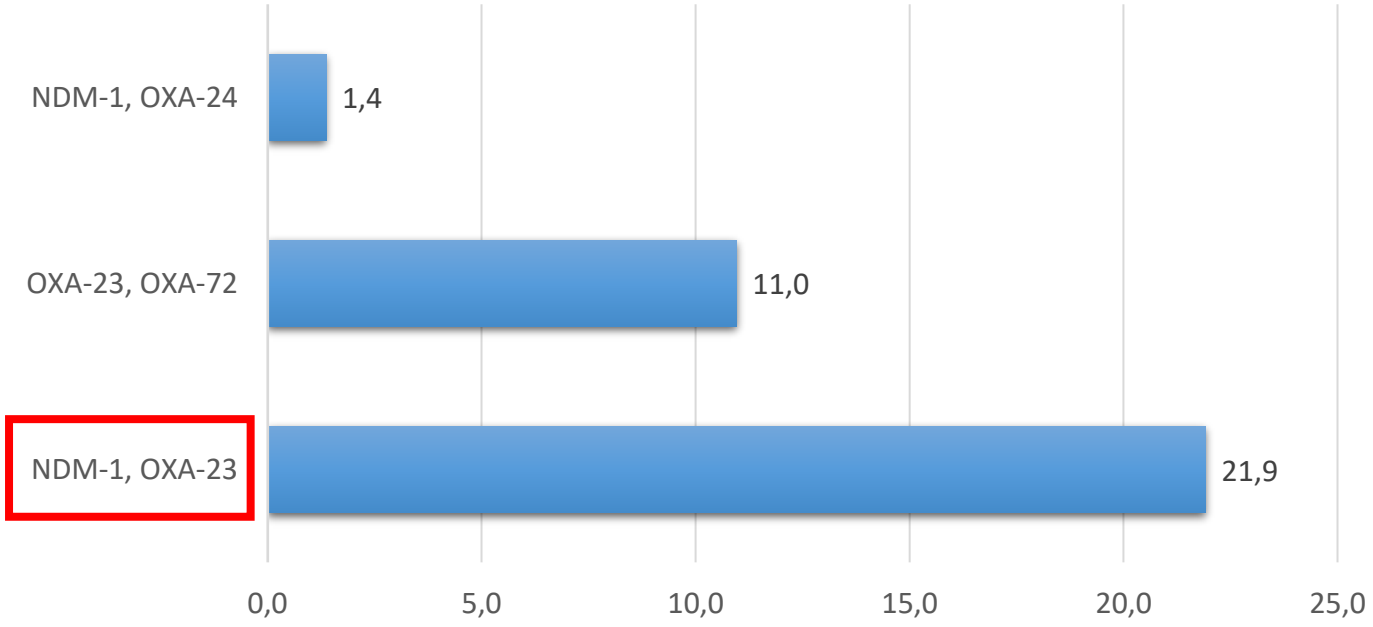
Distribution of Carbapenemases detected by regions

Grand Tunis: OXA 23;NDM1, OXA72, OXA 24

Sousse: OXA 23;NDM1, OXA72

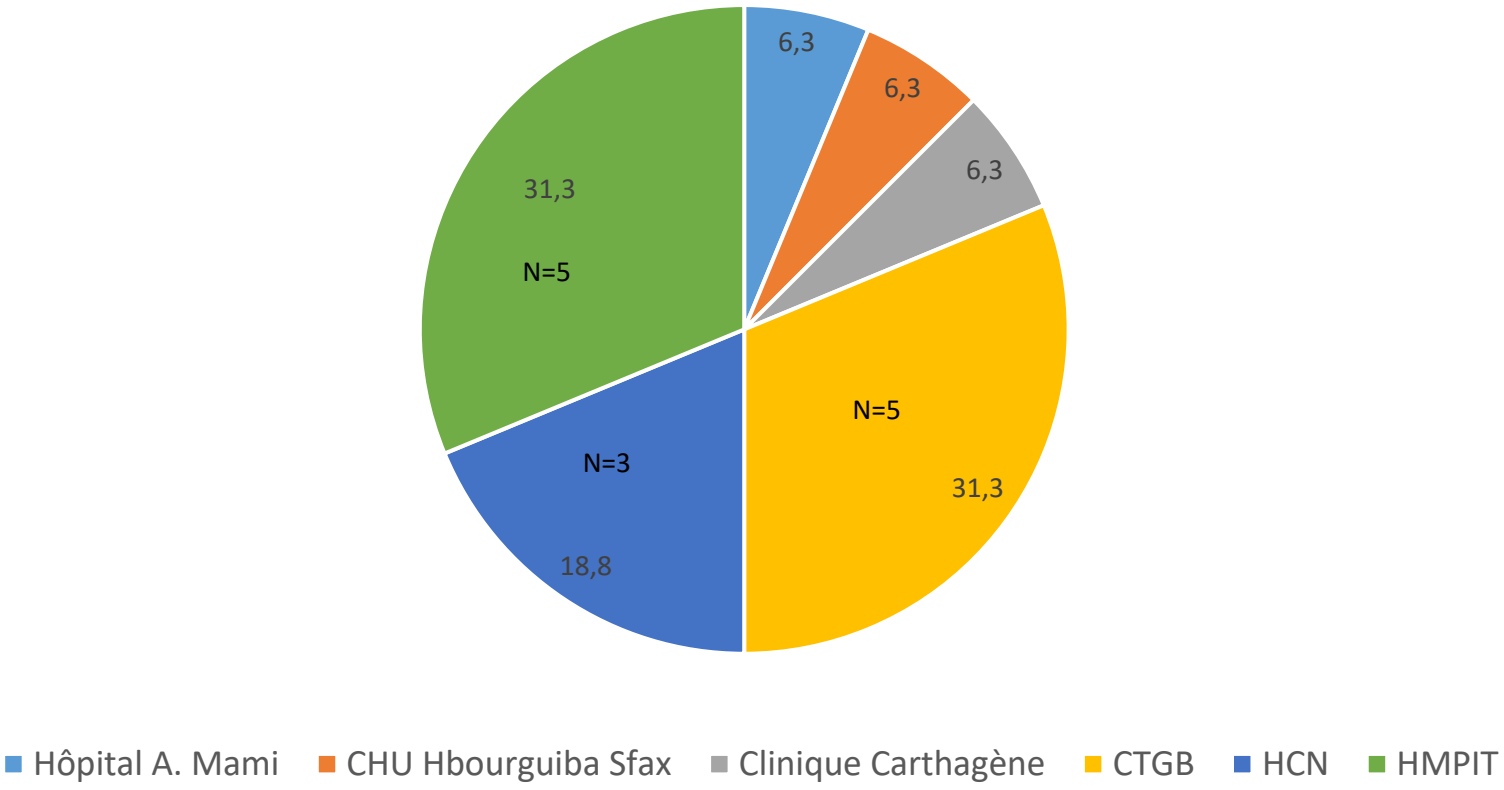
Sfax: OXA 23;NDM1

Carbapenemases combinations CARB Tunisian strains!

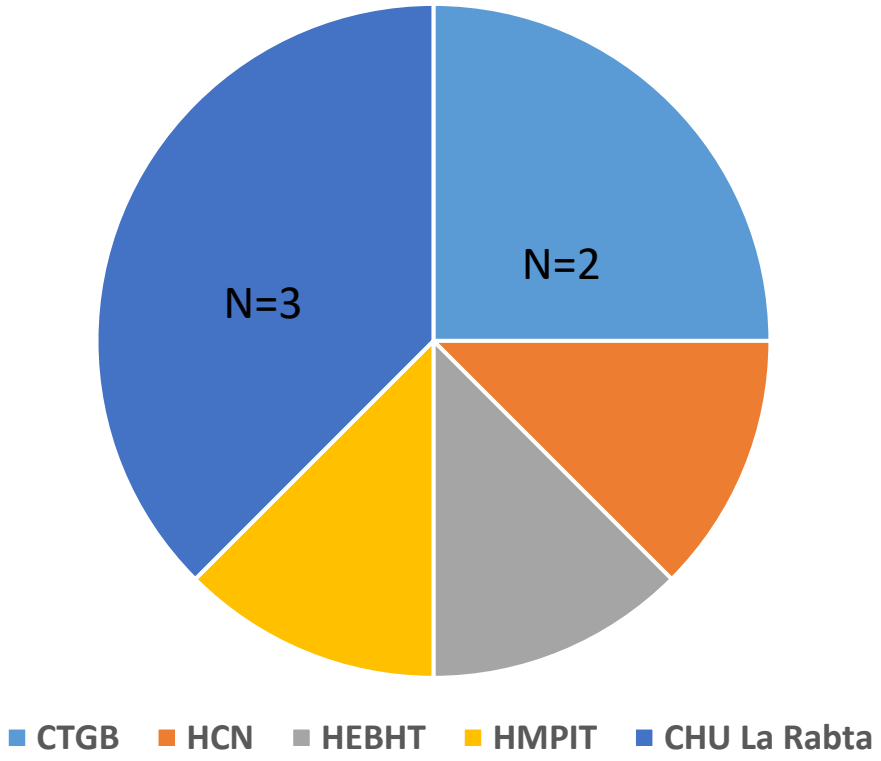


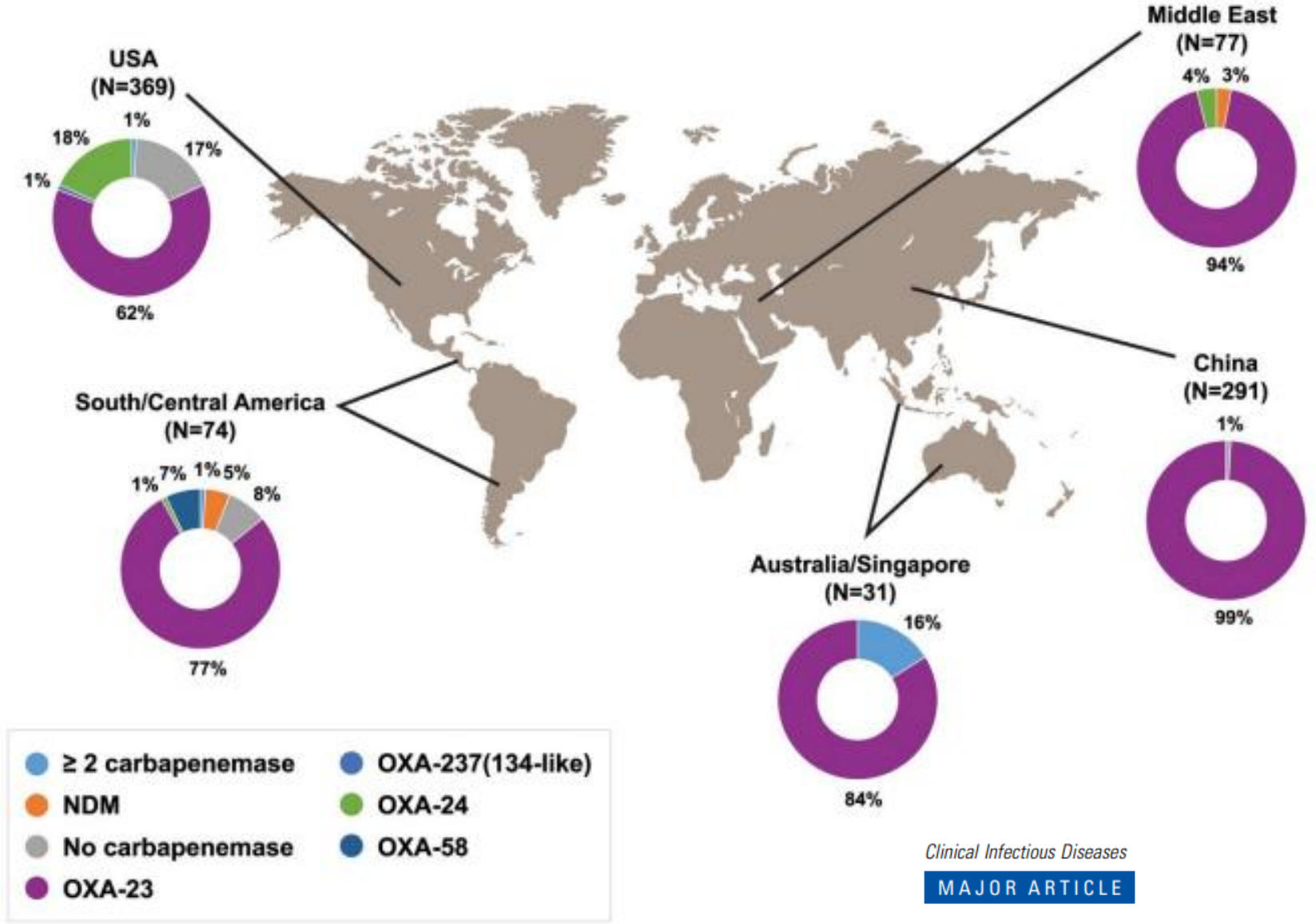
Percentage of Carbapenemases combinations

Combination NDM-1+OXA-23



Combination OXA-23, OXA-72



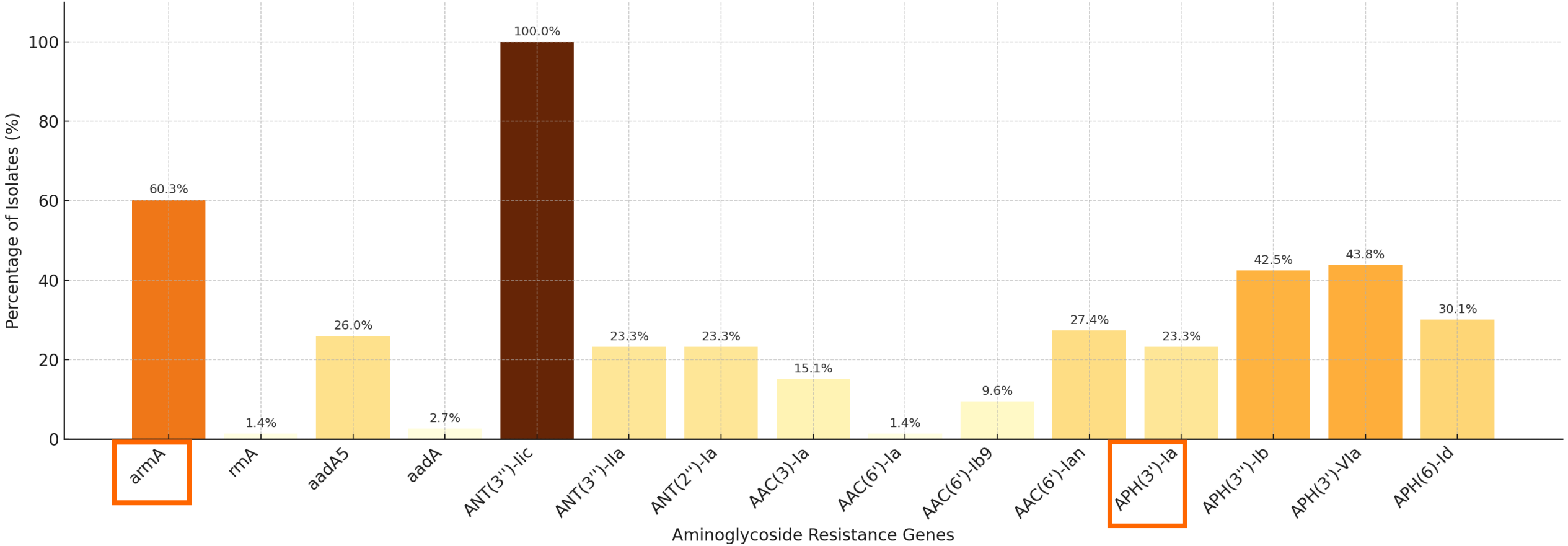


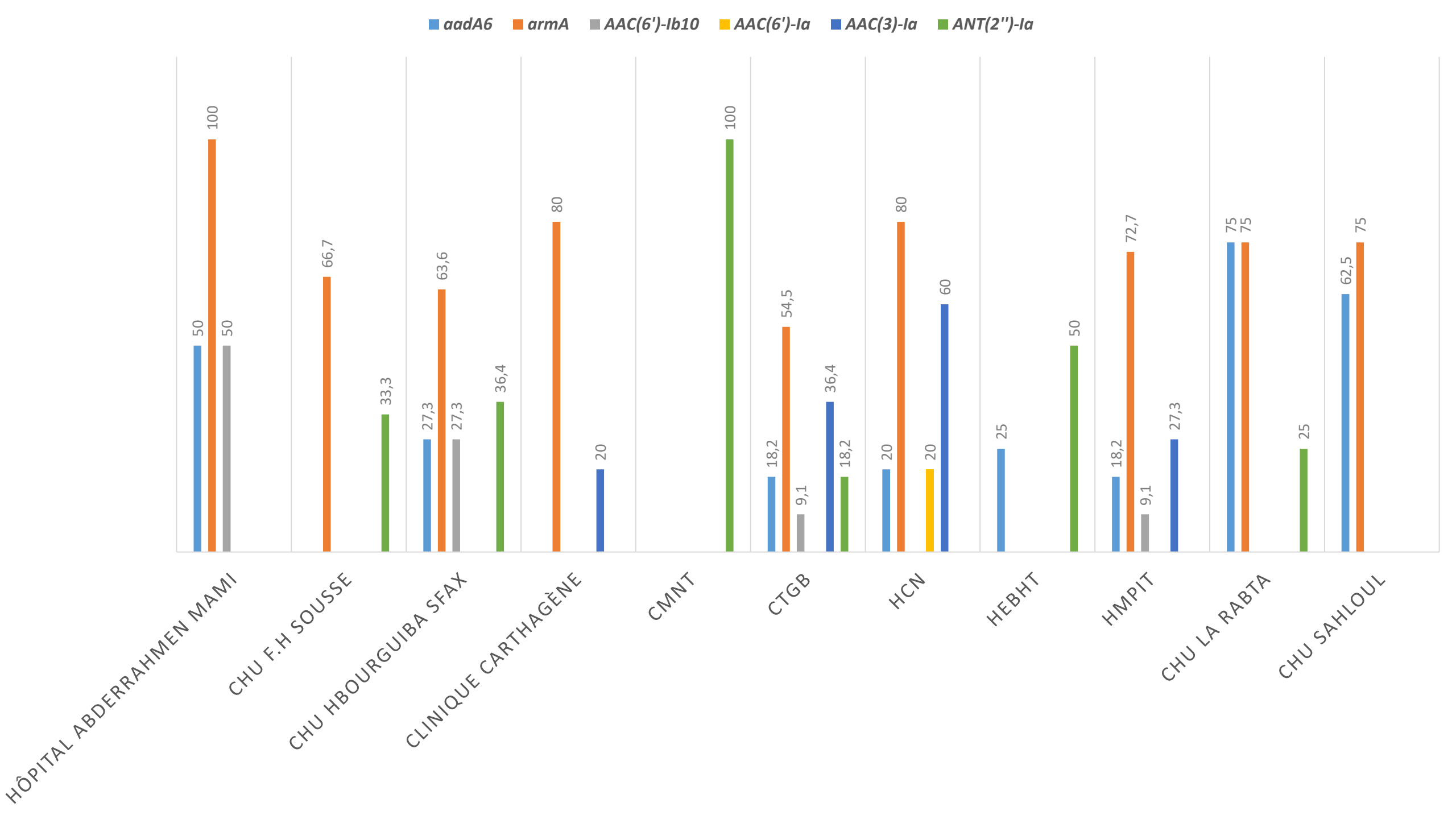
Clinical Infectious Diseases

MAJOR ARTICLE

Clinical Outcomes and Bacterial Characteristics of Carbapenem-resistant *Acinetobacter baumannii* Among Patients From Different Global Regions

Distribution of Aminoglycosides resistance genes




aadA6
armA
AAC(6')-Ib10
AAC(6')-Ia
AAC(3)-Ia
ANT(2'')-Ia
50
100
50
66,7
33,3
27,3
63,6
27,3
36,4
80
20
100
18,2
54,5
9,1
36,4
18,2
20
80
20
60
25
50
18,2
72,7
9,1
27,3
75
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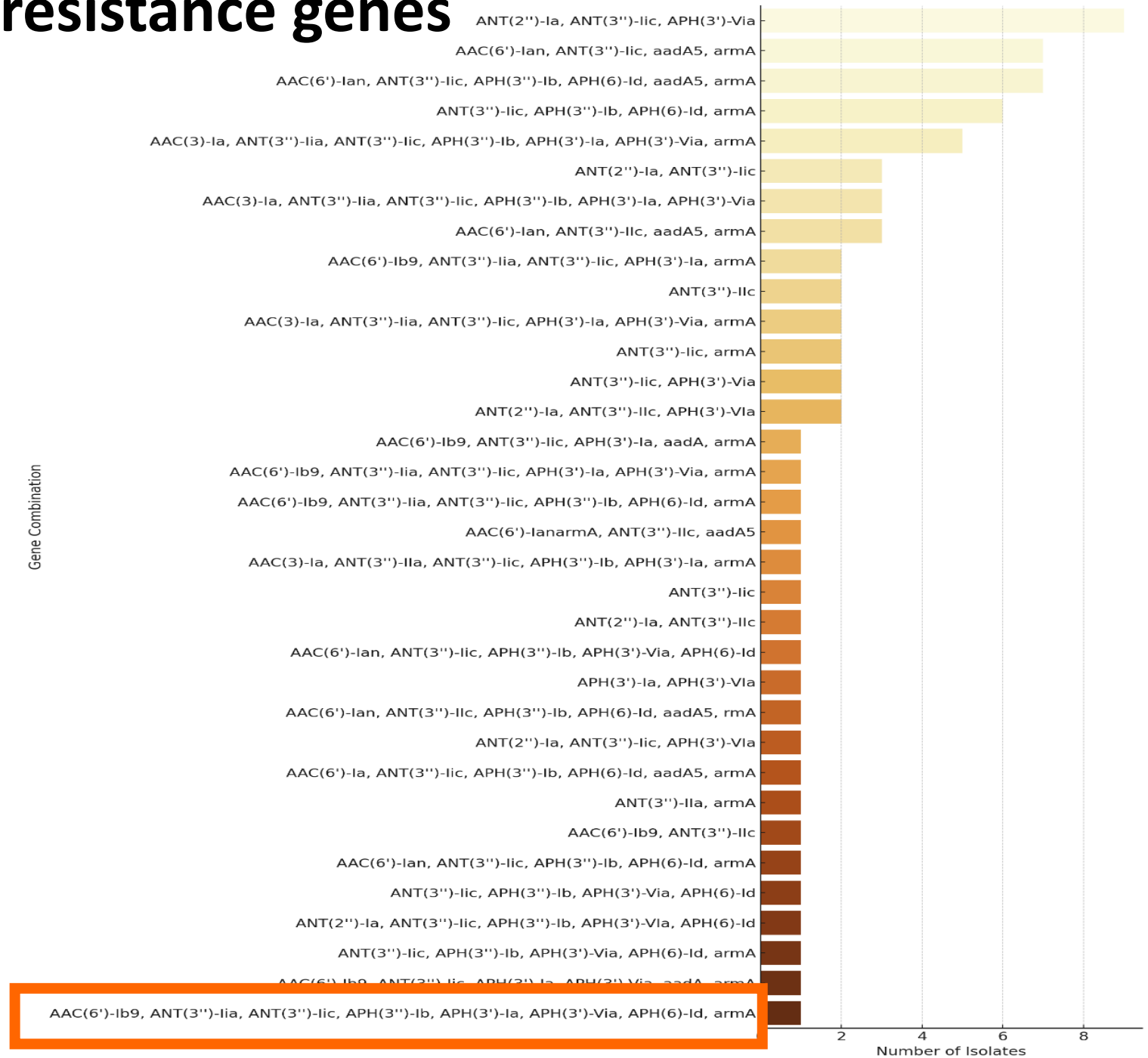
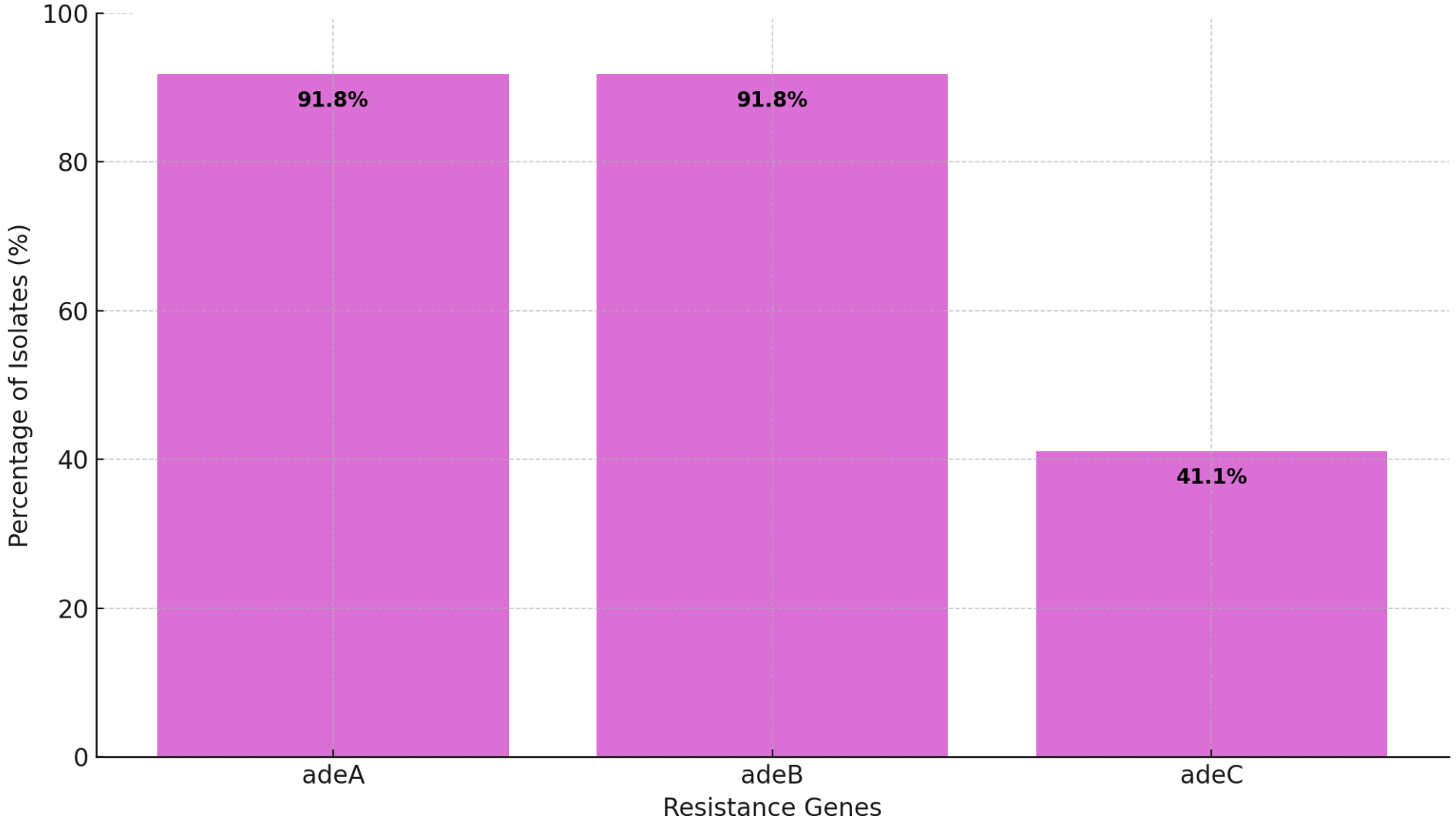
62,5
75
HÔPITAL ABDERRAHMEN MAMI
CHU F.H SOUSSE
CHU HBOURGUIBA SFAX
CLINIQUE CARTHAGÈNE
CMNT
CTGB
HCN
HEBHT
HMPIT
CHU LA RABTA
CHU SAHLOUL

Aminoglycosides resistance genes



Tigecyclin specific mechanisms resistance



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TYPE Review
PUBLISHED 22 January 2024
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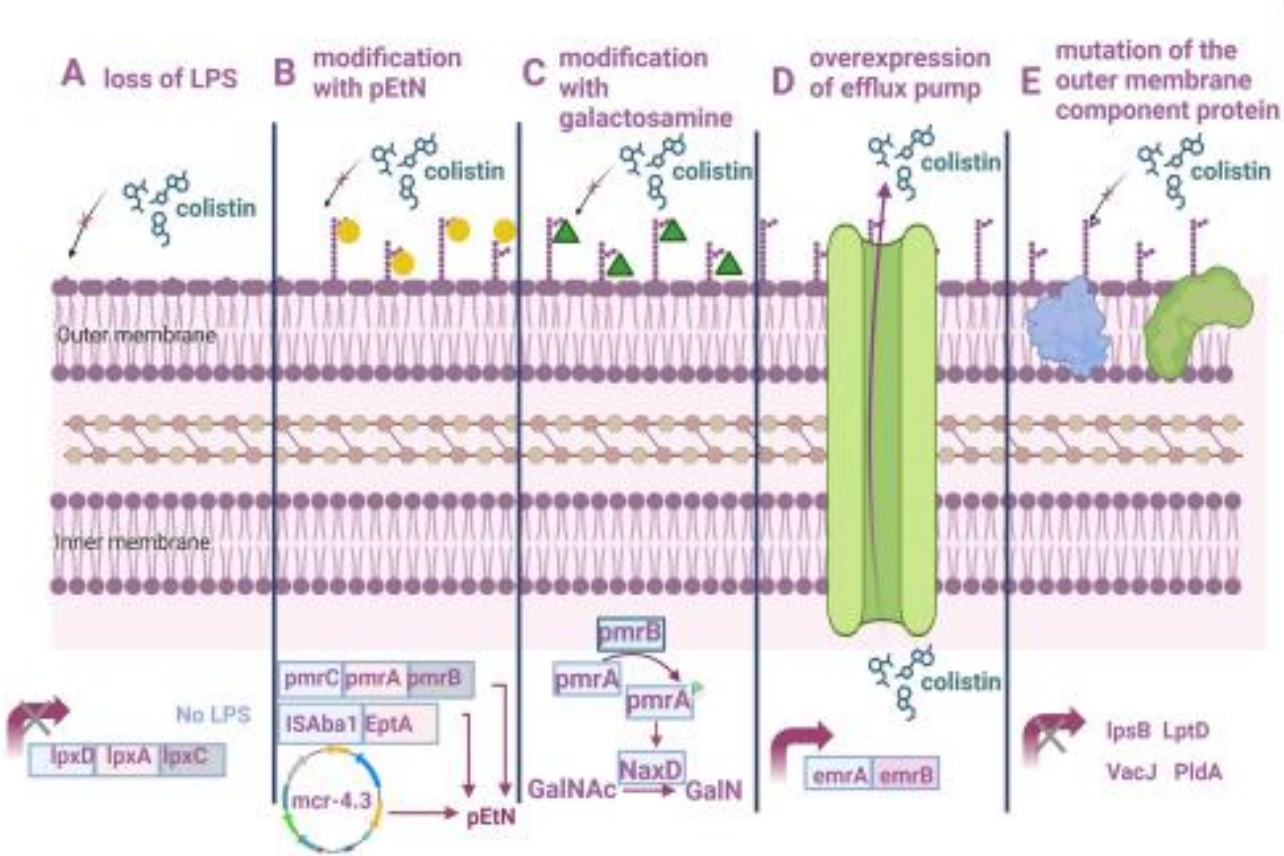
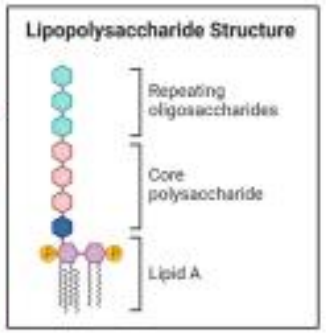
Resistance to colistin





Acinetobacter baumannii: an evolving and cunning opponent

Jingchao Shi[1,2,3], Jianghao Cheng[1], Shourong Liu[4], Yufeng Zhu[1] and Mingli Zhu[3*]

[1]Open Laboratory Medicine, Hangzhou Xixi Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, China, [2]Department of Clinical Laboratory, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, China, [3]Graduate School, Zhejiang Chinese Medical University, Hangzhou, China, [4]Department of Infectious Disease, Hangzhou Xixi Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, China

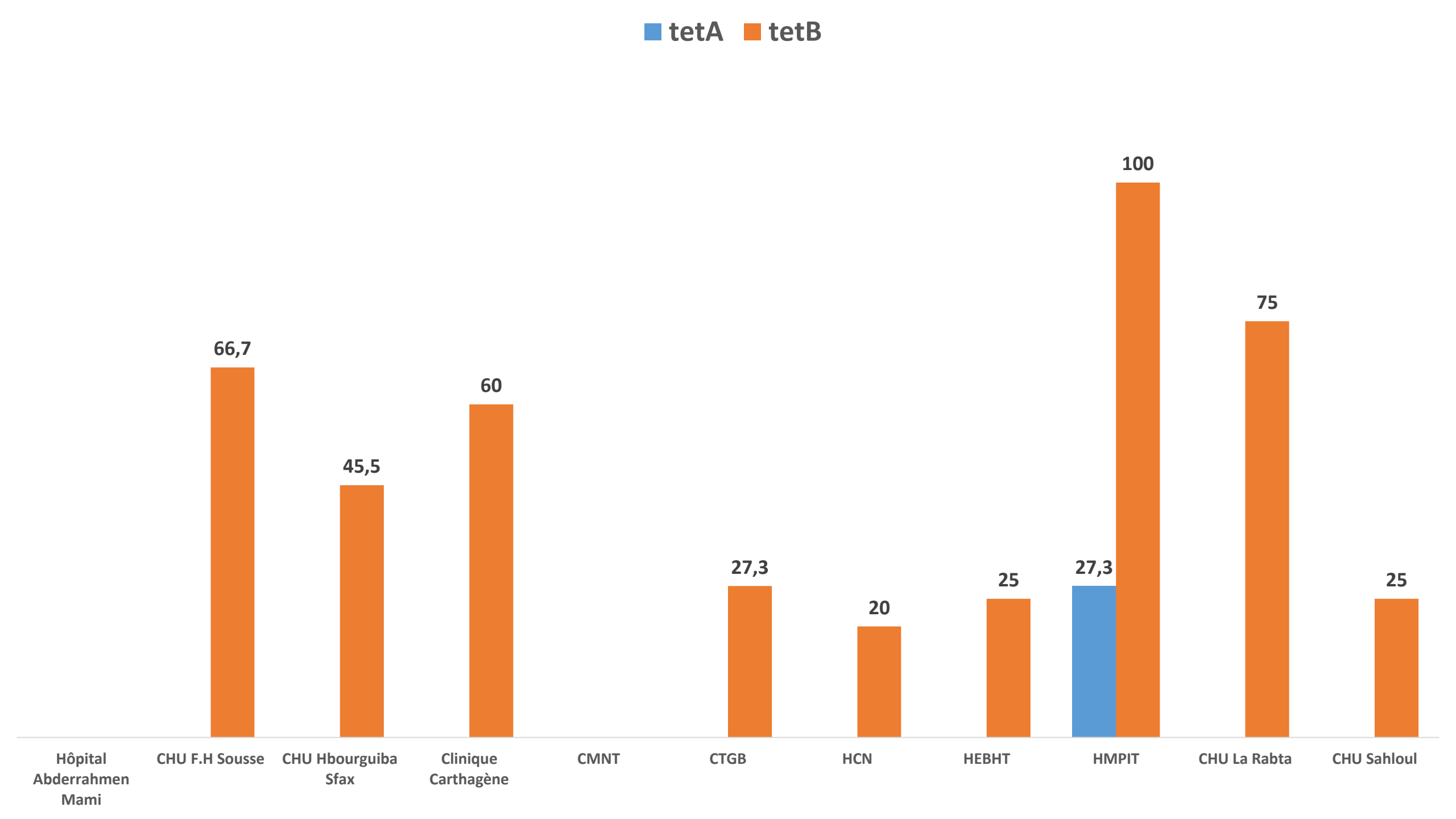
Mechanism underlying polymyxin resistance in *A. baumannii*. **(A)** LPS loss. **(B)** PEtN modification. **(C)** Galactosamine modification. **(D)** Efflux pump overexpression. **(E)** Outer membrane component protein mutations. Created with BioRender.com.

Resistance to colistin

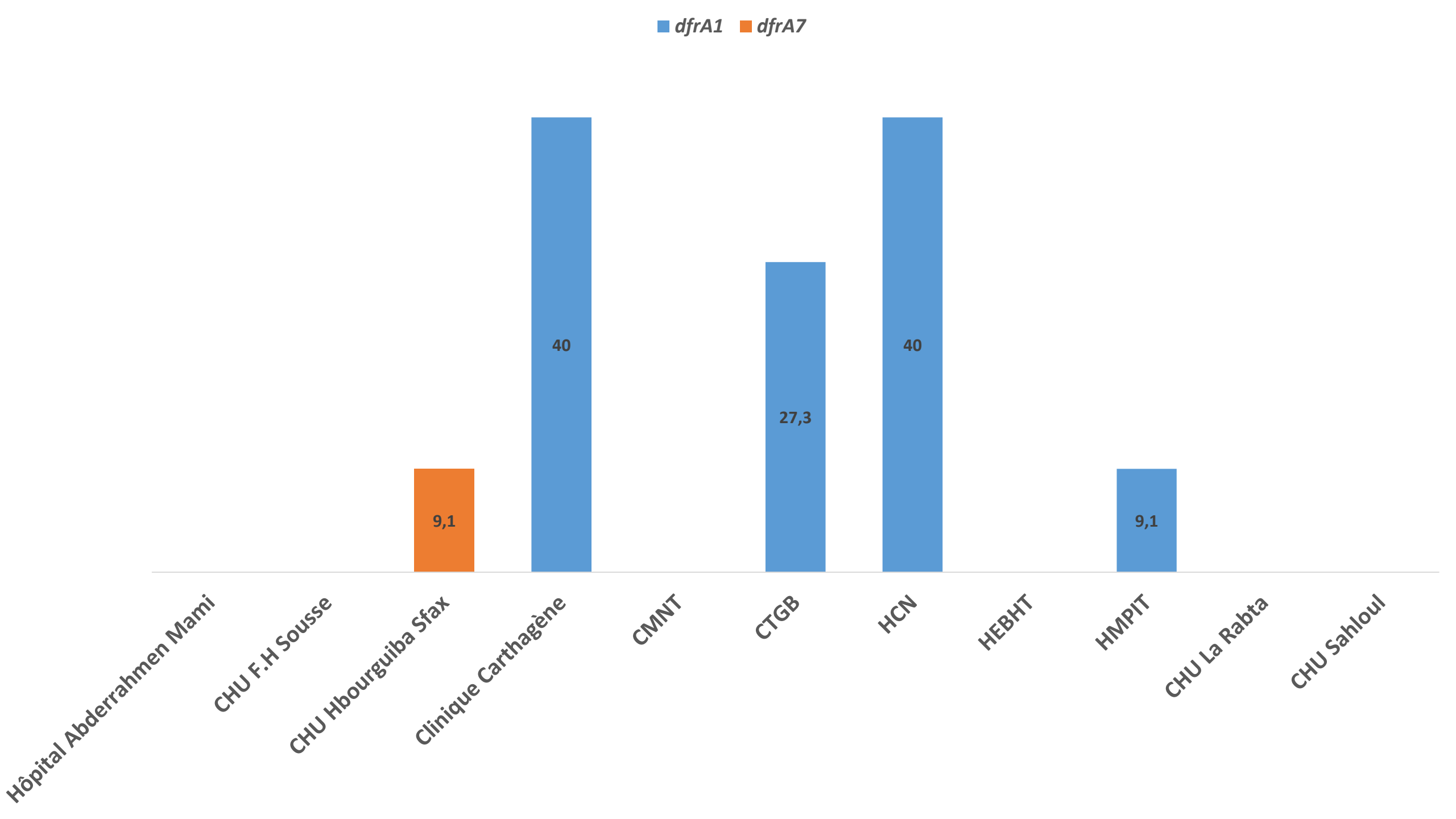
All colistin resistant strains:

- ✓ No plasmid genes (*mcr-1, mcr-2,mcr-3*)
- ✓ Mechanisms of colistin resistance: Mutations++++

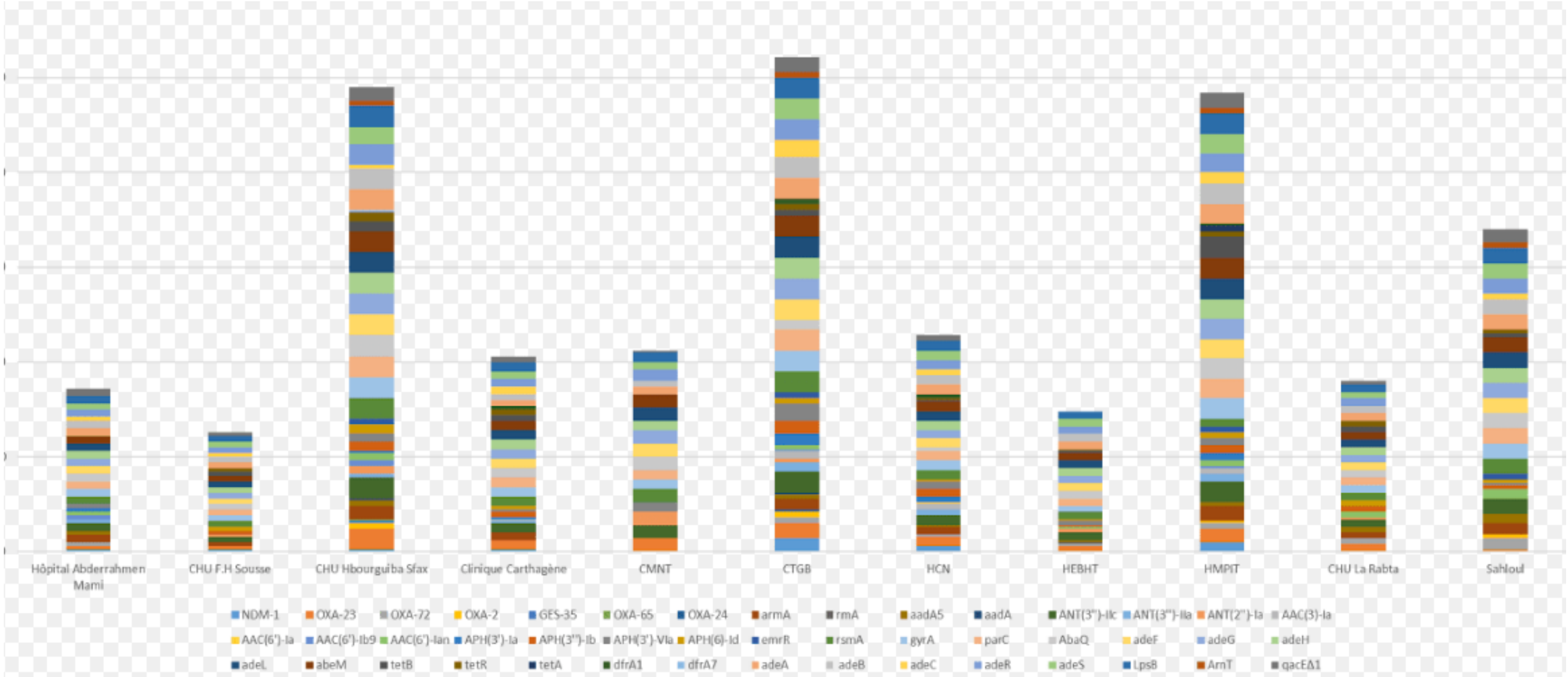
Tetracyclin Resistance


tetA
tetB
66,7
45,5
60
27,3
20
25
27,3
100
75
25
Hôpital Abderrahmen Mami
CHU F.H Sousse
CHU Hbourguiba Sfax
Clinique Carthagène
CMNT
CTGB
HCN
HEBHT
HMPIT
CHU La Rabta
CHU Sahloul

Trimethoprim Resistance


dfrA1
dfrA7
40
9,1
40
27,3
9,1
Hôpital Abderrahmen Mami
CHU F.H Sousse
CHU Hbourguiba Sfax
Clinique Carthagène
CMNT
CTGB
HCN
HEBHT
HMPIT
CHU La Rabta
CHU Sahloul

Distribution of Resistance genes by regions



Resistance to desinfectants !!!

- Gène *qacEΔ1* is detected *in* 63% of strains

▸ Am J Trop Med Hyg. 2023 Dec 11;110(1):136–141. doi: 10.4269/ajtmh.23-0247

Characterization of the Disinfectant Resistance Genes *qacEΔ1* and *cepA* in Carbapenem-Resistant *Klebsiella pneumoniae* Isolates

Xiaoli Liu [1,*], Lin Gong [1], Ernan Liu [1], Changfeng Li [2], Yimei Wang [1]

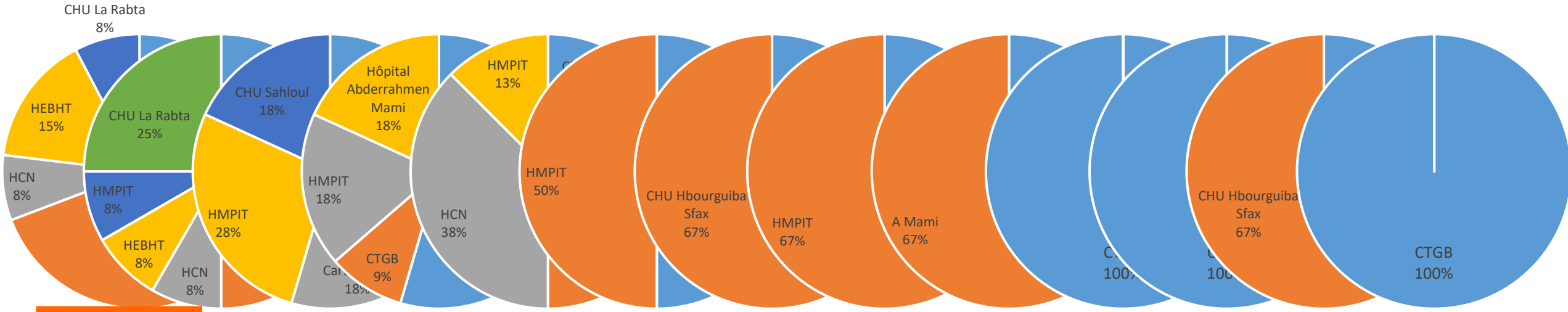
› Int J Food Microbiol. 2023 Nov 2:404:110319. doi: 10.1016/j.ijfoodmicro.2023.110319. Epub 2023 Jul 13.

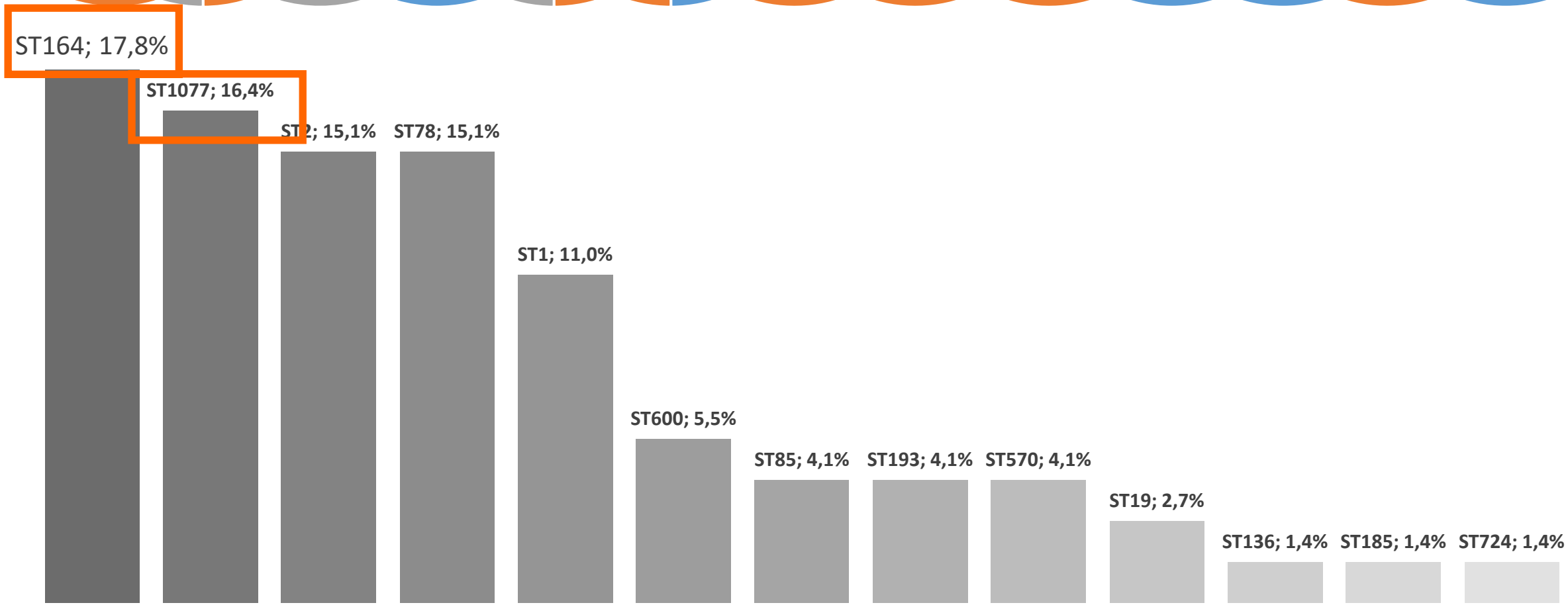
Class 1 integron carrying qacEΔ1 gene confers resistance to disinfectant and antibiotics in Salmonella

Shujuan Chen [1], Jingxia Fu [2], Ke Zhao [3], Shengzhi Yang [3], Chun Li [4], Petri Penttinen [3], Xiaolin Ao [3], Aiping Liu [1], Kaidi Hu [1], Jianlong Li [1], Yong Yang [1], Shuliang Liu [1], Li Bai [5], Likou Zou [6]

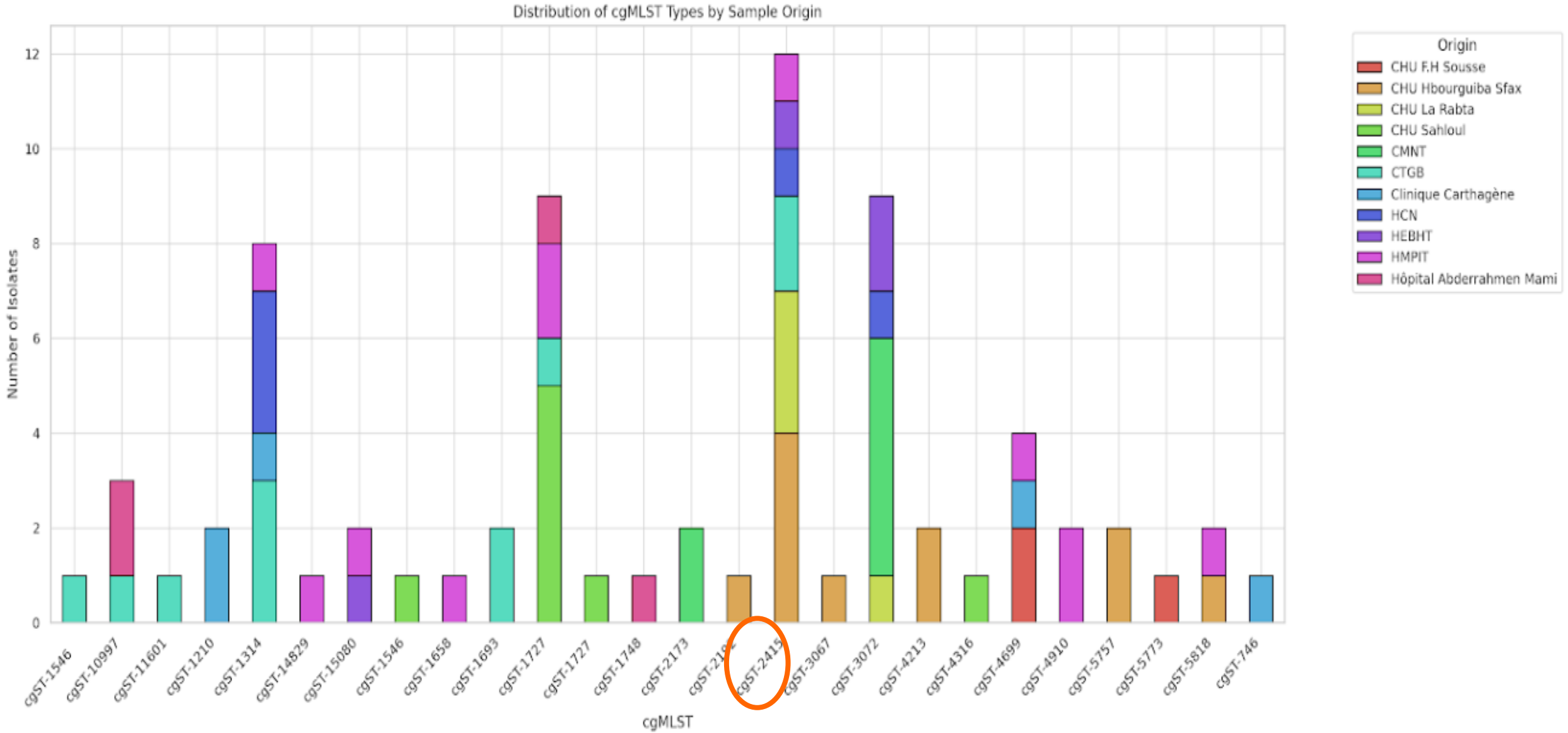
Sequence Type (ST) identification by MLST et cg MLST

MLST: Pasteur Scheme


CHU La Rabta
8%
HEBHT
15%
CHU La Rabta
25%
CHU Sahloul
18%
Hôpital
Abderrahmen
Mami
18%
HMPIT
13%
HCN
8%
HMPIT
8%
HMPIT
28%
HMPIT
18%
HMPIT
50%
HEBHT
8%
HCN
8%
CTGB
9%
HCN
38%
CHU Hbourguiba
Sfax
67%
HMPIT
67%
A Mami
67%
CHU Hbourguiba
Sfax
67%
CTGB
100%


ST164; 17,8%
ST1077; 16,4%
ST78; 15,1%
ST1; 11,0%
ST600; 5,5%
ST85; 4,1%
ST193; 4,1%
ST570; 4,1%
ST19; 2,7%
ST136; 1,4%
ST185; 1,4%
ST724; 1,4%

Cg-MLST


Distribution of cgMLST Types by Sample Origin
Number of Isolates
cgMLST
Origin
CHU F.H Sousse
CHU Hbourguiba Sfax
CHU La Rabta
CHU Sahloul
CMNT
CTGB
Clinique Carthagène
HCN
HEBHT
HMPIT
Hôpital Abderrahmen Mami
cgST-1546
cgST-10997
cgST-11601
cgST-1210
cgST-1314
cgST-14829
cgST-15080
cgST-1546
cgST-1658
cgST-1693
cgST-1727
cgST-1727
cgST-1748
cgST-2173
cgST-2415
cgST-3067
cgST-3072
cgST-4213
cgST-4316
cgST-4699
cgST-4910
cgST-5757
cgST-5773
cgST-5818
cgST-746

Take home messages

- ❑ The majority of Tunisian *A baumannii* strains are CRAB
- ❑ The resistance to other antibiotics is very important
- ❑ Acquired carbapenemases: OXA23+++, emerging of NDM-1
- ❑ Resistance to colistin (5.4%): Mutations++
- ❑Resistance to desinfectants (63%)
- ❑ Circulating strains are belonging essentially to ST 164 and ST 1077
- ❑Prevention and control measures must be applied

The way forward…

- Virulence genes
- Molecular epidemiology and the global distribution of CRAB strains circulating in Tunisia: Phylogenetic study
- Publication

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O. Gargouri, K Mefteh, M, Hamdoun, Y, Chebbi, Z. Hamzaoui.

Thank you for your attention