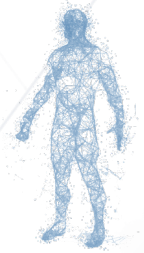
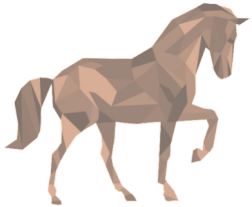


Decreased susceptibility to a major component of disinfectant among equine *Pseudomonas* *aeruginosa* (PSAE) isolates

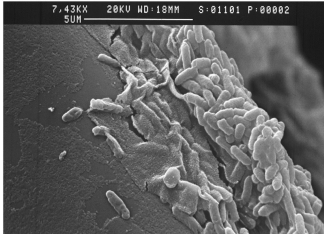


Marine Pottier^{1, 2, 3, @}, Sophie Castagnet^{2, 3}, François Gravey¹, Michel Auzou⁴, Guillaume Leduc⁴,
Simon Le Hello^{1, 4, 5, 6}, Albertine Léon^{2, 3, 6}





Background

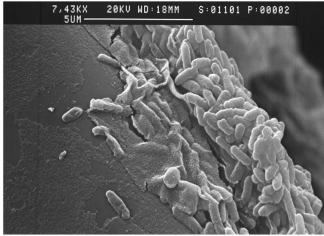


SEM observation of Pseudomonas aeruginosa biofilm, (Benamara et al. 2014).

- Gram-negative, mesophilic and strictly aerobic bacilli
- **Opportunistic** pathogen (human/animal)

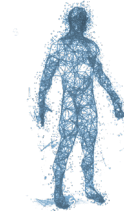


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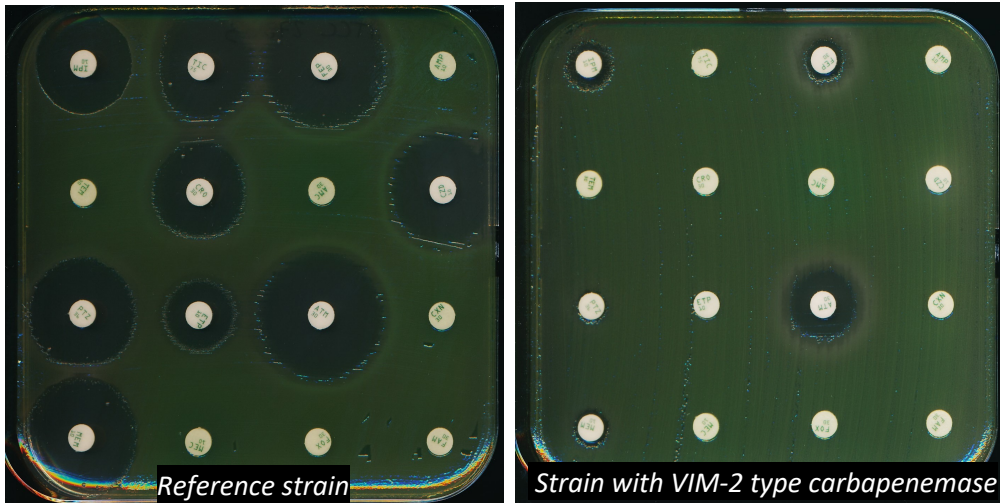


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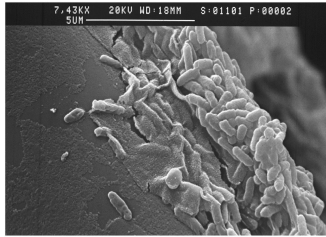
- 4th leading cause of HCAI in 2017 (*Santé Publique France*)
- **Therapeutic failures** : strains close to total resistance to antibiotics (*Alnour et al. 2017*)



WGS antibiogram

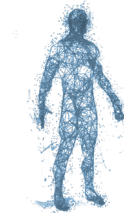


Background

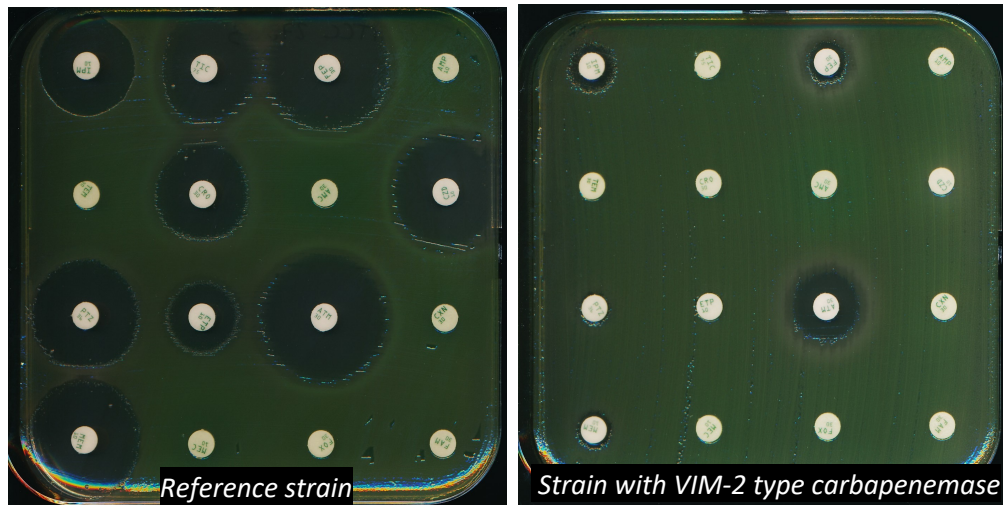


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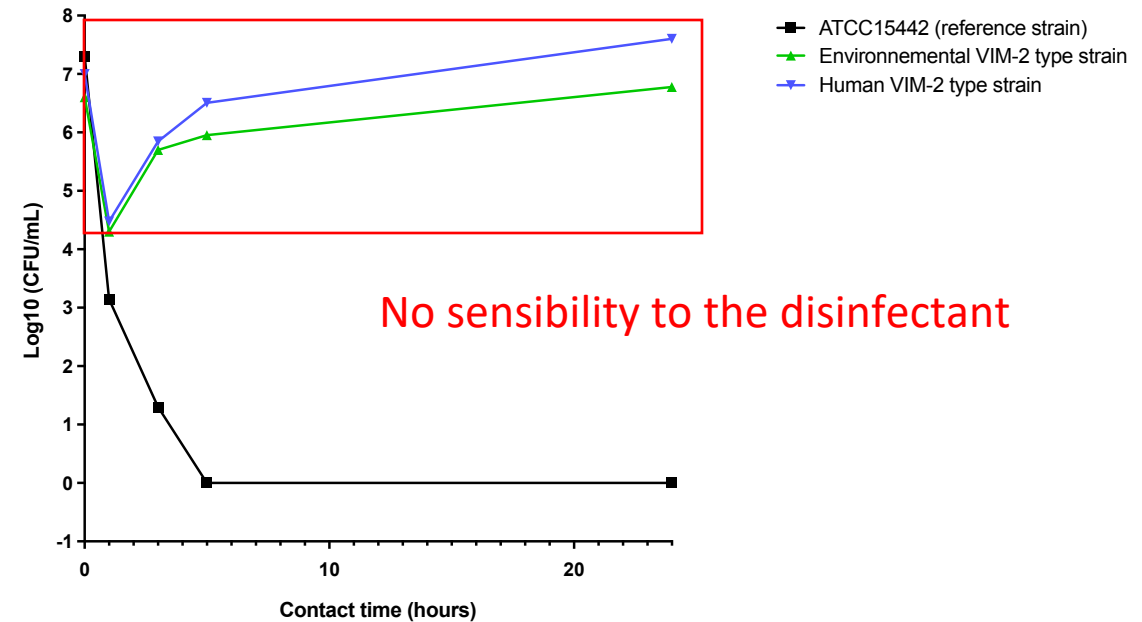
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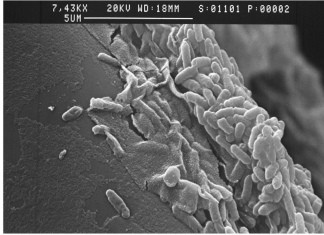


WGS antibiogram





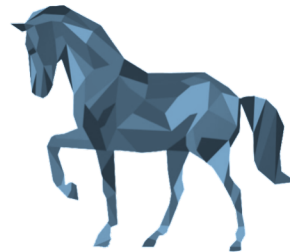
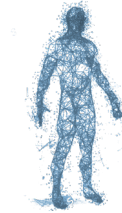
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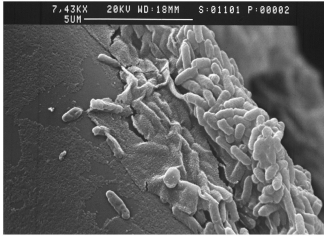
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- **Opportunistic** pathogen (human/animal)



- **STI** = sexually transmitted infections like contagious equine metritis (*Rapport de monte 2021, RESPE*)
- Mandatory screening for before breeding in thoroughbreds
- **Resistance selection** through antibiotic / disinfectant misuse

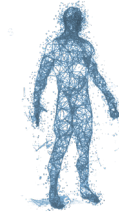


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- **Decrease sensibility to disinfectant**



RESPE : 2021 Breeding Reports

TESTING FOR OTHER PATHOGENS CAUSING METRITIS

The PS and AQPS studbooks require stallions and mares to be tested for the following two bacteria

Pseudomonas aeruginosa - Category 3* health hazard

- 13,840 horses tested

- 102 positive, i.e. 0.1% of horses tested

- **STI** = sexually transmitted infections like contagious equine metritis (*Rapport de monte 2021, RESPE*)
- Mandatory screening for before breeding in thoroughbreds
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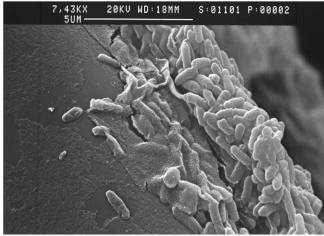
RESPE

<https://respe.net/reproduction-saison-de-monte-2021-rapport-numero-1/>





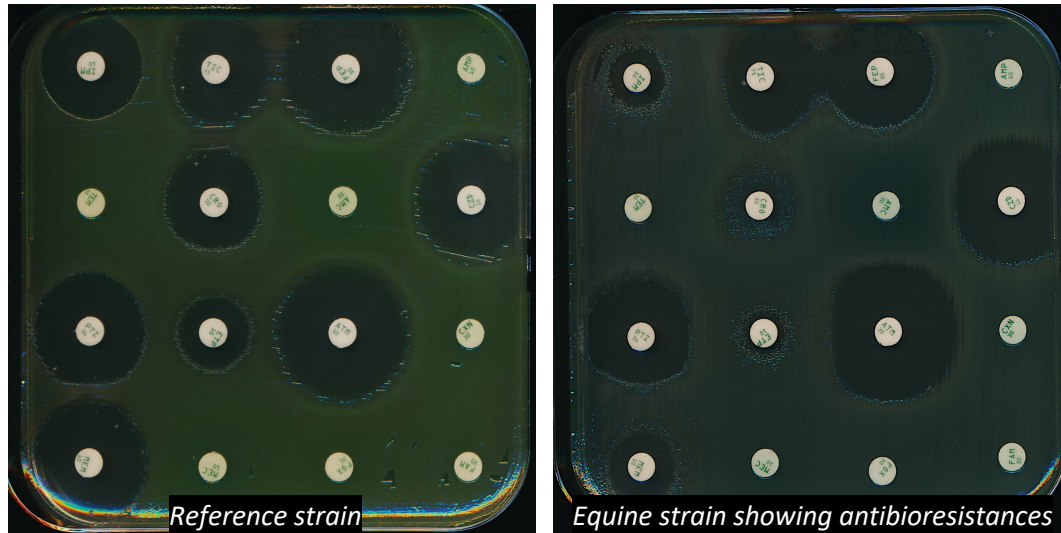
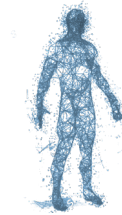
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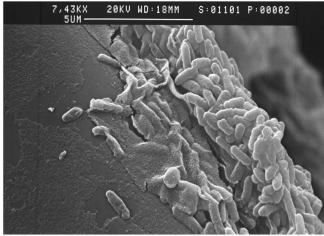
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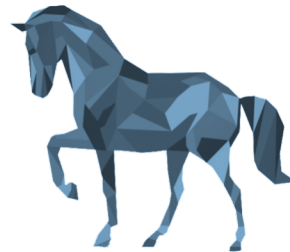
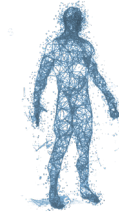


Background



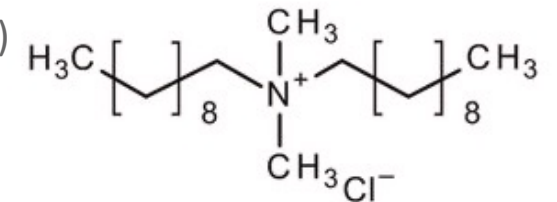
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- Mandatory screening for before breeding in thoroughbreds
- **Resistance selection** through antibiotic / disinfectant misuse

- **DDAC** (didecyltrimethylammonium chloride) = **QACs family** (Quaternary ammonium compound)
- Used in **disinfection products**
- Used in human and veterinary medicine



Chemical formula for DDAC (Sigma-Aldrich)



Objective

Evaluate the existence of PSAE strains with DDAC decreased susceptibility among animals in Normandy

I.1

Materials and methods

Sampling

183 PSAE strains

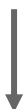


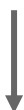
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Occular				1	1	2
Genital					1	1
Wound / cutaneous			1			1
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Auricular					1	1
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Total	23	10	19	77	54	183

I.1

Materials and methods Sampling

183 PSAE strains

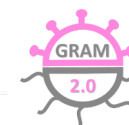


isolated from samples
received for analysis



Table I: Repartition of strains origin.

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	1996 - 2015	2017	2018	2019	2020	
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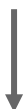


I.1

Materials and methods

Sampling

183 PSAE strains



Period 1996 - 2020

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Genital				1		1
Total	23	10	19	77	54	183

I.1

Materials and methods Sampling

183 PSAE strains



Period 1996 - 2020



Mainly in **2019** and **2020**

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Auricular					1	1
Digestive					1	1
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Genital				1		1
Total	23	10	19	77	54	183

1

2

I.1

Materials and methods

Sampling

183 PSAE strains



Various animal origin

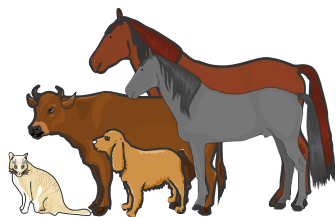


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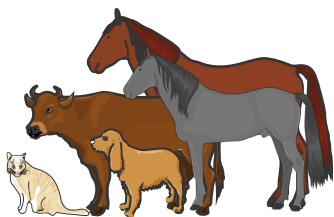
I.1

Materials and methods Sampling

183 PSAE strains



Various animal origin



Most represented :

- **Equine (146/183 → 80%)**

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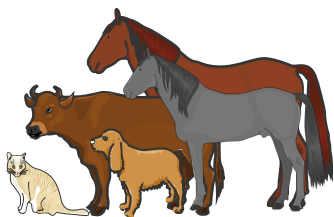
I.1

Materials and methods Sampling

183 PSAE strains



Various animal origin



Most represented :

- **Equine (146/183 → 80%)**
- **Canine (33/183 → 18%)**

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Sample origine	Isolation year					Total
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Auricular		5	8	7	9	29
Ocular				1	1	2
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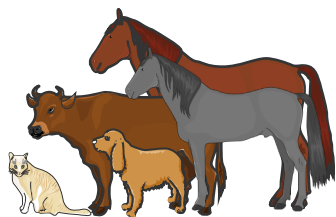
I.1

Materials and methods Sampling

183 PSAE strains



Various animal origin



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- **Canine (33/183 → 18%)**

For others n=4 → 2%

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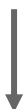
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Occular			1	1	1	3
Lymphatic					1	1
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Auricular					1	1
Digestive					1	1
4 Asinine				1		1
Genital				1		1
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I.1

Materials and methods

Sampling

183 PSAE strains



Strains from various samples

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I.1

Materials and methods Sampling

183 PSAE strains



Strains from various samples



Most represented :

- Genital (101/183 → 55%)
mainly associated at equines samples

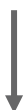
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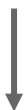
I.1

Materials and methods Sampling

183 PSAE strains



Strains from various samples



Most represented :

- Genital (101/183 → 55%)
mainly associated at equines samples
- Auricular (30/183 → 16%)
mainly associated at canines samples

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Lymphatic					1	1
Feline					2	2
Auricular					1	1
Digestive					1	1
Asinine				1		1
Genital				1		1
Total	23	10	19	77	54	183

I.1

Materials and methods

Sampling

183 PSAE strains



Strains from various samples



Most represented :

- Genital (101/183 → 55%)
mainly associated at equines samples
- Auricular (30/183 → 16%)
mainly associated at canines samples
- Respiratory (12/183 → 7%)
only equine

Table I: Repartition of strains origin.

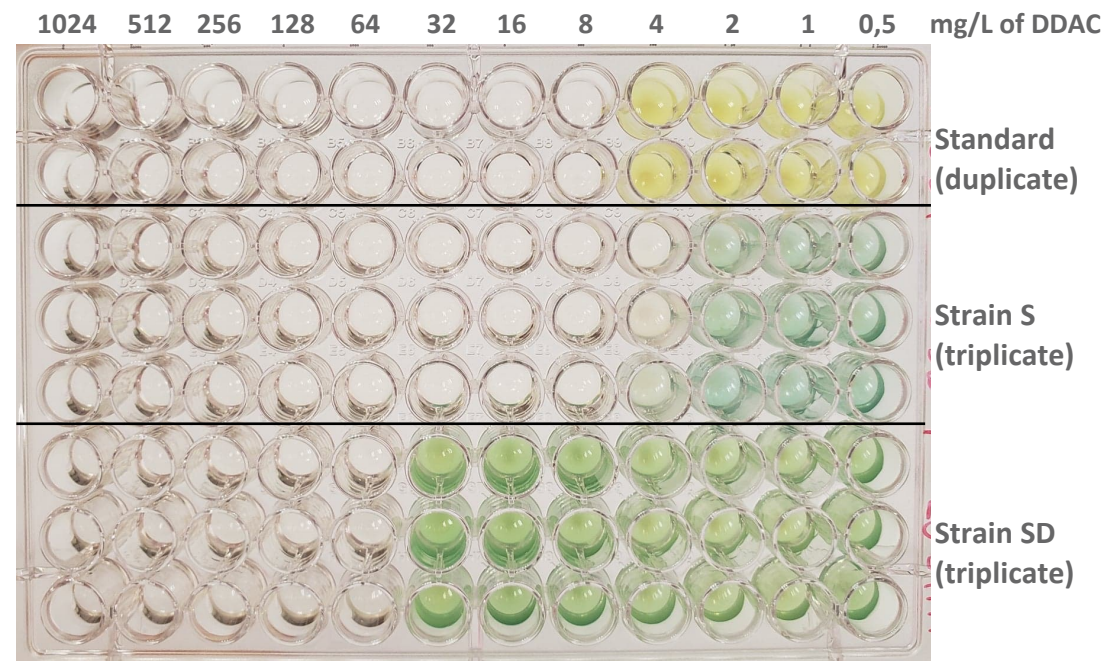
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Asinine				1		1
Genital				1		1
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1.2

Materials and methods

DDAC decrease sensibility test

- **MIC** (minimal inhibitory concentration) determination



Picture of DDAC MIC determination plate

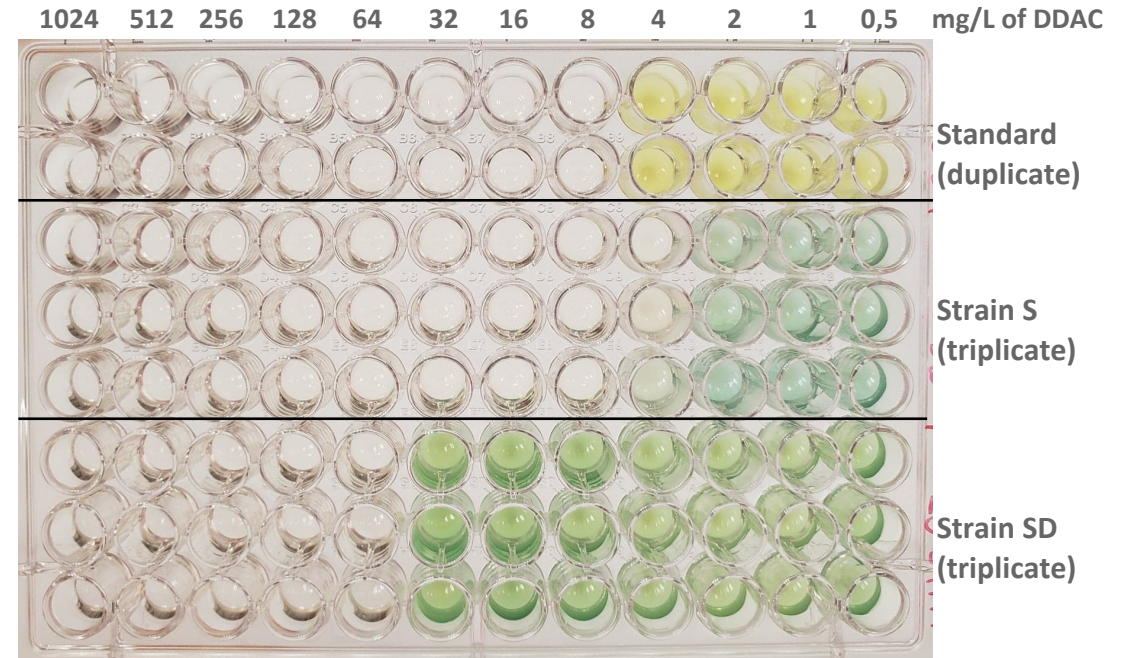
- If MIC < 64 mg/L → susceptible strain (S)
- If MIC ≥ 64 mg/L → decrease sensibility strain (SD)

1.2

Materials and methods

DDAC decrease sensibility test

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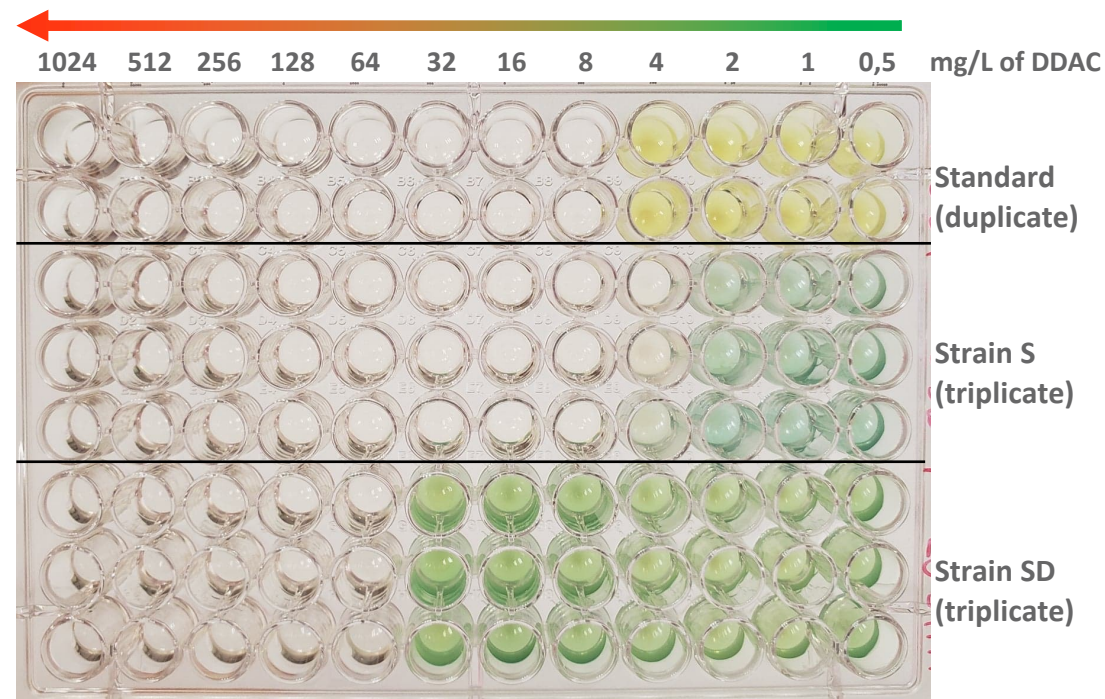
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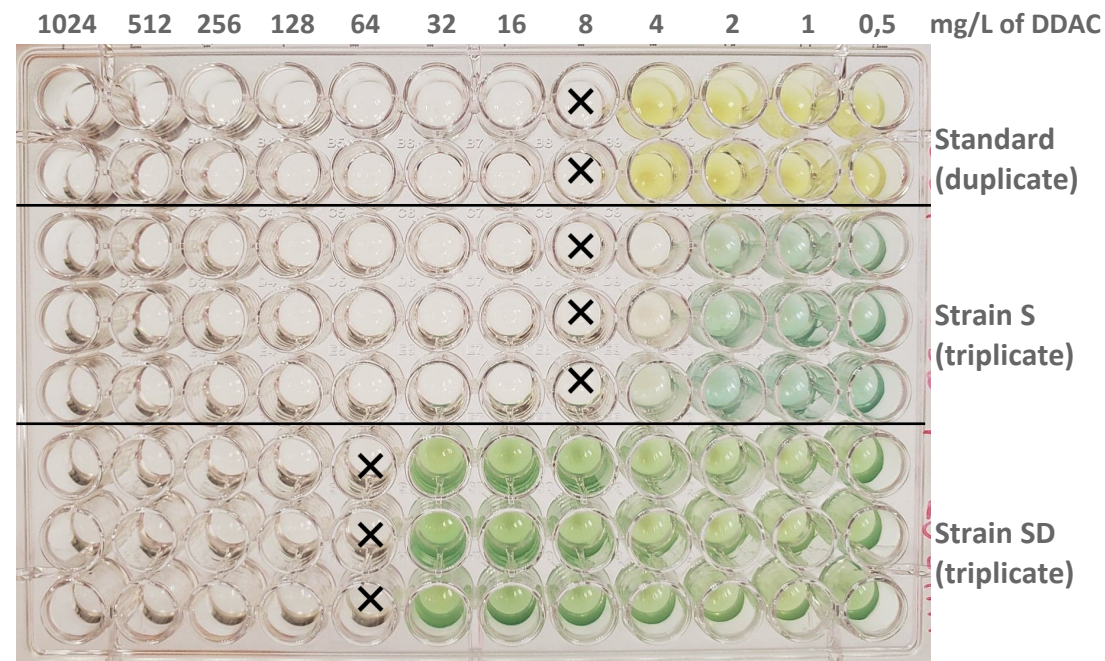
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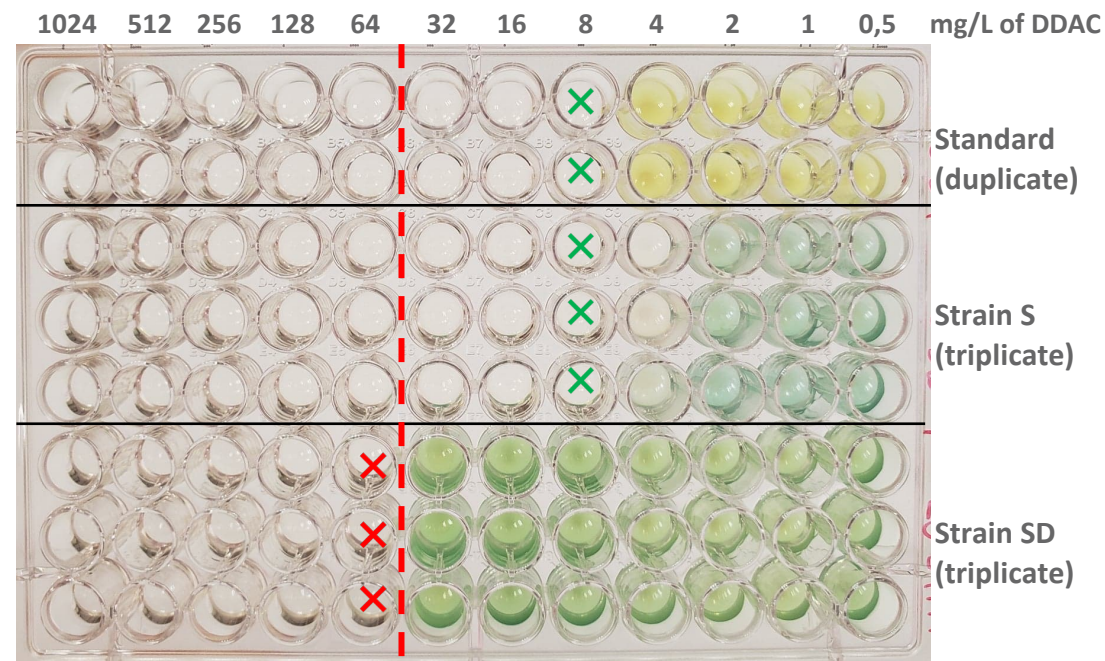
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➔ *concentration in disinfectant in use conditions*



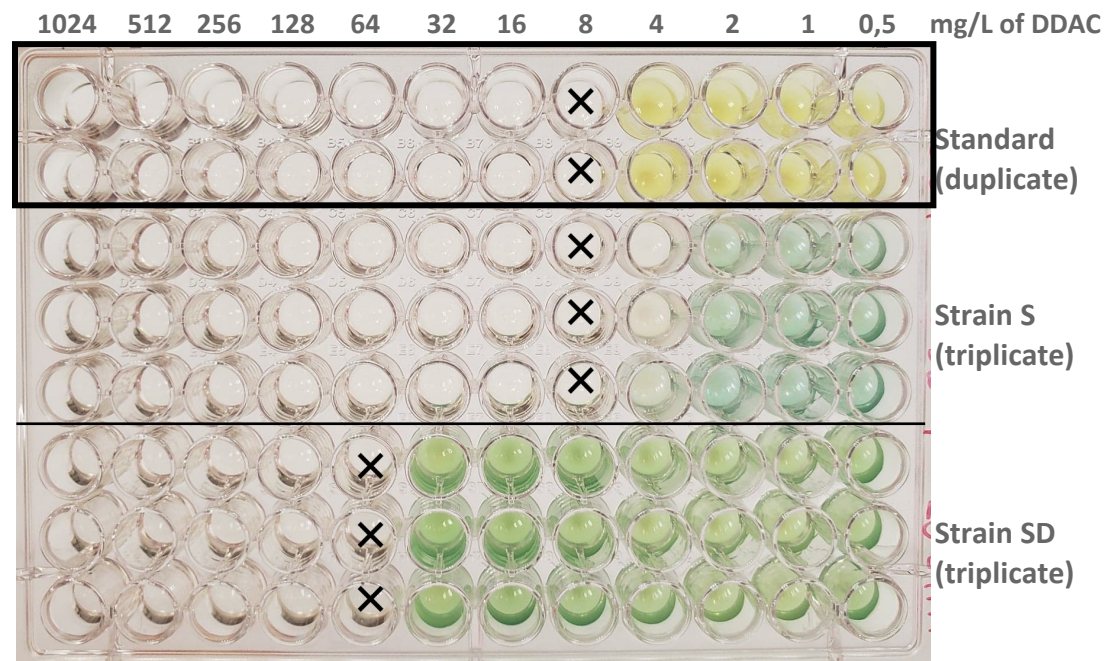
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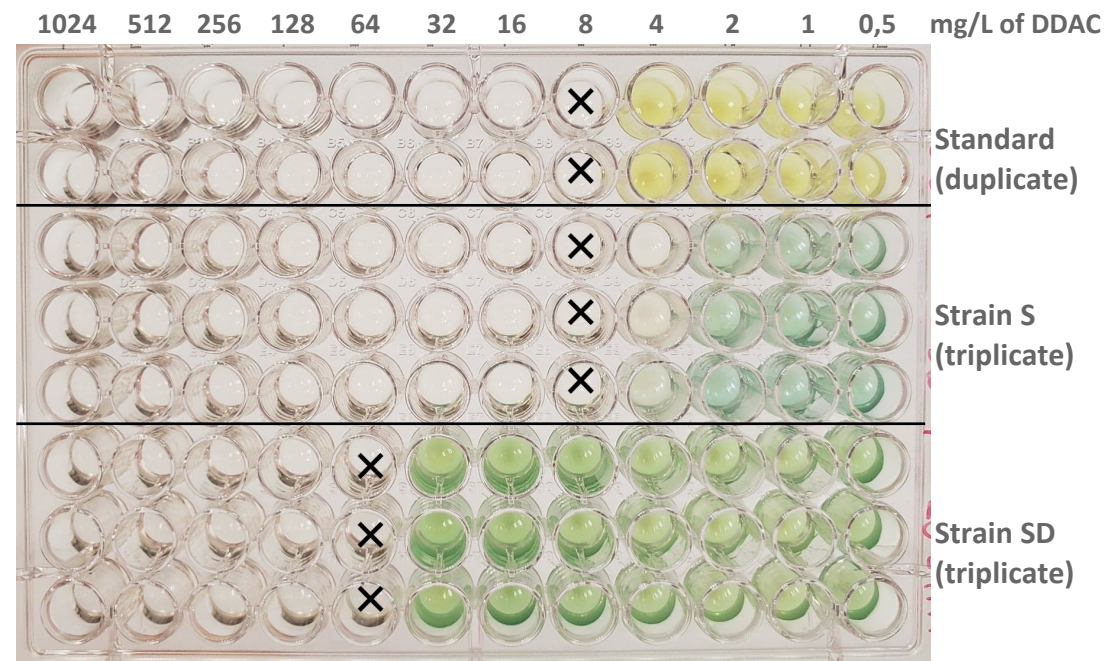
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➔ *concentration in disinfectant in use conditions*
- Comparison with standard at MIC = 8 mg/L ➔ sensitive
- Work in triplicate



Picture of DDAC MIC determination plate

- If MIC < 64 mg/L ➔ susceptible strain (S)
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II.1

Results Decrease sensibility over time

Green dot = sensitive strain
Red dot = SD to DDAC
Dot size → sample size

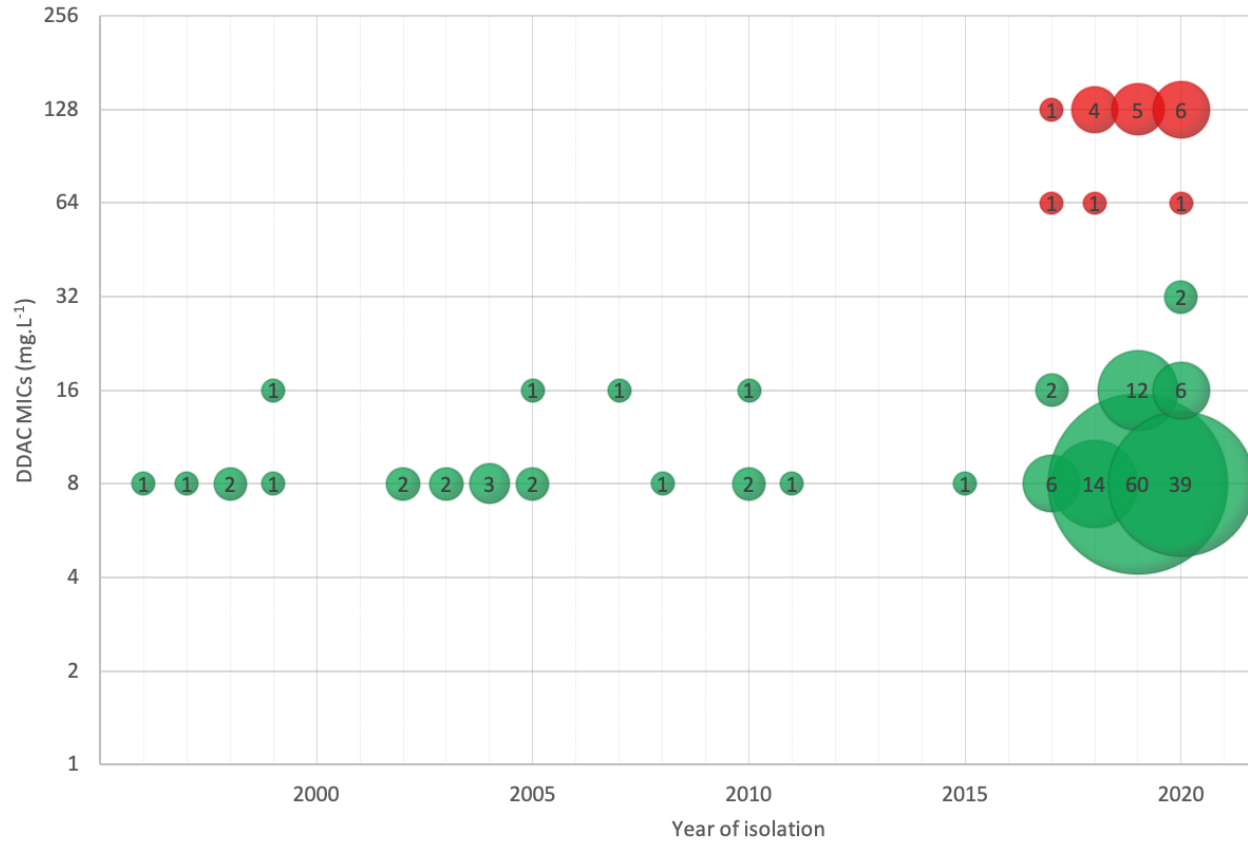


Figure I : Temporal evolution of DDAC MICs for PSAE strains isolated from animals (n=183)
Red : sample with DDAC decreased susceptibility ; Green : DDAC sensitive sample ; inside the bubbles number of strains tested

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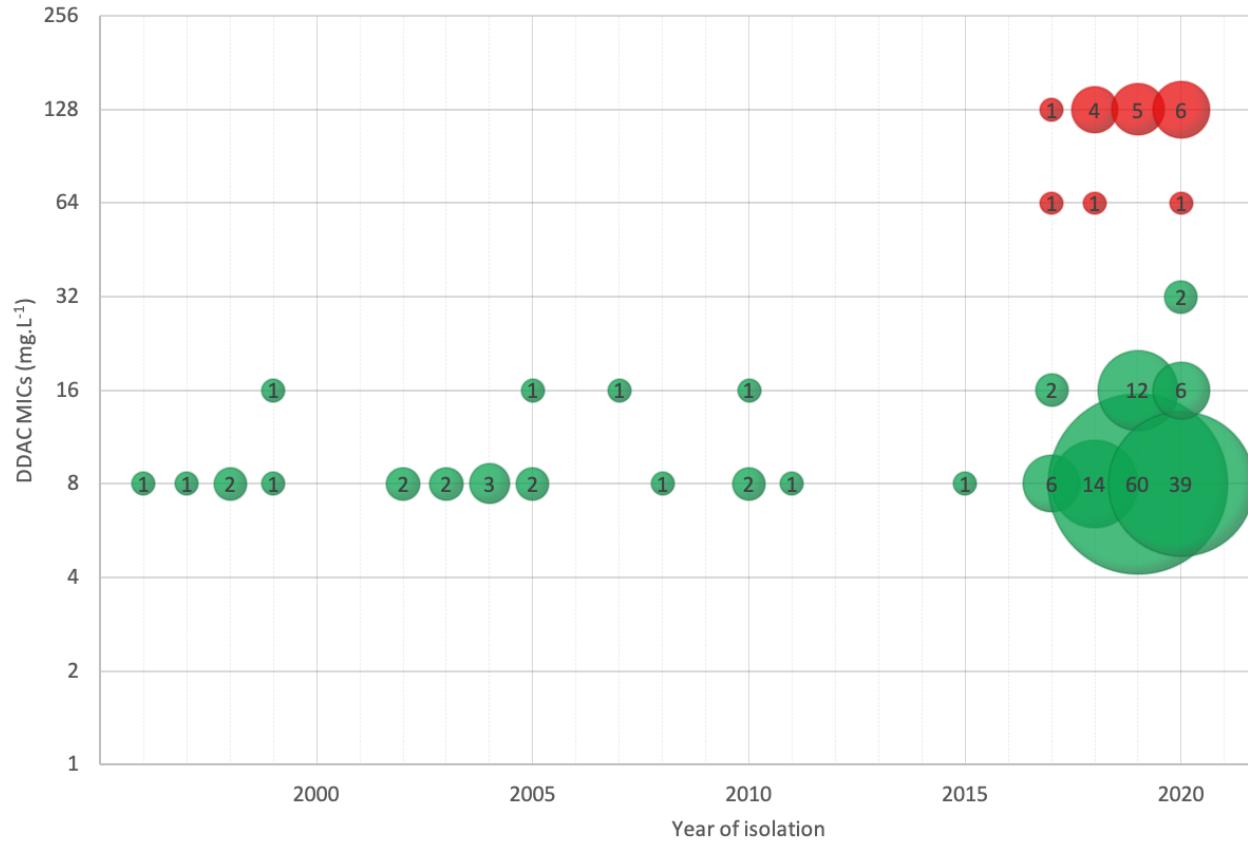
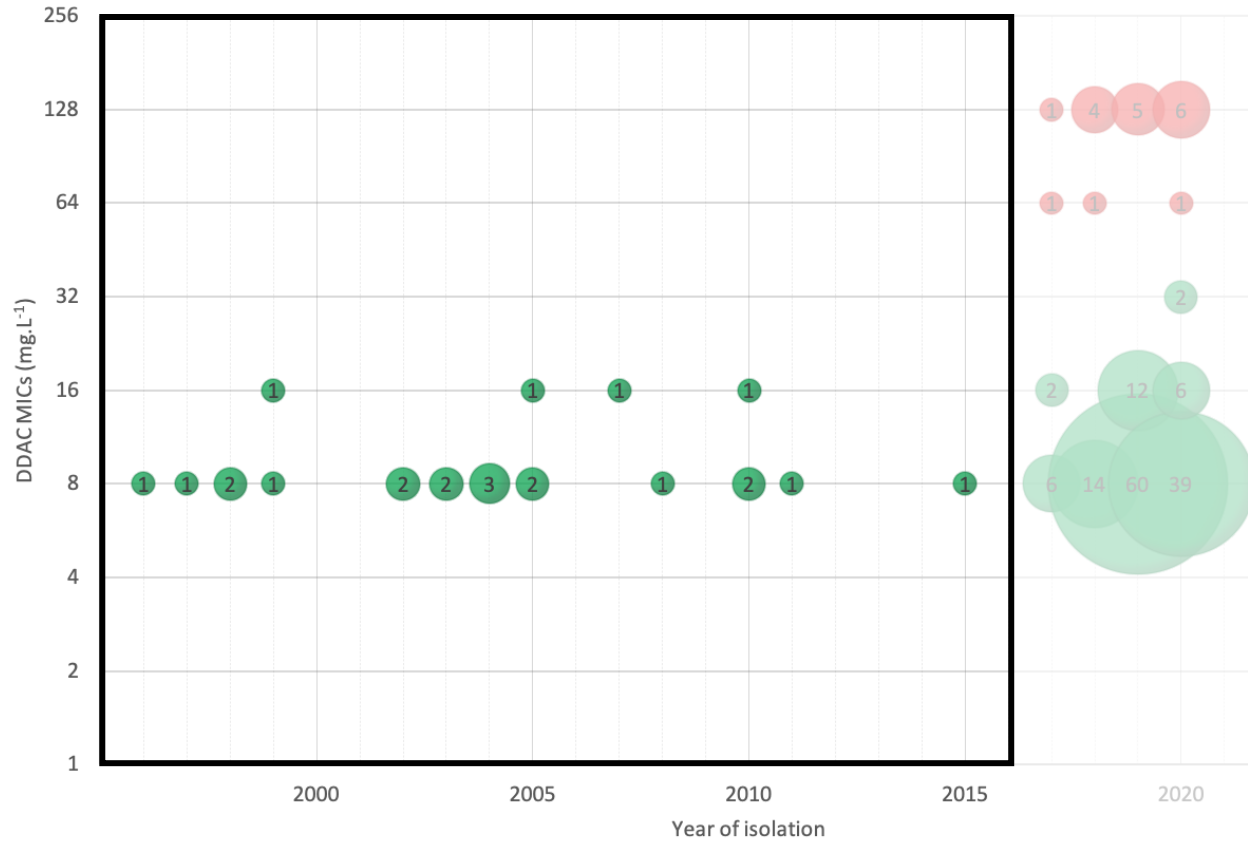


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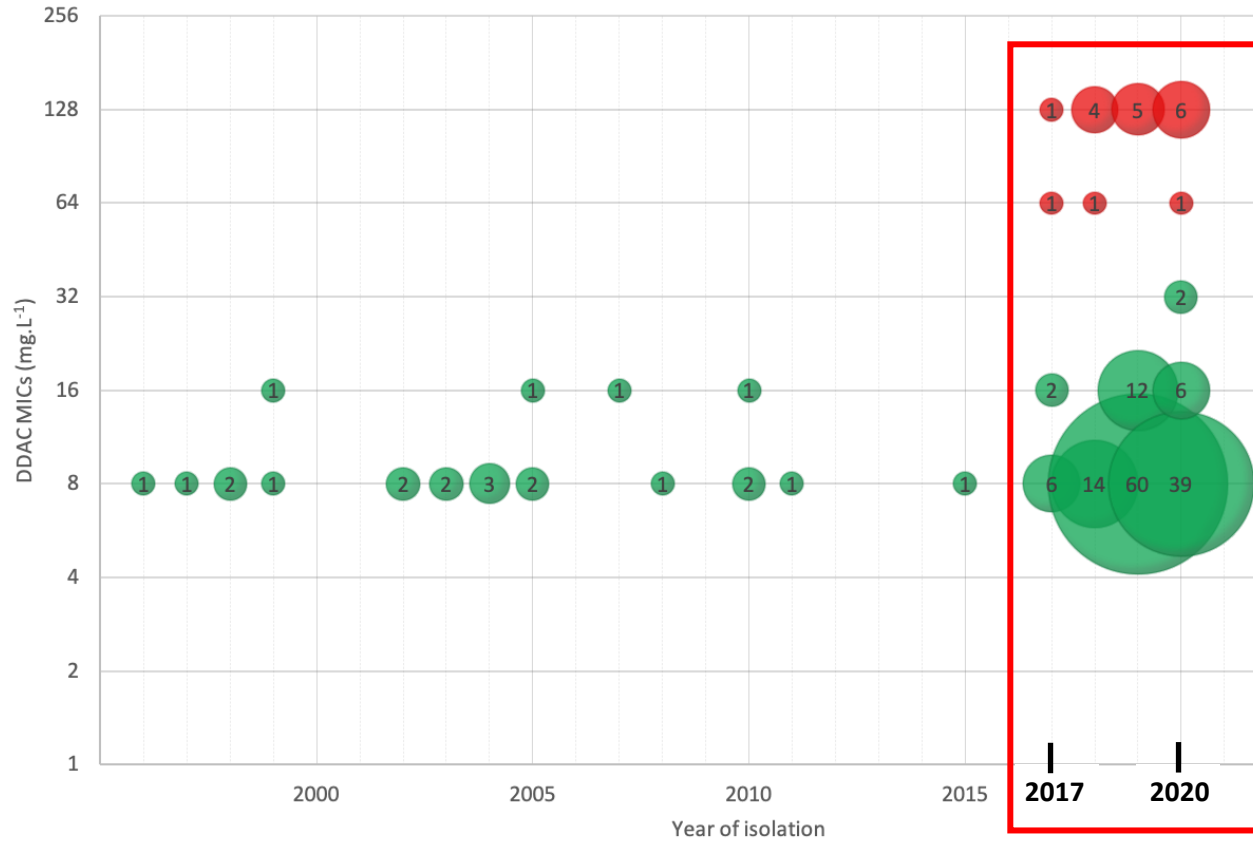
Not found before 2017

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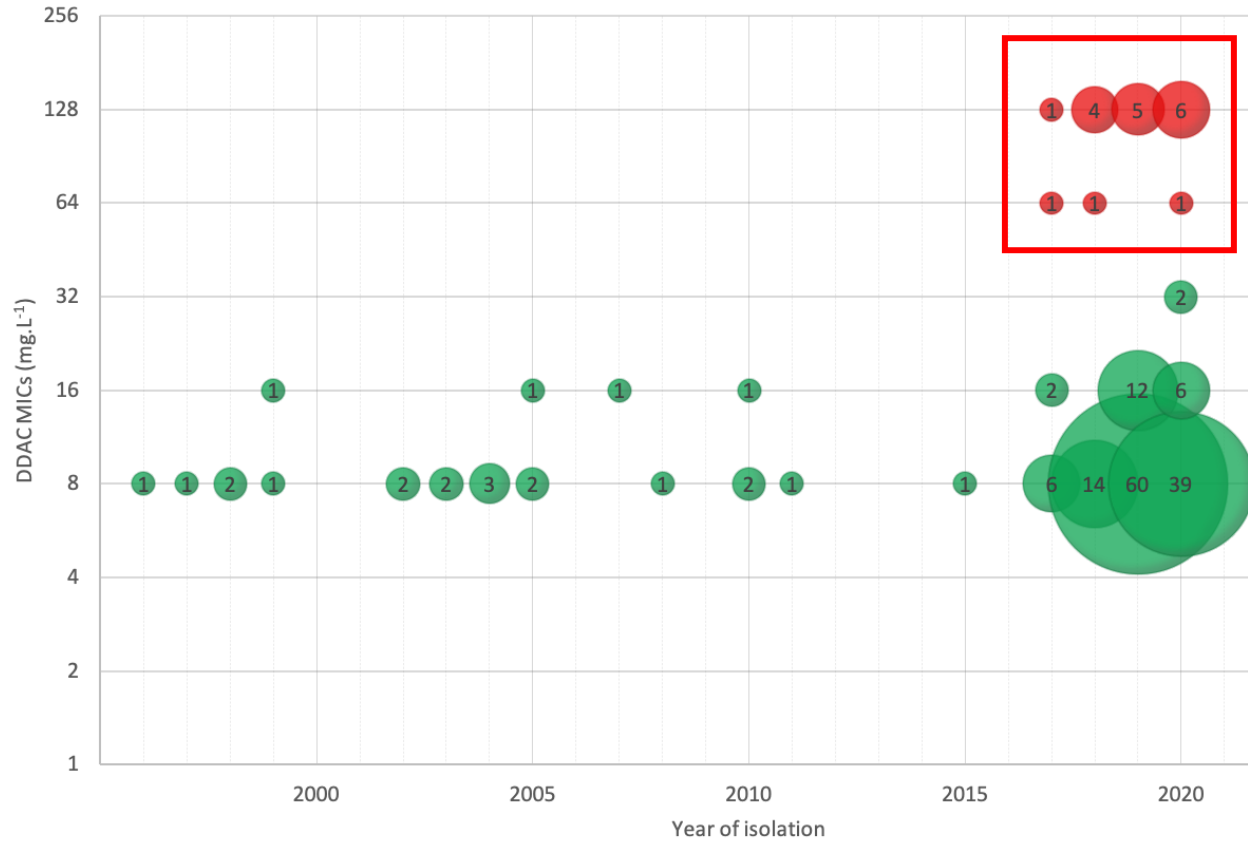
Only found between 2017 - 2020

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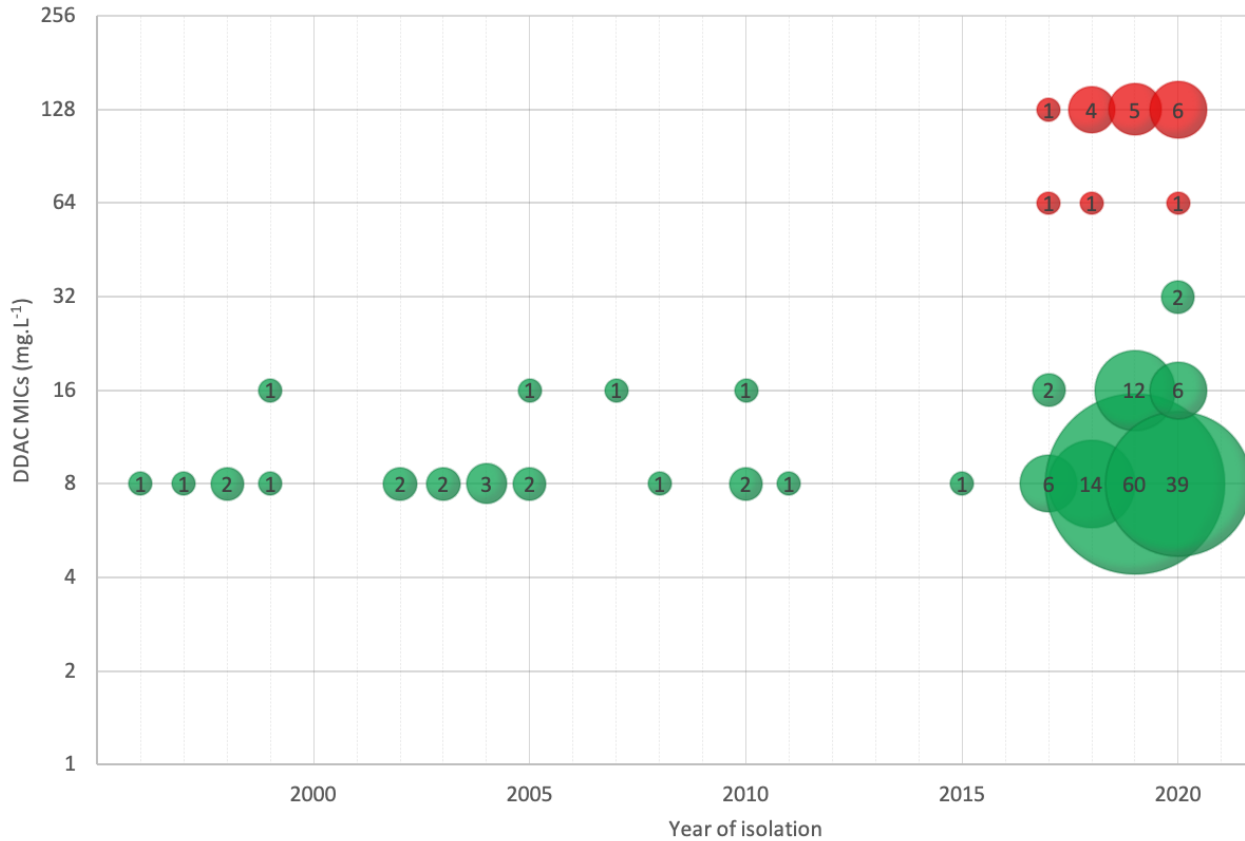
Observed at **19** strains over the 183 tested → **10%**

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→ According to our study, SD to DDAC exists since 2017 in veterinary medicine
→ This represent 10% of the tested strains.

II.2

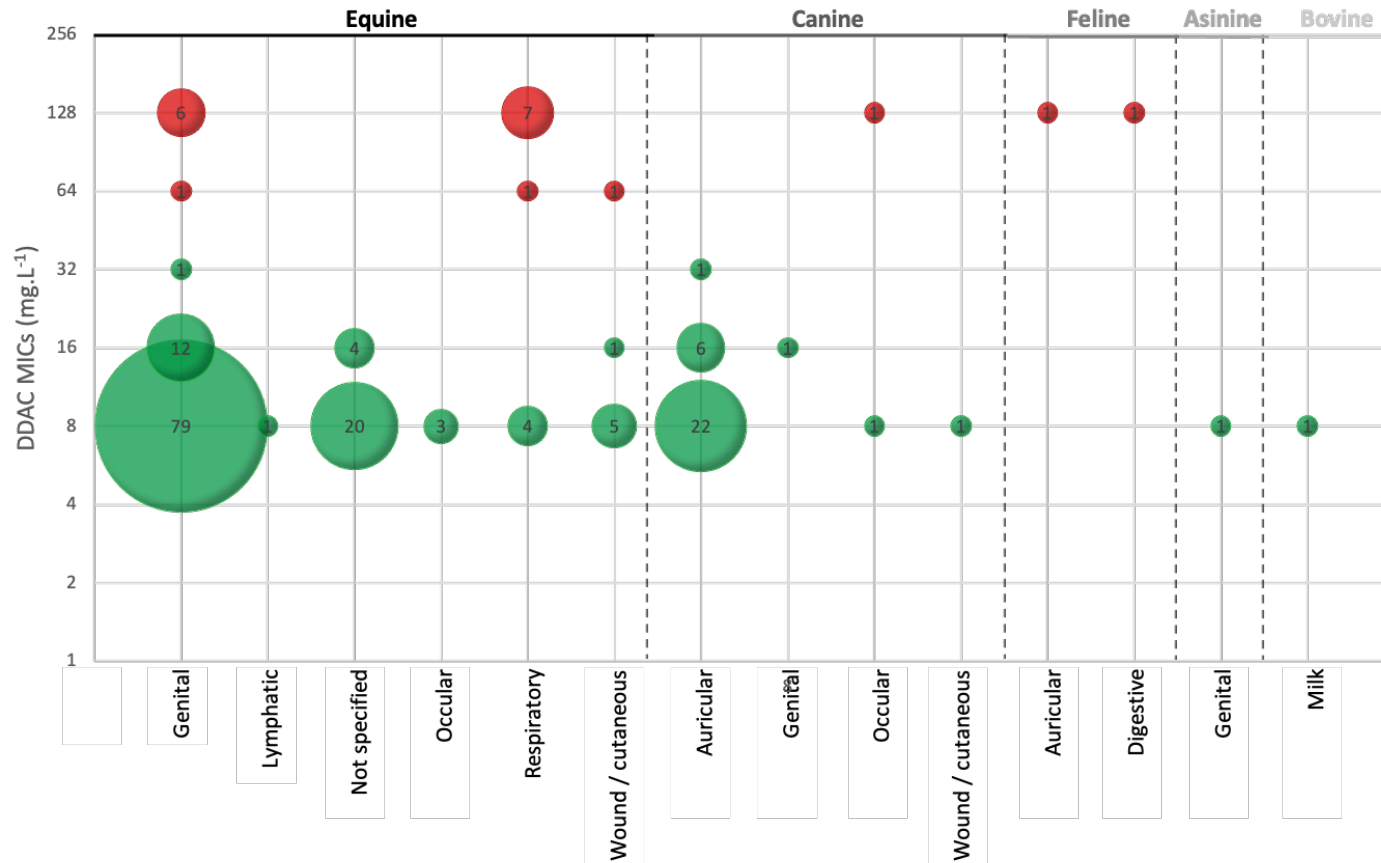
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Decrease sensibility according strain origin

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Dot size → sample size



SD to DDAC was found on :

Figure II : Dispersion of DDAC MICs as a function of sample origin (n=183)

Red : sample with DDAC decreased susceptibility ; Green : DDAC sensitive sample ; inside the bubbles number of strains tested

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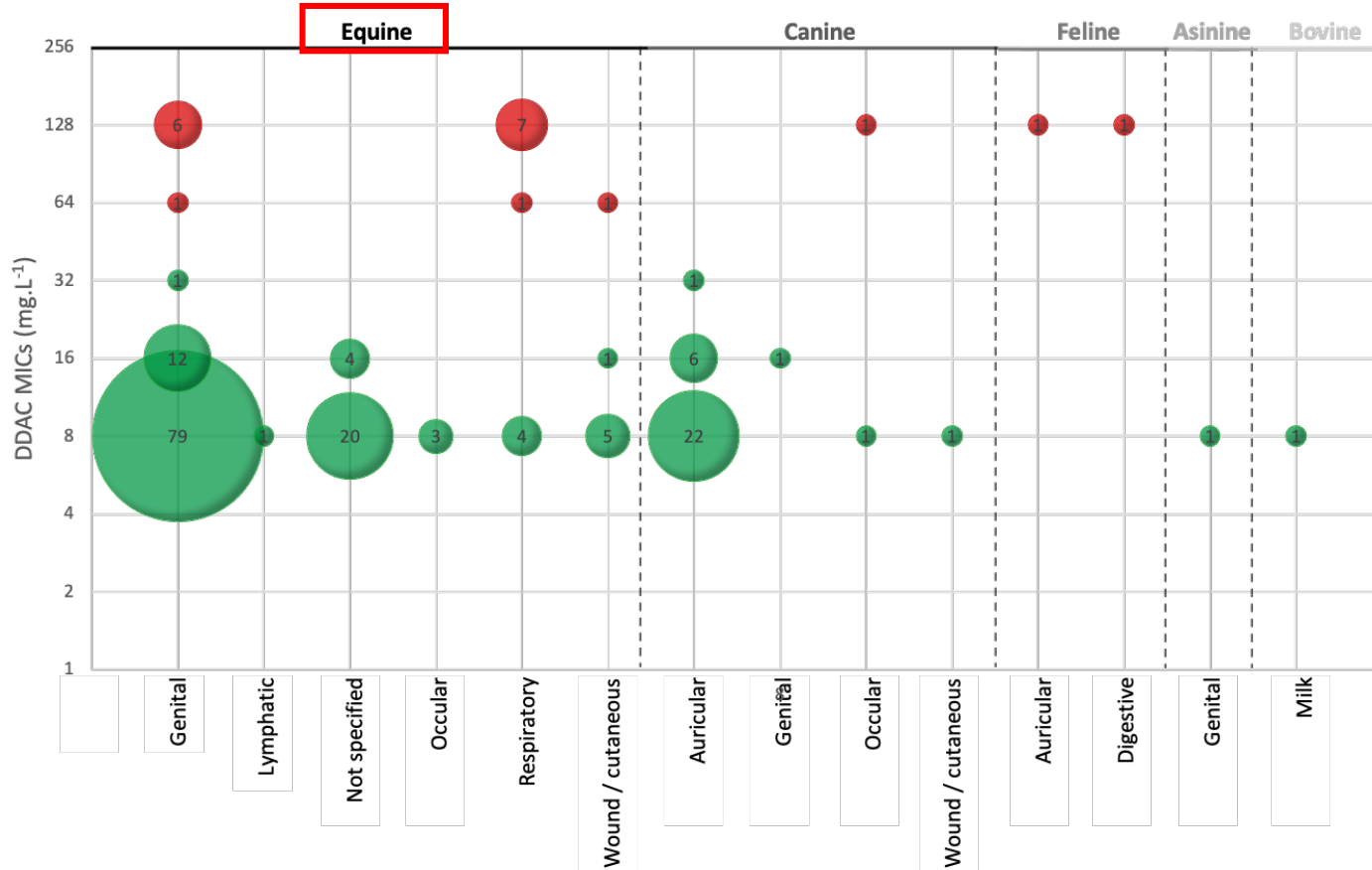
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- Equine n=16/146 → 11%

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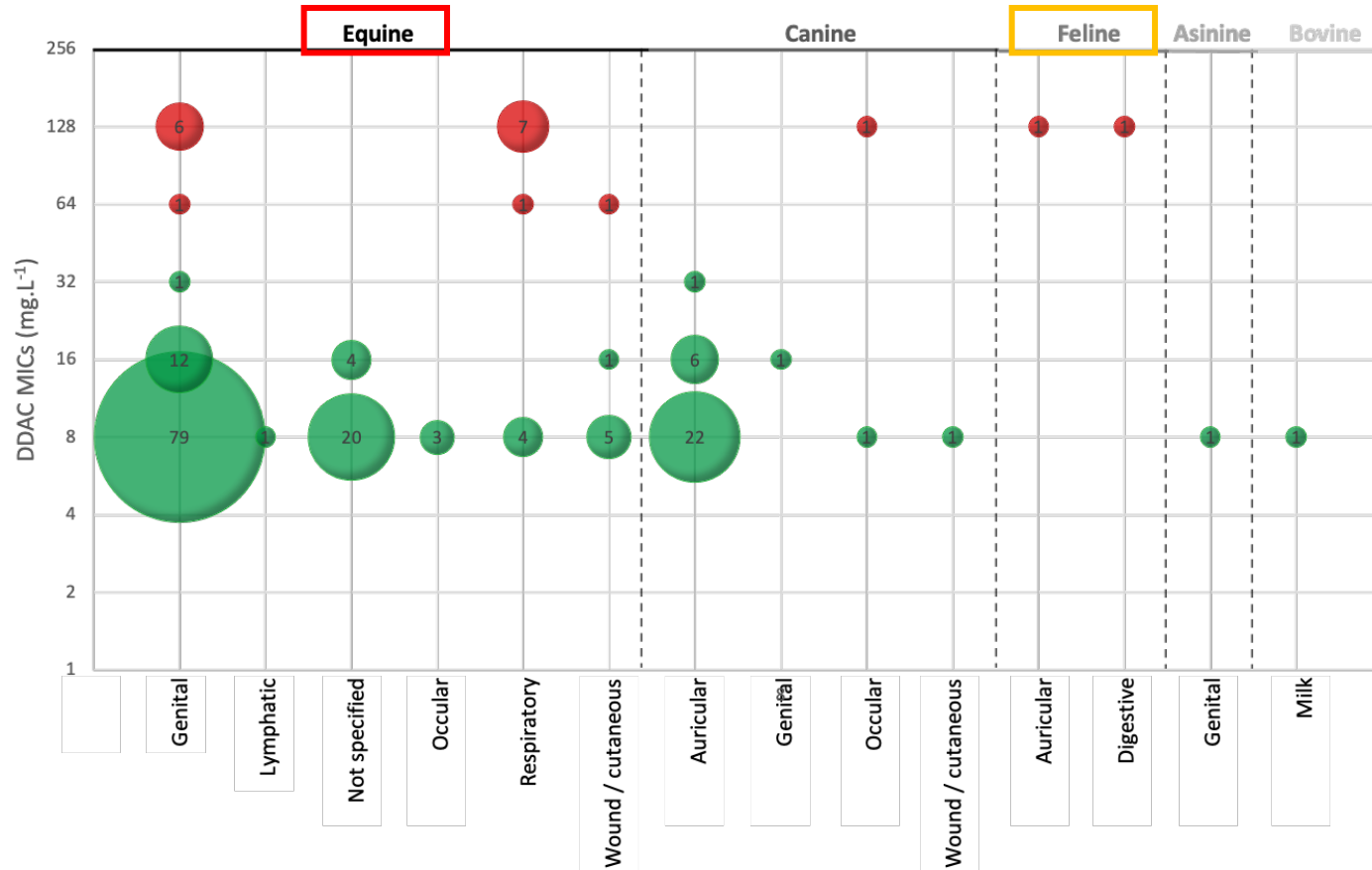
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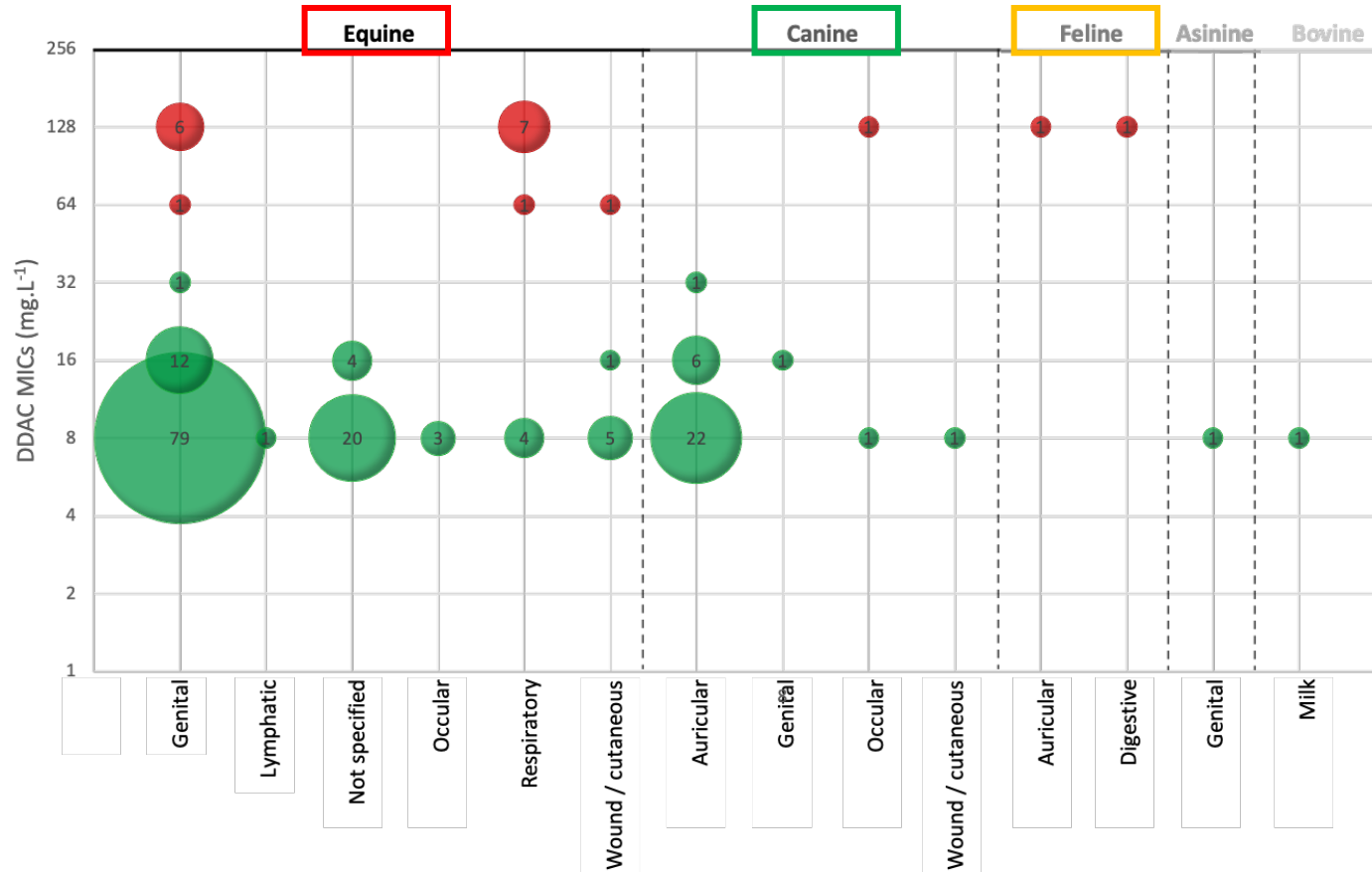
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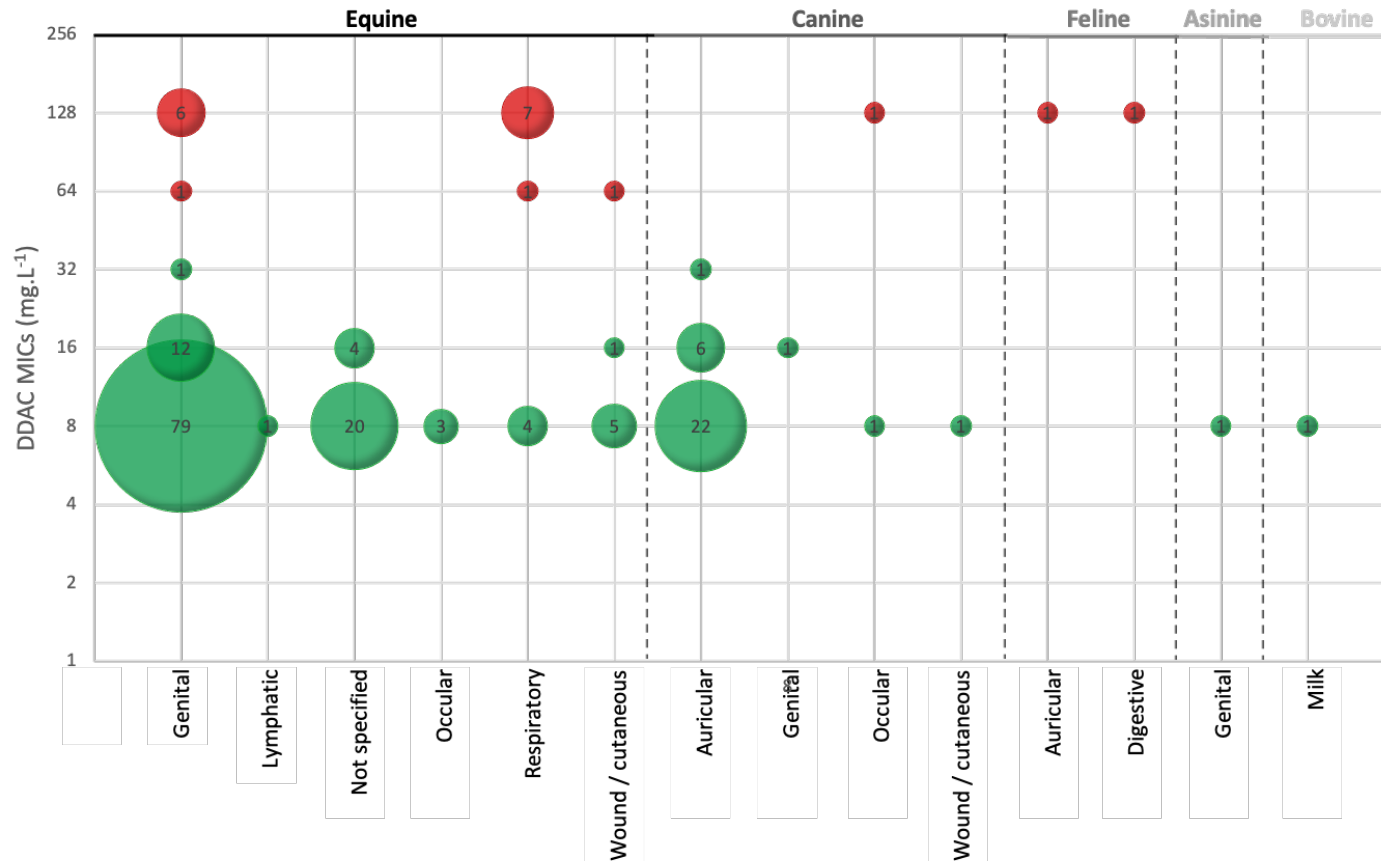
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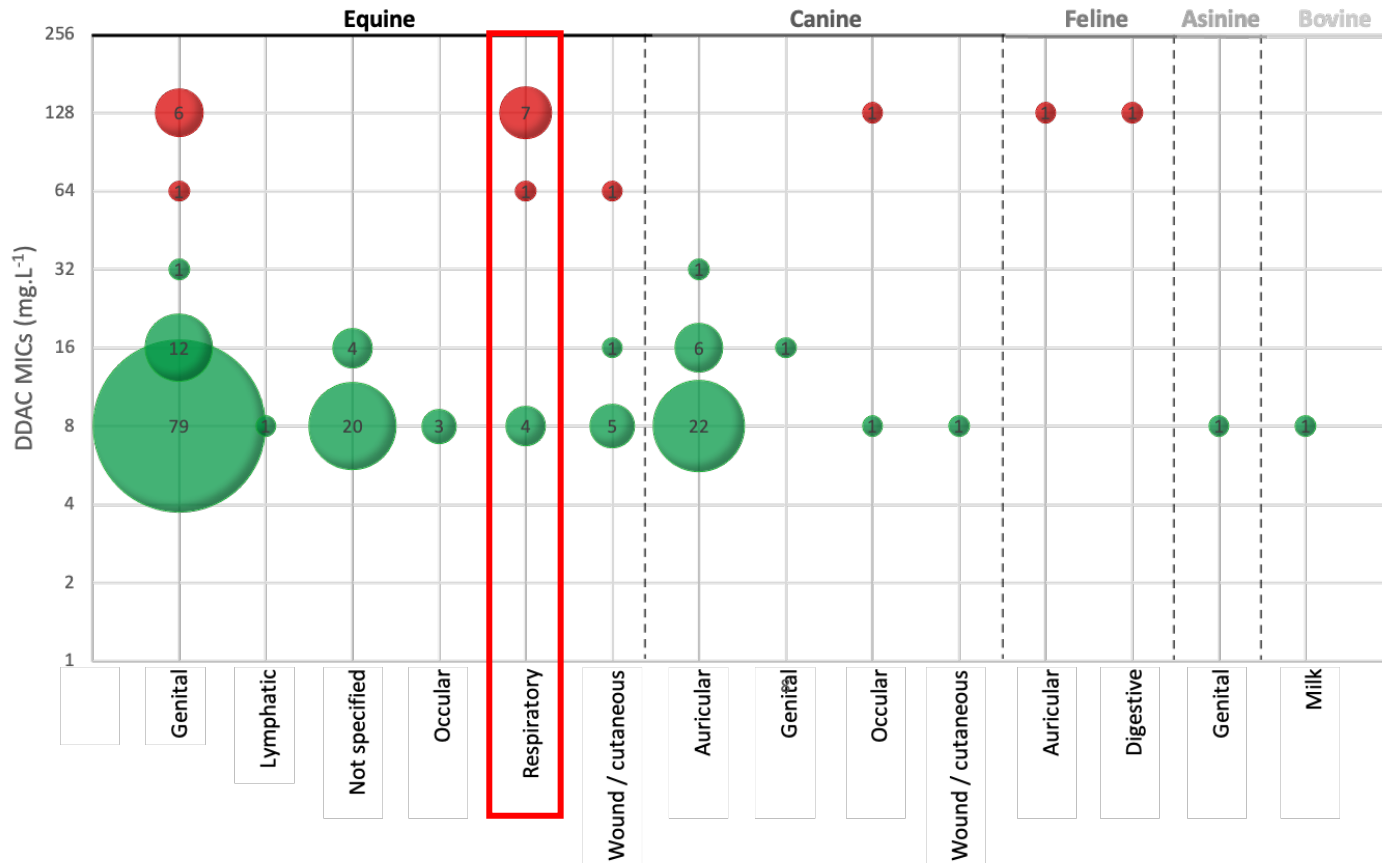
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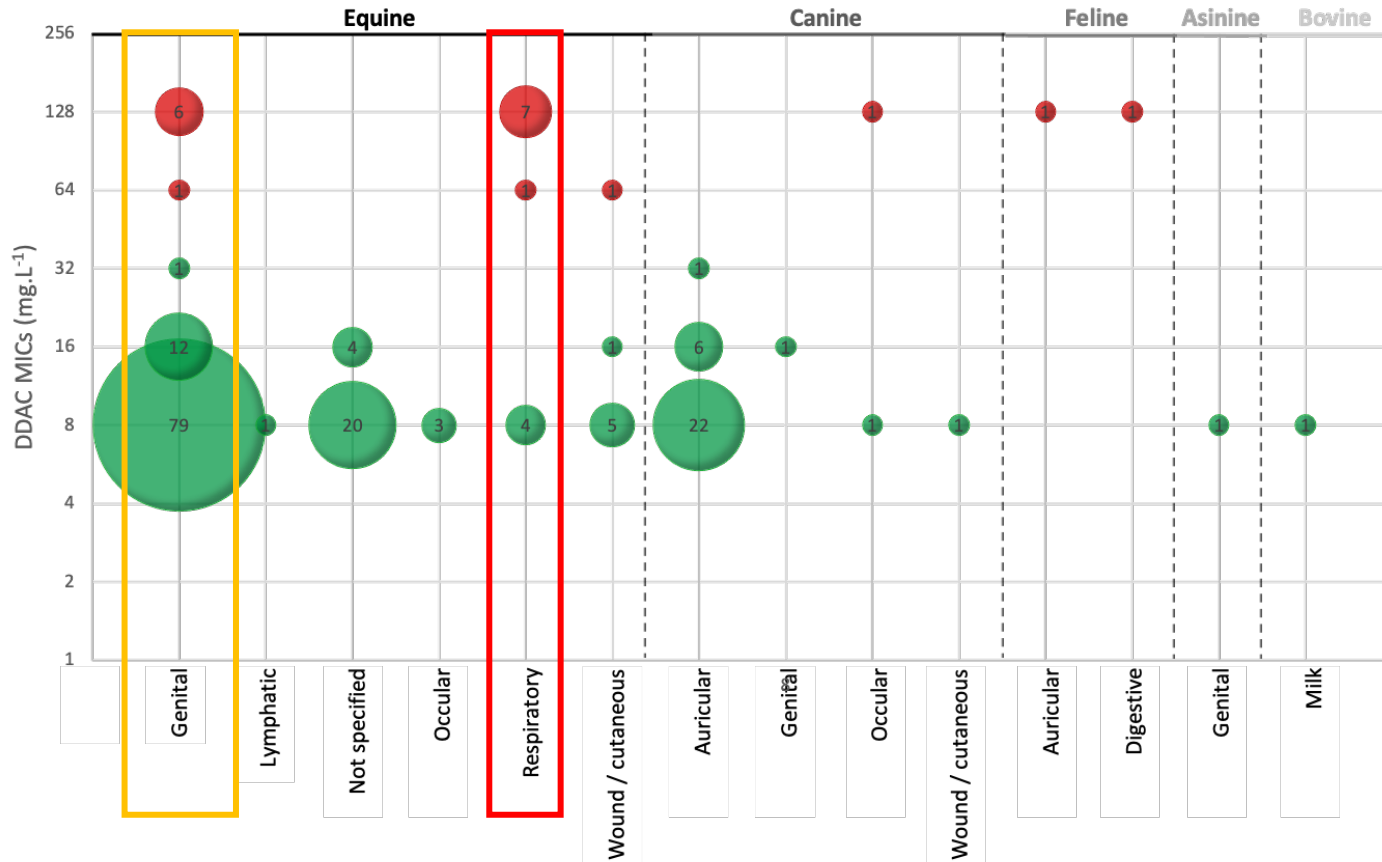
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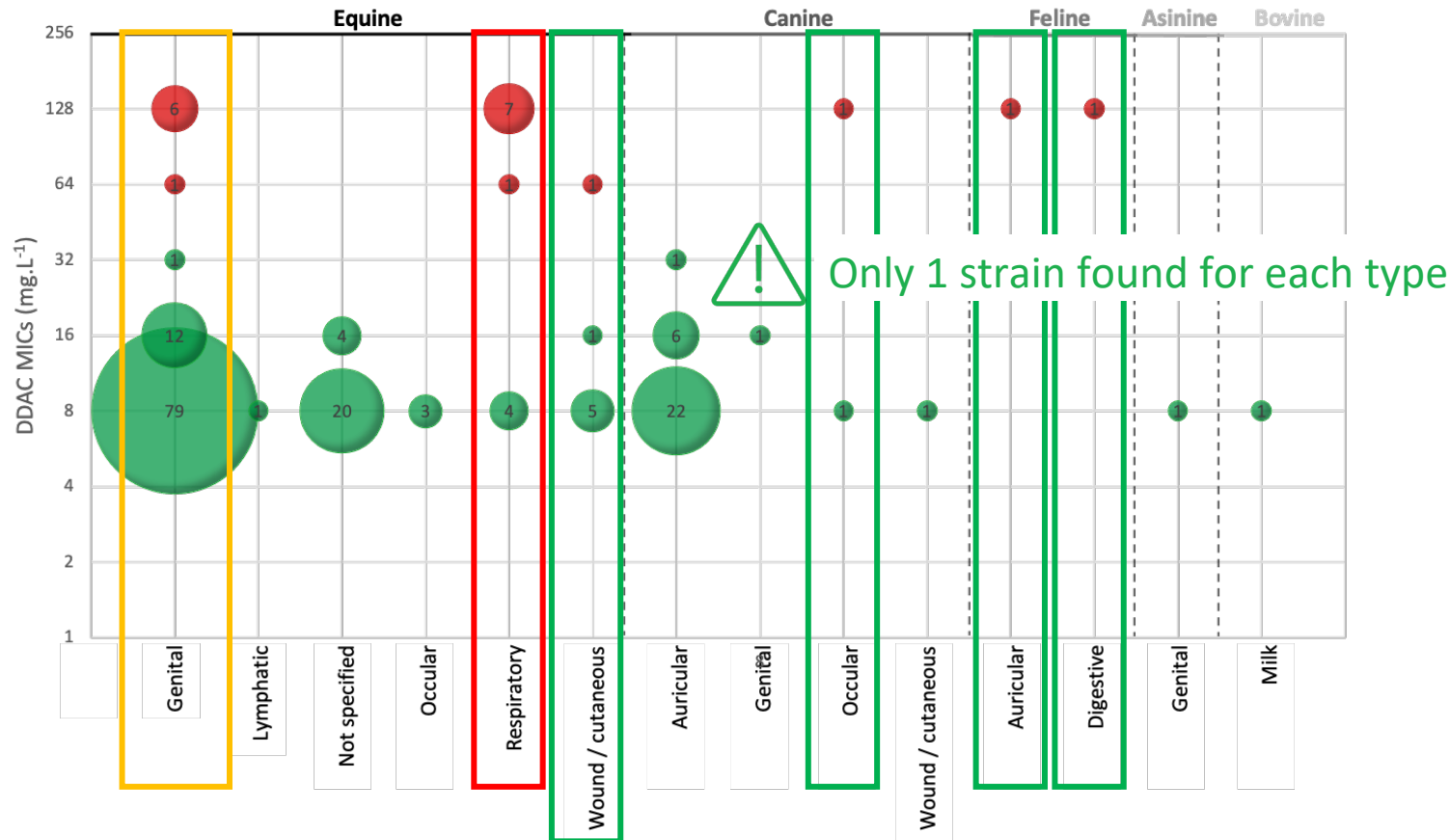
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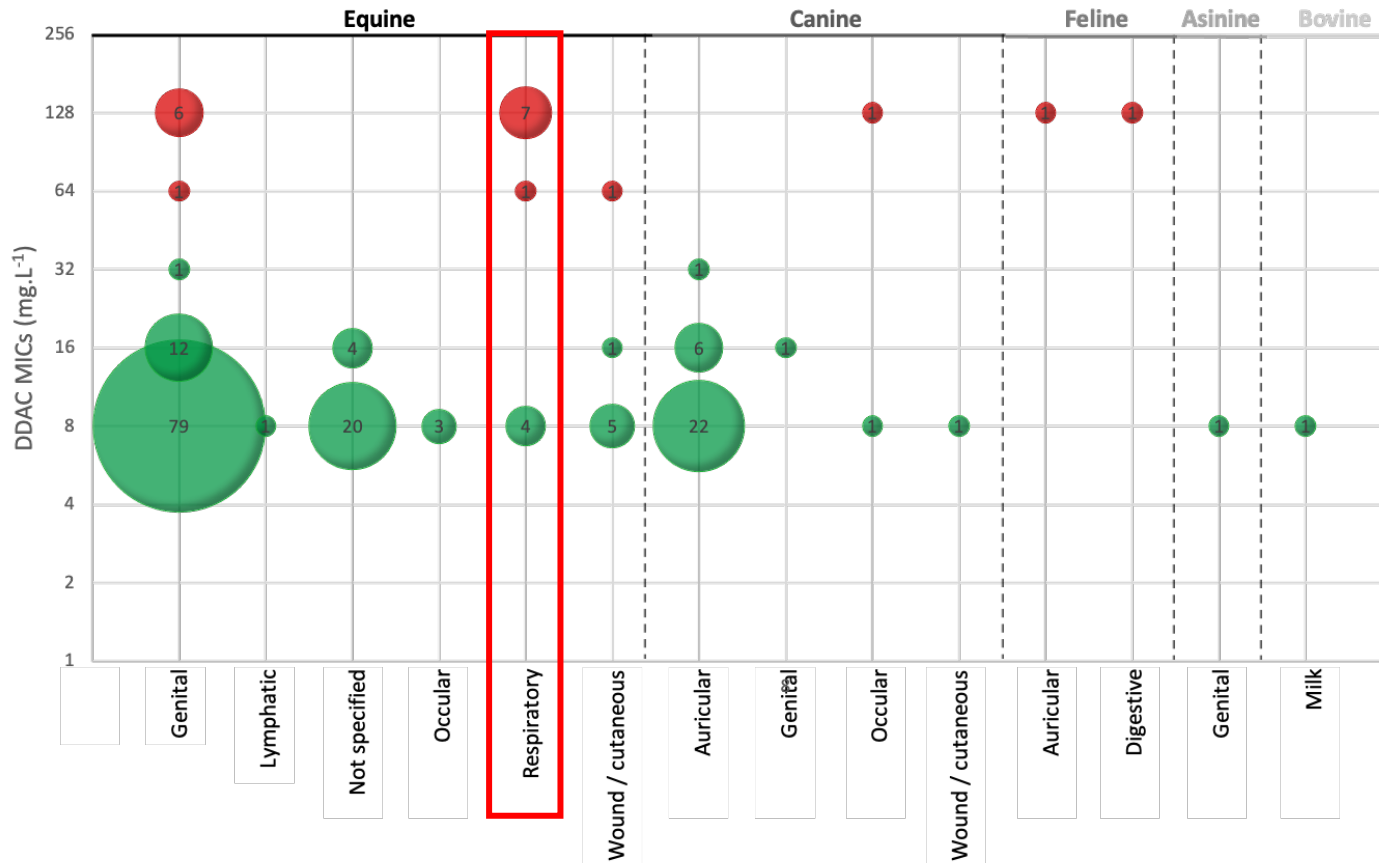


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Decreased susceptibility to DDAC is both present in human (27%) and in veterinary (10%) medicine

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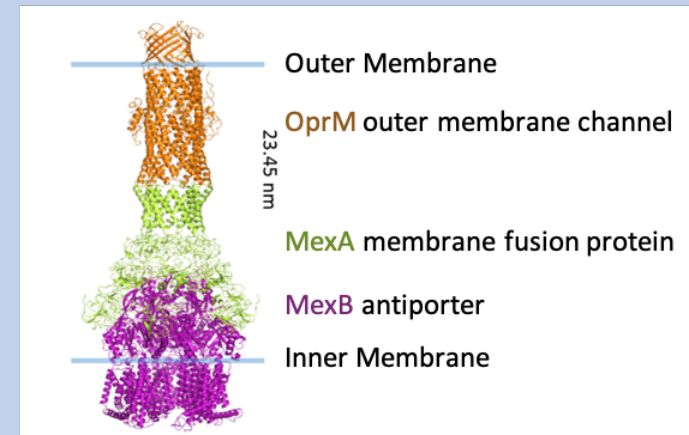
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Which mechanism(s) is(are) implicated in this phenotype ?

→ Exploration of the overexpression of the RND MexA/B-OprM efflux pump
(Master results on humans samples)



The efflux pump MexA/B-OprM constructed by optimized pipeline for building atomistic models of complete Gram-negative tripartite efflux pumps (according to Travers et al., 2018)

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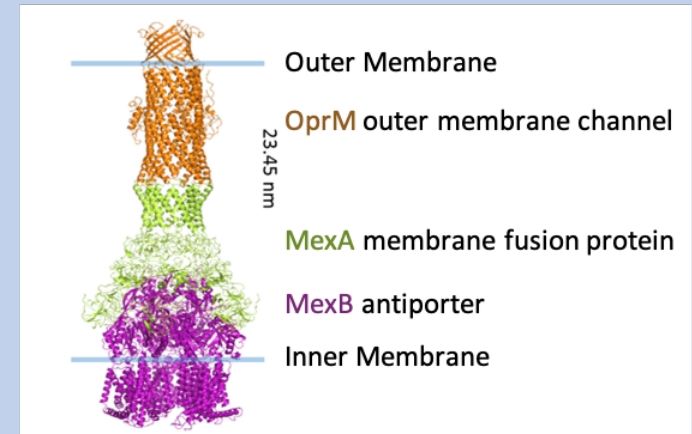
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Is SD to DDAC associated with antibiotic resistance ?

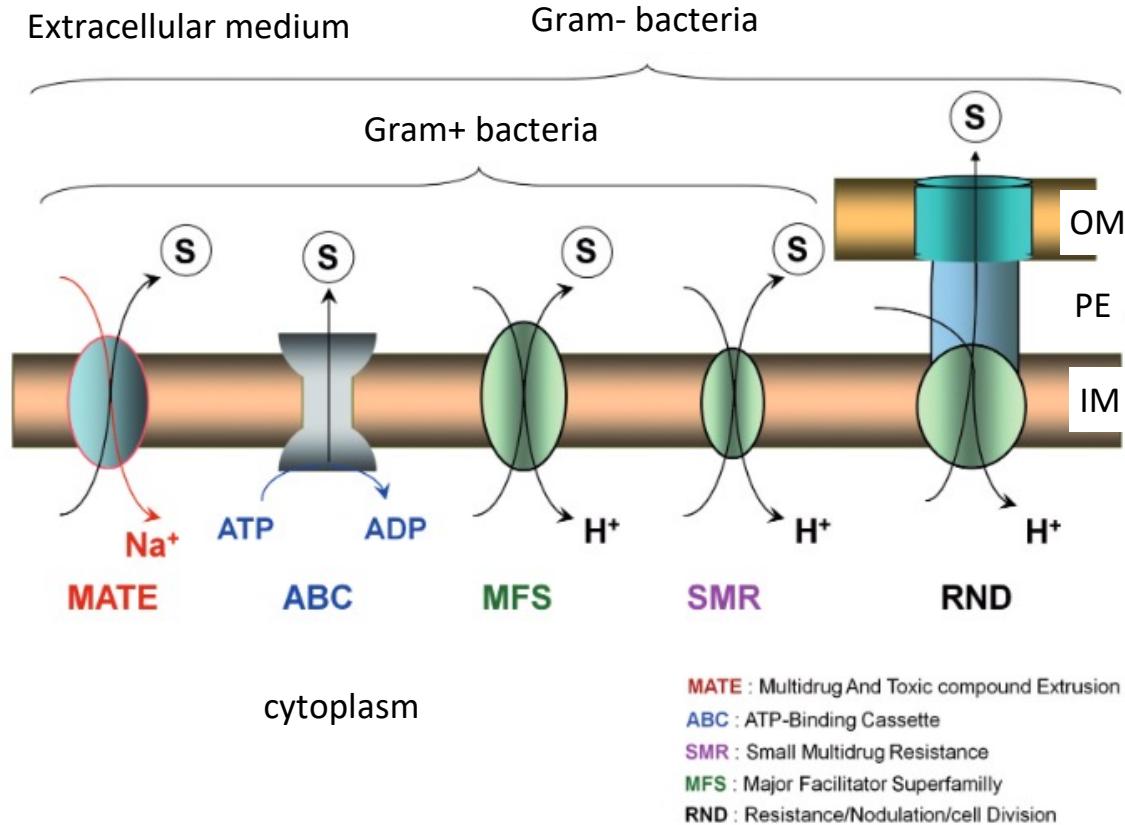


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Thank for your e-attention

En Normandie on est à cheval sur l'hygiène des mains !





Representation of the five families of bacterial efflux pumps. ME, outer membrane; EP, periplasmic space; MC, cytoplasmic membrane; S, substrate. After Bull. Acad. Vét. France - 2011 - Volume 164

5 large family :

1. MATE (*Multidrug and toxic compound extrusion*)
2. SMR (*Small multidrug resistance*)
3. MFS (*Major facilitator superfamily*)
4. ABC (*ATP-binding cassette*)
5. RND (**Résistance-Nodulation-Division**)
 - G- specific
 - IM and OM crossing
 - Energy = proton gradient

Operon	Component	Function ^a	Regulator ^b		Substrate(s)	
			Primary ^b	Secondary ^c	Antibiotics	Additional compounds
<i>mexAB-oprM</i>	MexA MexB OprM	MFP RND OMF	MexR NalD	NalC	Fluoroquinolones, β -lactams, β -lactamase inhibitors, tetracyclines, chloramphenicol, macrolides, novobiocin, trimethoprim, sulfonamides	Biocides (e.g., triclosan), detergents, dyes, HSL, ^f aromatic hydrocarbons
<i>mexCD-oprJ</i>	MexC MexD OprJ	MFP RND OMF	NfxB		Fluoroquinolones, β -lactams, tetracycline, chloramphenicol, macrolides, trimethoprim, novobiocin	Biocides (e.g., triclosan), detergents, dyes, aromatic hydrocarbons
<i>mexEF-oprN</i>	MexE MexF OprN	MFP RND OMF	MexT	MexS MvaT	Fluoroquinolones, chloramphenicol, trimethoprim	Biocides (e.g., triclosan), aromatic hydrocarbons
<i>mexXY</i>	MexX MexY OprM/Opm ^d	MFP RND OMF	MexZ		Fluoroquinolones, β -lactams, tetracycline, aminoglycosides, macrolides, chloramphenicol	
<i>mexJK</i>	MexJ MexK OprM/OpmH	MFP RND OMF	MexL		Tetracycline, erythromycin	Biocides (e.g., triclosan)
<i>mexGHI-opmD</i>	MexG MexH MexI OpmD	? MFP RND OMF	SoxR		Fluoroquinolones	Vanadium
<i>mexVW</i>	MexV MexW OprM	MFP RND OMF	—		Fluoroquinolones, tetracycline, chloramphenicol, erythromycin	
<i>mexPQ-opmE</i>	MexP MexQ OpmE	MFP RND OMF	—		Fluoroquinolones, tetracycline, chloramphenicol, macrolides	
<i>mexMN</i>	MexM MexN OprM	MFP RND OMF	—		Chloramphenicol, thiamphenicol	
<i>triABC</i>	TriA TriB TriC OpmH	MFP MFP RND OMF	—			Triclosan

^a MFP, membrane fusion protein; RND, resistance-nodulation-division transporter; OMF, outer membrane factor; ?, encodes a protein of unknown function.

^b Regulatory proteins that directly control expression of the efflux operons.

^c Proteins that indirectly activate operon expression when mutated.

^d MexXY may utilize OpmB, OpmG, OpmH, and/or OpmI as OMFs.

^e —, not identified.

^f HSL, homoserine lactones.

• 5 RND pump involved in biocide resistance :

- MexA/B-OprM → multidrug efflux pump
- MexC/B-OprJ → multidrug efflux pump
- MexE/F-OprN → multidrug efflux pump
- MexVW-OprM → multidrug efflux pump
- TriABC-OpmH

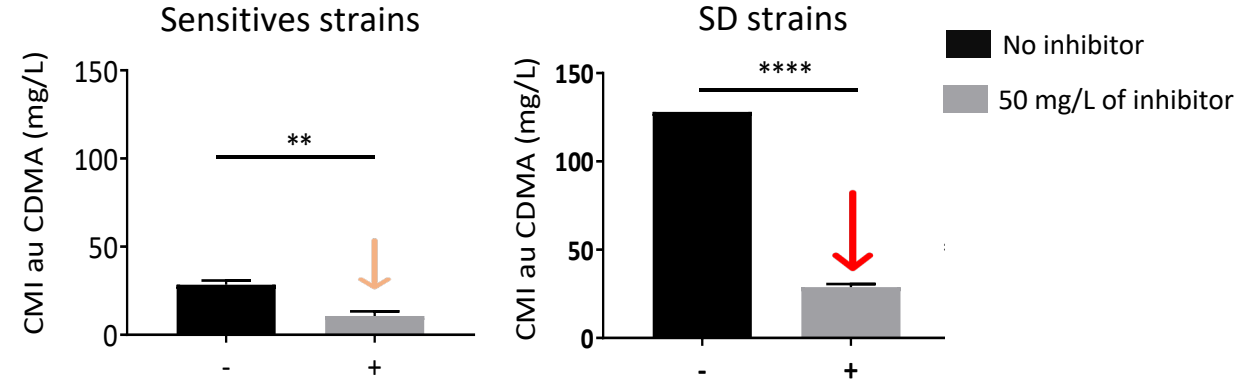
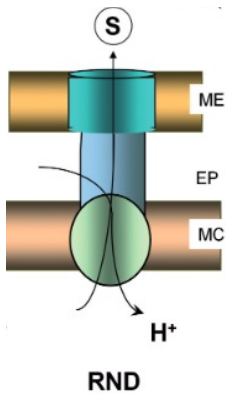
RND pumps inhibition
(Résistance-Nodulation-Division)



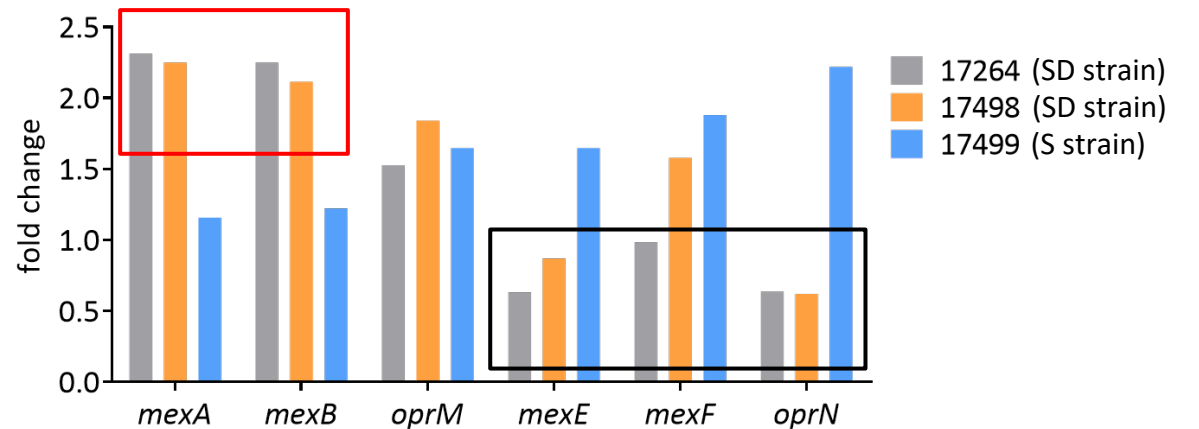
Return to a sensitive phenotype to
DDAC

Which genes implicated ?

Over-expression of *mexA* and *mexB*
genes in two resistant strains



Determination of the MIC of CDMA in the absence or presence of an RND pump inhibitor (50 mg/L PBNA).



Expression variation of the genes encoding the RND pumps MexA/B-OprM and MexE/F-OprN compared to a reference strain.

➤ An overexpression of the RND **MexA/B-OprM** pump could be involved in this SD phenotype



A4

Main limitations

Strains between 1996 – 2016 are few represented (n = 23 / 183)

Equines samples are majoritary (n = 146 / 183)

2017 - 2018 : only strains showing antibiotic resistances phenotype were selected (RESAPATH collection)