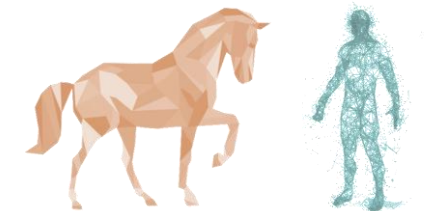


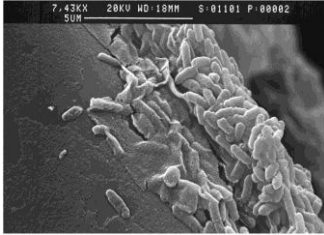
## Decreased susceptibility to a major component of disinfectant among equine *Pseudomonas aeruginosa* (PSAE) isolates.

Marine Pottier<sup>1, 2, 3, @</sup>, Sophie Castagnet<sup>2, 3</sup>, François Gravey<sup>1</sup>, Michel Auzou<sup>4</sup>, Guillaume Leduc<sup>4</sup>, Simon Le Hello<sup>1, 4, 5, 6</sup>, Albertine Léon<sup>2, 3, 6</sup>

- 1: Normandie Univ, UNICAEN, GRAM 2.0 - Caen (France) Normandie Univ, UNICAEN;
- 2: LABÉO, Saint Contest - Caen (France);
- 3: Normandie Univ, UNICAEN, U2RM - Caen (France) Normandie Univ, UNICAEN;
- 4: Service de microbiologie, CHU - Caen (France);
- 5: Service d'hygiène hospitalière, CHU - Caen (France);
- 6: Equivalent coordination



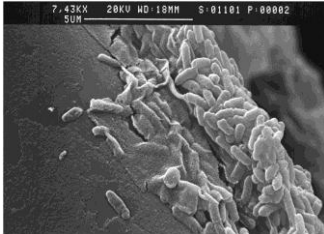
# Background



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

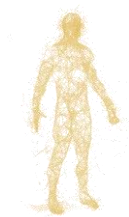
- Gram-negative, mesophilic and strictly aerobic bacilli
- **Opportunistic** pathogen (human / animal)

# Background

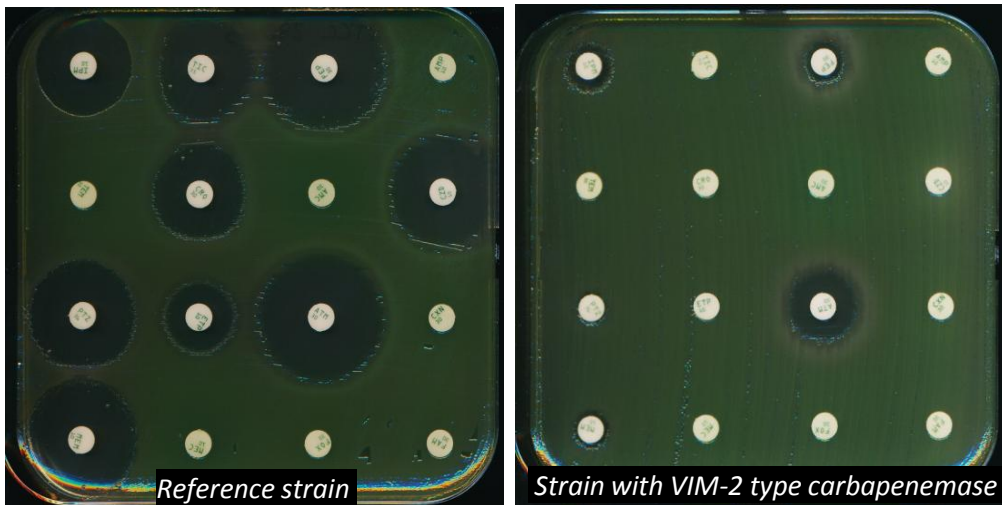


SEM observation of *Pseudomonas aeruginosa* biofilm, (Benamara et al. 2014).

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

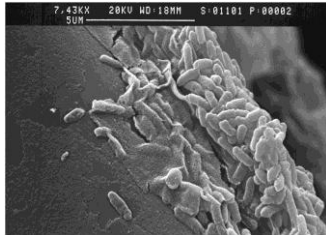


- 4th leading cause of HCAI in 2017 (*Santé Publique France*)
- **Therapeutic failures:** strains close to total resistance to antibiotics (*Alnour et al. 2017*)



Antibiograms

# Background

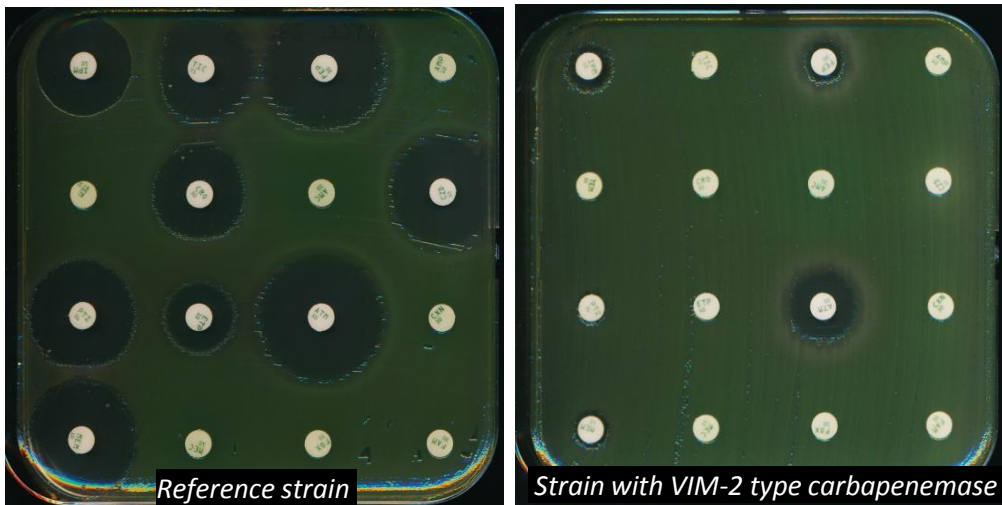


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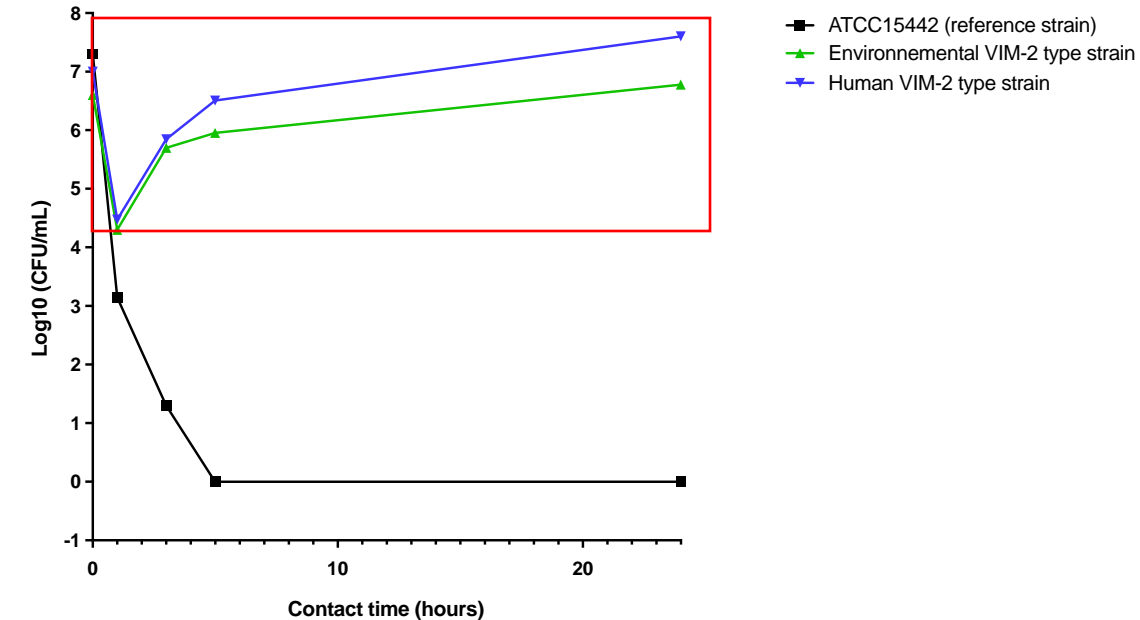
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- **Decrease susceptibility to quaternary ammonium compound**

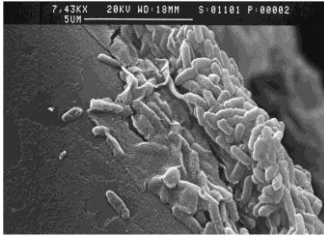


Antibiograms



Time kill curve in presence of a quaternary ammonium compound

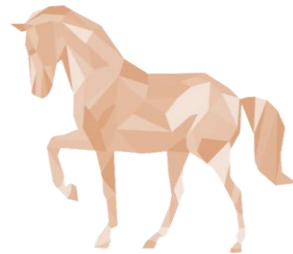
# Background



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

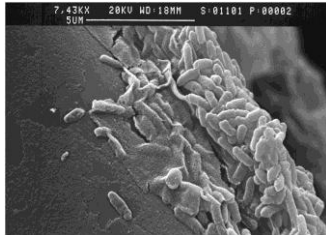
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- **Opportunistic** pathogen (human / animal)



- **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

# Background



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

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



## 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

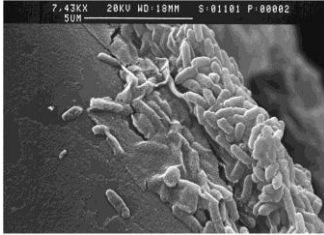
<https://respe.net/reproduction-saison-de-monte-2021-rapport-numero-1/>



RESPE

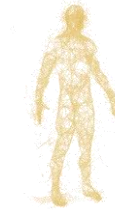
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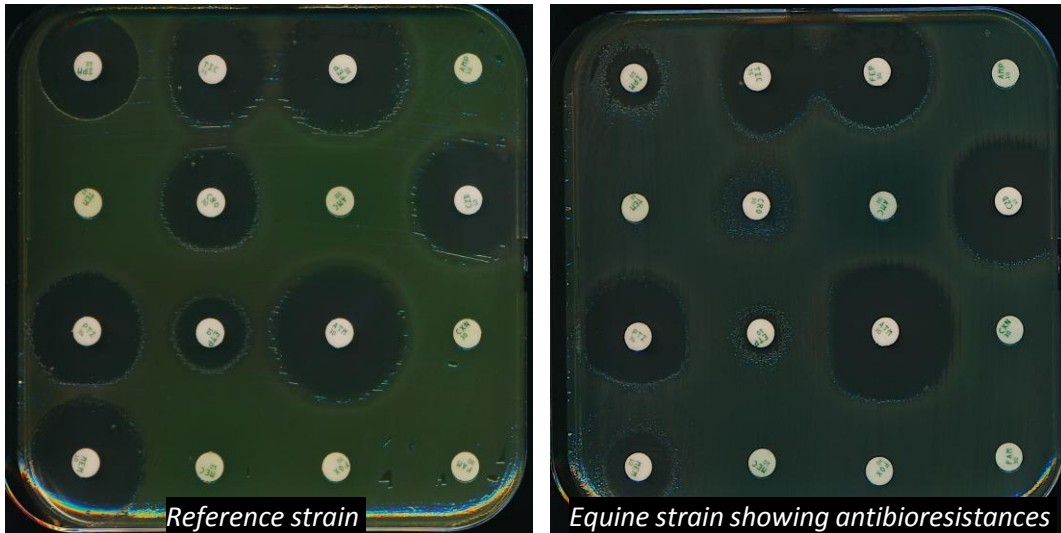


SEM observation of *Pseudomonas aeruginosa* biofilm, (Benamara et al. 2014).

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- **Therapeutic failures:** strains close to total resistance to antibiotics (*Alnour et al. 2017*)
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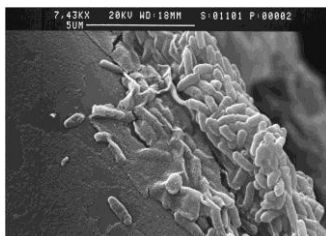
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Antibiograms

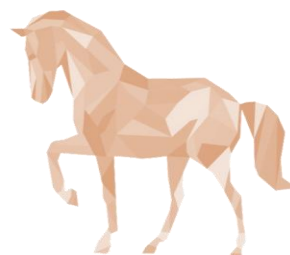
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# Background



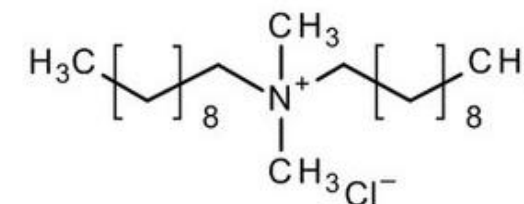
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- **STI** = sexually transmitted infections like contagious equine metritis (*Rapport de monte 2021, RESPE*)
- Mandatory screening for before breeding in thoroughbreds
- **Resistance selection** through antibiotic/disinfectant misuse

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



Chemical formula for DDAC (Sigma-Aldrich)





## Objective

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

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183 PSAE isolates



Isolated from samples received for analysis



**Table I: Repartition of strains origin.**

Sample origine	Isolation year					Total
	1996 - 2015	2017	2018	2019	2020	
<b>Bovine</b>					<b>1</b>	<b>1</b>
<b>Milk</b>					<b>1</b>	<b>1</b>
<b>Canine</b>		<b>5</b>	<b>9</b>	<b>8</b>	<b>11</b>	<b>33</b>
<b>Auricular</b>		<b>5</b>	<b>8</b>	<b>7</b>	<b>9</b>	<b>29</b>
<b>Occular</b>				<b>1</b>	<b>1</b>	<b>2</b>
<b>Genital</b>					<b>1</b>	<b>1</b>
<b>Wound / cutaneous</b>			<b>1</b>			<b>1</b>
<b>Equine</b>	<b>23</b>	<b>5</b>	<b>10</b>	<b>68</b>	<b>40</b>	<b>146</b>
<b>Genital</b>		<b>1</b>	<b>2</b>	<b>63</b>	<b>33</b>	<b>99</b>
<b>Not specified</b>	<b>23</b>	<b>1</b>				<b>24</b>
<b>Respiratory</b>		<b>1</b>	<b>6</b>	<b>3</b>	<b>2</b>	<b>12</b>
<b>Wound / cutaneous</b>		<b>2</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>7</b>
<b>Occular</b>			<b>1</b>	<b>1</b>	<b>1</b>	<b>3</b>
<b>Lymphatic</b>					<b>1</b>	<b>1</b>
<b>Feline</b>					<b>2</b>	<b>2</b>
<b>Auricular</b>					<b>1</b>	<b>1</b>
<b>Digestive</b>					<b>1</b>	<b>1</b>
<b>Asinine</b>				<b>1</b>		<b>1</b>
<b>Genital</b>				<b>1</b>		<b>1</b>
<b>Total</b>	<b>23</b>	<b>10</b>	<b>19</b>	<b>77</b>	<b>54</b>	<b>183</b>

# Materials and methods

## Sampling

183 PSAE isolates



Period 1996 - 2020

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<b>Asinine</b>				1		1
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# Materials and methods

## Sampling

183 PSAE isolates



Period 1996 - 2020



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

**Table I: Repartition of strains origin.**

Sample origine	Isolation year					Total
	1996 - 2015	2017	2018	2019	2020	
<b>Bovine</b>					1	1
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<b>Auricular</b>					1	1
<b>Digestive</b>					1	1
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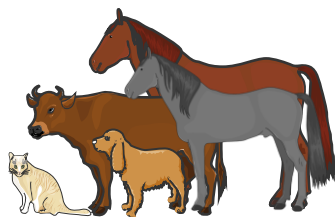
# Materials and methods

## Sampling

183 PSAE isolates



Various animal origin



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<b>Ocular</b>				<b>1</b>	<b>1</b>	<b>2</b>
<b>Genital</b>					<b>1</b>	<b>1</b>
<b>Wound / cutaneous</b>			<b>1</b>			<b>1</b>
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<b>Wound / cutaneous</b>		<b>2</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>7</b>
<b>Ocular</b>			<b>1</b>	<b>1</b>	<b>1</b>	<b>3</b>
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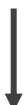
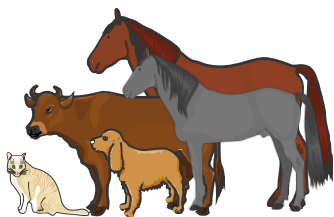
# Materials and methods

## Sampling

183 PSAE isolates



Various animal origin



Most represented:

- **Equine (146/183 → 80%)**

**Table I: Repartition of strains origin.**

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	1996 - 2015	2017	2018	2019	2020	
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<b>Genital</b>					<b>1</b>	<b>1</b>
<b>Wound / cutaneous</b>			<b>1</b>			<b>1</b>
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<b>Digestive</b>					<b>1</b>	<b>1</b>
<b>Asinine</b>				<b>1</b>		<b>1</b>
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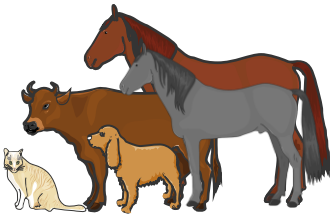
# Materials and methods

## Sampling

183 PSAE isolates



Various animal origin



Most represented:

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

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Ocular			1	1	1	3
Lymphatic					1	1
Feline					2	2
Auricular					1	1
Digestive					1	1
Asinine				1		1
Genital				1		1
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183 PSAE isolates



Various animal origin



Most represented:

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

For the others n=4 → 2%

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# Materials and methods

## Sampling

183 PSAE isolates



Isolates from various samples

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	1996 - 2015	2017	2018	2019	2020	
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<b>Occular</b>				<b>1</b>	<b>1</b>	<b>2</b>
<b>Genital</b>					<b>1</b>	<b>1</b>
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<b>Lymphatic</b>					<b>1</b>	<b>1</b>
<b>Feline</b>					<b>2</b>	<b>2</b>
<b>Auricular</b>					<b>1</b>	<b>1</b>
<b>Digestive</b>					<b>1</b>	<b>1</b>
<b>Asinine</b>				<b>1</b>		<b>1</b>
<b>Genital</b>				<b>1</b>		<b>1</b>
<b>Total</b>	<b>23</b>	<b>10</b>	<b>19</b>	<b>77</b>	<b>54</b>	<b>183</b>

# Materials and methods

## Sampling

183 PSAE isolates



Isolates from various samples



Most represented:

- Genital (101/183 → 55%)  
*mainly associated with equine samples*

**Table I: Repartition of strains origin.**

Sample origine	Isolation year					Total
	1996 - 2015	2017	2018	2019	2020	
<b>Bovine</b>					<b>1</b>	<b>1</b>
<b>Milk</b>					<b>1</b>	<b>1</b>
<b>Canine</b>		<b>5</b>	<b>9</b>	<b>8</b>	<b>11</b>	<b>33</b>
<b>Auricular</b>		<b>5</b>	<b>8</b>	<b>7</b>	<b>9</b>	<b>29</b>
<b>Occular</b>				<b>1</b>	<b>1</b>	<b>2</b>
<b>Genital</b>					<b>1</b>	<b>1</b>
<b>Wound / cutaneous</b>			<b>1</b>			<b>1</b>
<b>Equine</b>	<b>23</b>	<b>5</b>	<b>10</b>	<b>68</b>	<b>40</b>	<b>146</b>
<b>Genital</b>		<b>1</b>	<b>2</b>	<b>63</b>	<b>33</b>	<b>99</b>
<b>Not specified</b>	<b>23</b>	<b>1</b>				<b>24</b>
<b>Respiratory</b>		<b>1</b>	<b>6</b>	<b>3</b>	<b>2</b>	<b>12</b>
<b>Wound / cutaneous</b>		<b>2</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>7</b>
<b>Occular</b>			<b>1</b>	<b>1</b>	<b>1</b>	<b>3</b>
<b>Lymphatic</b>					<b>1</b>	<b>1</b>
<b>Feline</b>					<b>2</b>	<b>2</b>
<b>Auricular</b>					<b>1</b>	<b>1</b>
<b>Digestive</b>					<b>1</b>	<b>1</b>
<b>Asinine</b>				<b>1</b>		<b>1</b>
<b>Genital</b>				<b>1</b>		<b>1</b>
<b>Total</b>	<b>23</b>	<b>10</b>	<b>19</b>	<b>77</b>	<b>54</b>	<b>183</b>

# Materials and methods

## Sampling

183 PSAE isolates



Isolates from various samples



Most represented:

- Genital (101/183 → 55%)  
*mainly associated with equine samples*
- Auricular (30/183 → 16%)  
*mainly associated with canine samples*

**Table I: Repartition of strains origin.**

Sample origine	Isolation year					Total
	1996 - 2015	2017	2018	2019	2020	
<b>Bovine</b>					1	1
<b>Milk</b>					1	1
<b>Canine</b>		5	9	8	11	33
<b>Auricular</b>		5	8	7	9	29
<b>Occular</b>				1	1	2
<b>Genital</b>					1	1
<b>Wound / cutaneous</b>			1			1
<b>Equine</b>	23	5	10	68	40	146
<b>Genital</b>		1	2	63	33	99
<b>Not specified</b>	23	1				24
<b>Respiratory</b>		1	6	3	2	12
<b>Wound / cutaneous</b>		2	1	1	3	7
<b>Occular</b>			1	1	1	3
<b>Lymphatic</b>					1	1
<b>Feline</b>					2	2
<b>Auricular</b>					1	1
<b>Digestive</b>					1	1
<b>Asinine</b>				1		1
<b>Genital</b>				1		1
<b>Total</b>	<b>23</b>	<b>10</b>	<b>19</b>	<b>77</b>	<b>54</b>	<b>183</b>

# Materials and methods

## Sampling

183 PSAE isolates



Isolates from various samples



Most represented:

- Genital (101/183 → 55%)  
*mainly associated with equine samples*
- Auricular (30/183 → 16%)  
*mainly associated with canine samples*
- Respiratory (12/183 → 7%)  
*only equine*

**Table I: Repartition of strains origin.**

Sample origine	Isolation year					Total
	1996 - 2015	2017	2018	2019	2020	
<b>Bovine</b>					1	1
<b>Milk</b>					1	1
<b>Canine</b>		5	9	8	11	33
<b>Auricular</b>		5	8	7	9	29
<b>Occular</b>				1	1	2
<b>Genital</b>					1	1
<b>Wound / cutaneous</b>			1			1
<b>Equine</b>	23	5	10	68	40	146
<b>Genital</b>		1	2	63	33	99
<b>Not specified</b>	23	1				24
<b>Respiratory</b>		1	6	3	2	12
<b>Wound / cutaneous</b>		2	1	1	3	7
<b>Occular</b>			1	1	1	3
<b>Lymphatic</b>					1	1
<b>Feline</b>					2	2
<b>Auricular</b>					1	1
<b>Digestive</b>					1	1
<b>Asinine</b>				1		1
<b>Genital</b>				1		1
<b>Total</b>	<b>23</b>	<b>10</b>	<b>19</b>	<b>77</b>	<b>54</b>	<b>183</b>

# Materials and methods

## Sampling DDAC decrease susceptibility test

Determination of **Minimum inhibitory concentrations (MICs)**, in triplicate  
by broth dilution method.

*According to the International Organization for Standardization.*



# Materials and methods

## Sampling DDAC decrease susceptibility test

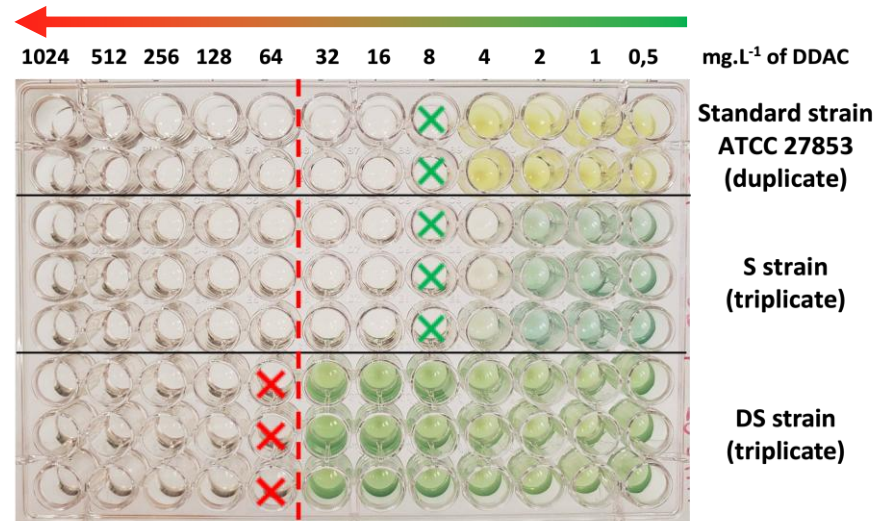
Determination of **Minimum inhibitory concentrations (MICs)**, in triplicate  
by broth dilution method.

*According to the International Organization for Standardization.*

**Decrease susceptibility cutoff** corresponding to the **concentration of DDAC in a disinfectant solution**  
regarding the manufacturer's instructions.

MIC > 62.9 mg.L<sup>-1</sup>  
**Decreased susceptibility strain (DS)**

MIC < 62.9 mg.L<sup>-1</sup>  
**Susceptible strain (S)**



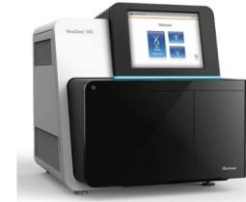
*Determination of DDAC MICs on a microplate*

# Materials and methods

## Multilocus Sequence Typing (MLST) and Core Genome MLST

**WGS – *Illumina*® technology**  
**In total 40/183 isolates sequenced**

<ul style="list-style-type: none"> <li>- 30 equines</li> <li>- 7 canines</li> <li>- 1 asinine</li> </ul>	-----	<ul style="list-style-type: none"> <li>- 1 bovine</li> <li>- 1 feline</li> </ul>
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*NextSeq500 (Illumina®)*

# Materials and methods

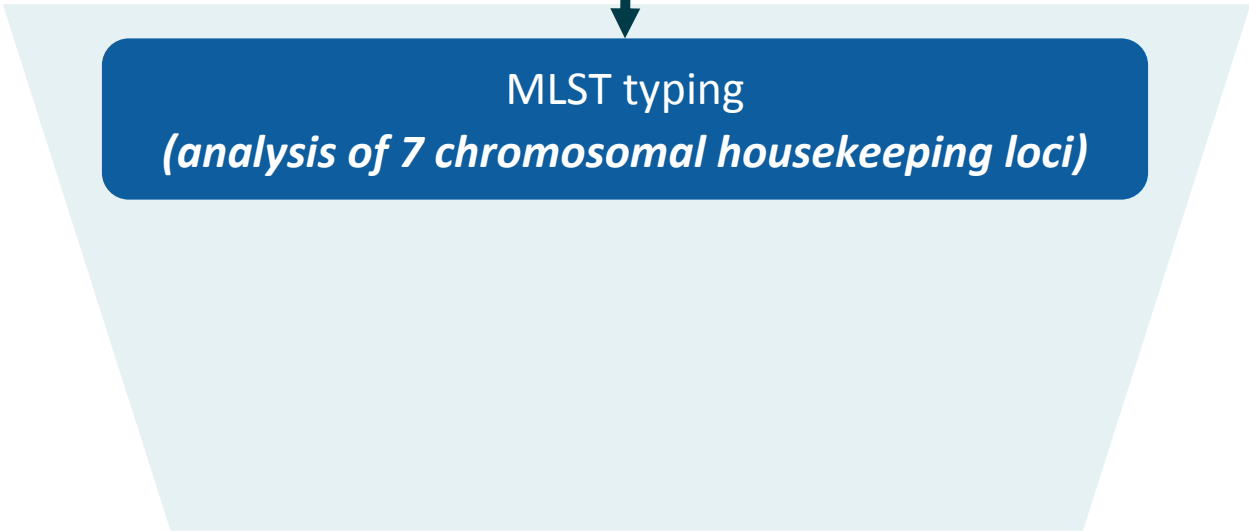
## Multilocus Sequence Typing (MLST) and Core Genome MLST

**WGS – *Illumina*® technology**  
**In total 40/183 isolates sequenced**

- 30 equines	- 1 bovine
- 7 canines	- 1 feline
- 1 asinine	



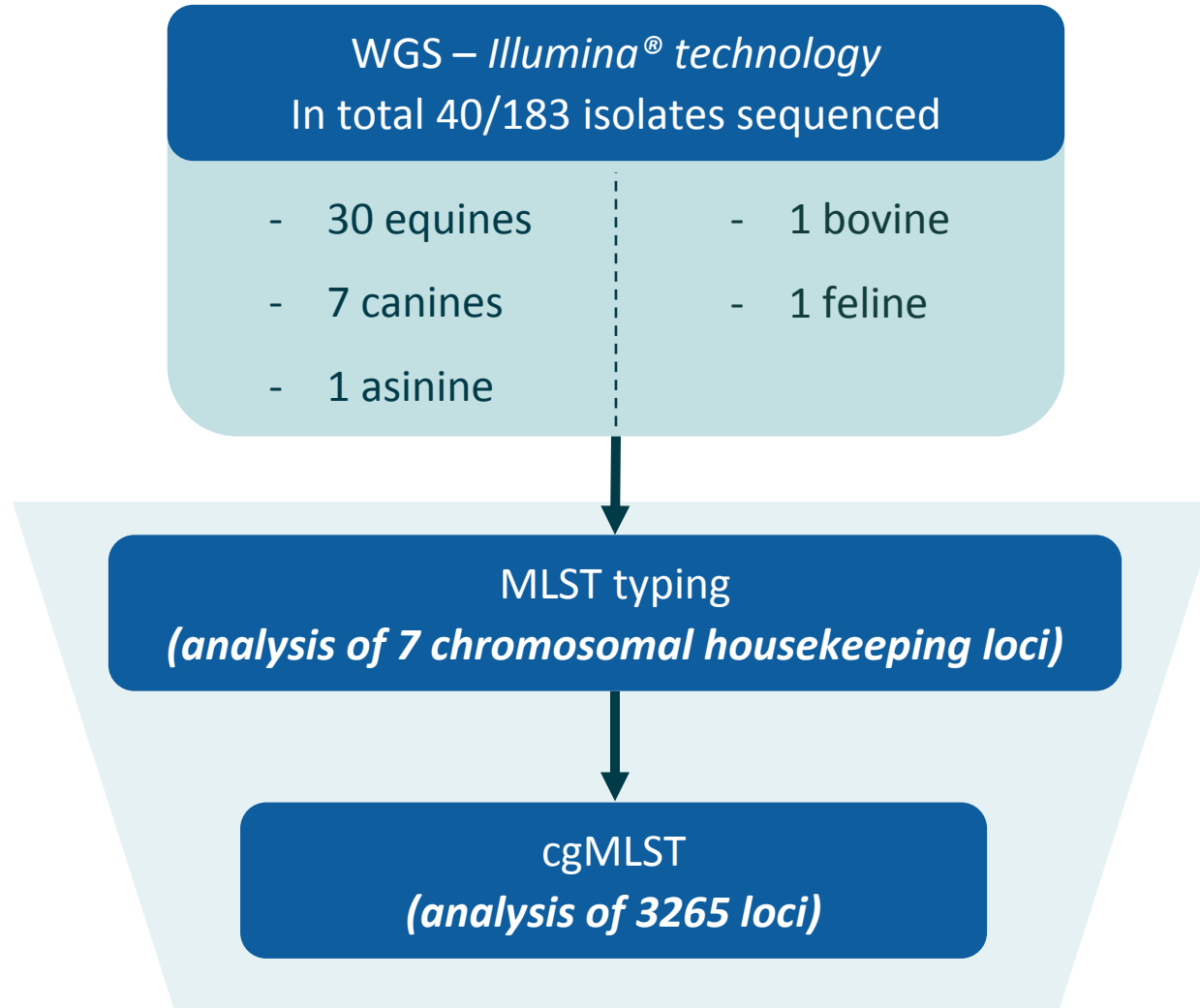
*NextSeq500 (Illumina®)*





# Materials and methods

## Multilocus Sequence Typing (MLST) and Core Genome MLST

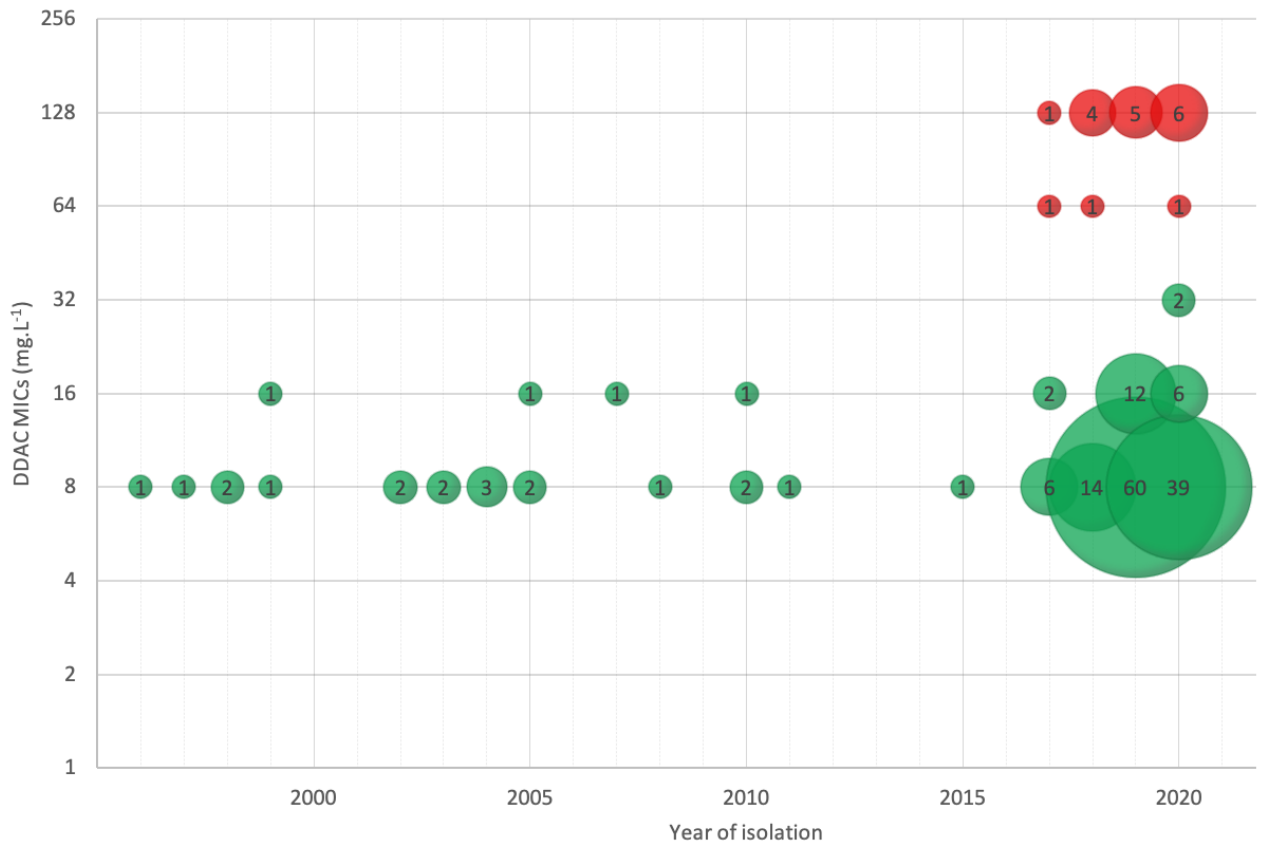


NextSeq500 (Illumina®)



# Results

## Decrease susceptibility over time

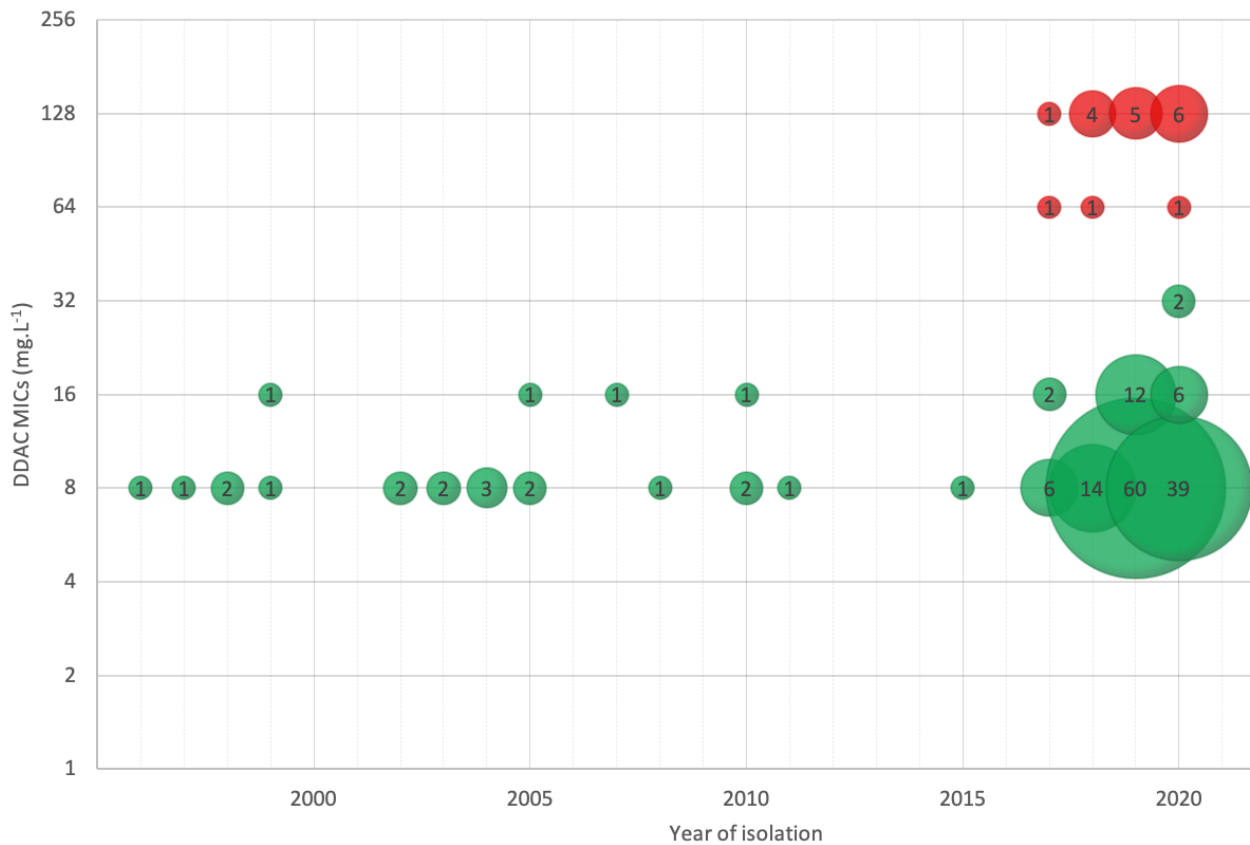


**Figure I: Temporal evolution of DDAC MICs for PSAE strains isolated from animals (n=183)**

*Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested*

# Results

## Decrease susceptibility over time



Green dot = susceptible isolate  
Red dot = DS to DDAC  
Dot size → sample size

DS to DDAC was:

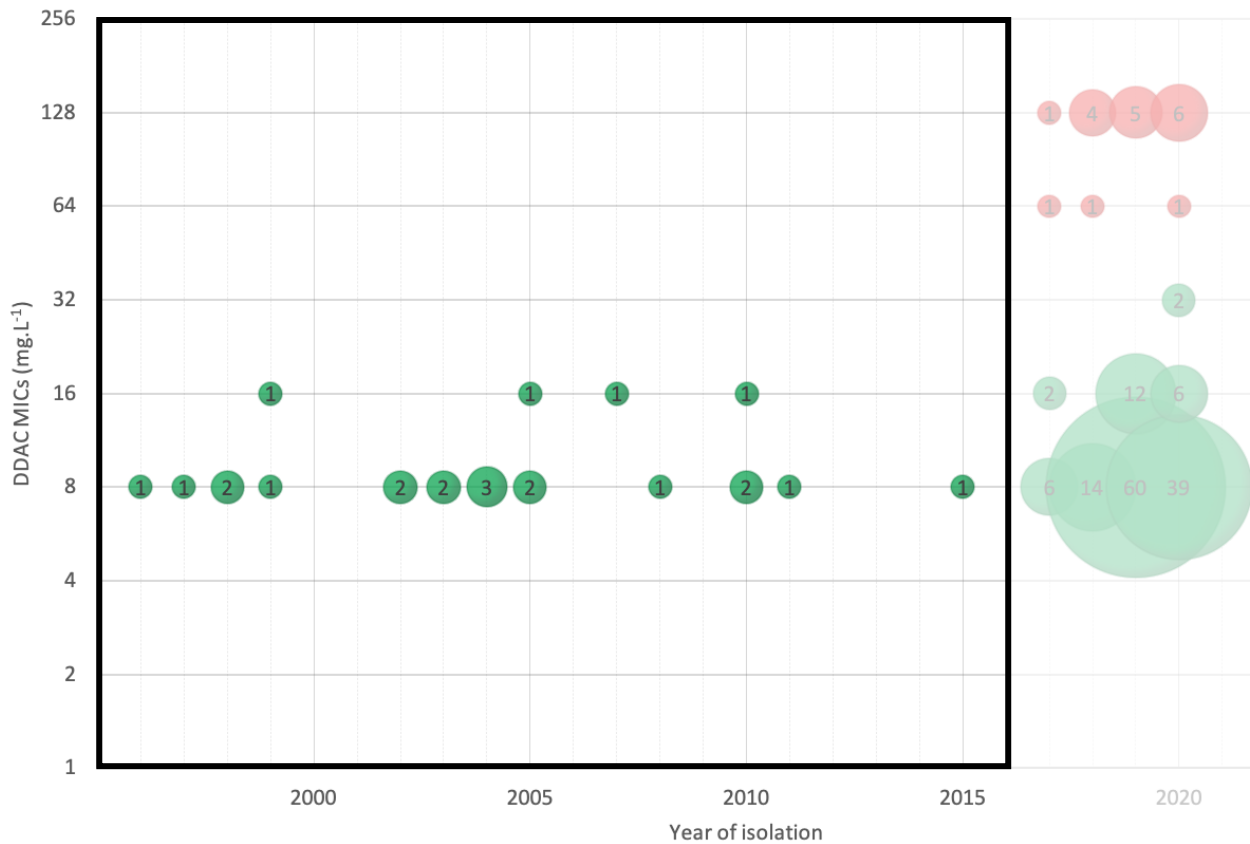
Not found before 2017

**Figure I: Temporal evolution of DDAC MICs for PSAE strains isolated from animals (n=183)**

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility over time



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

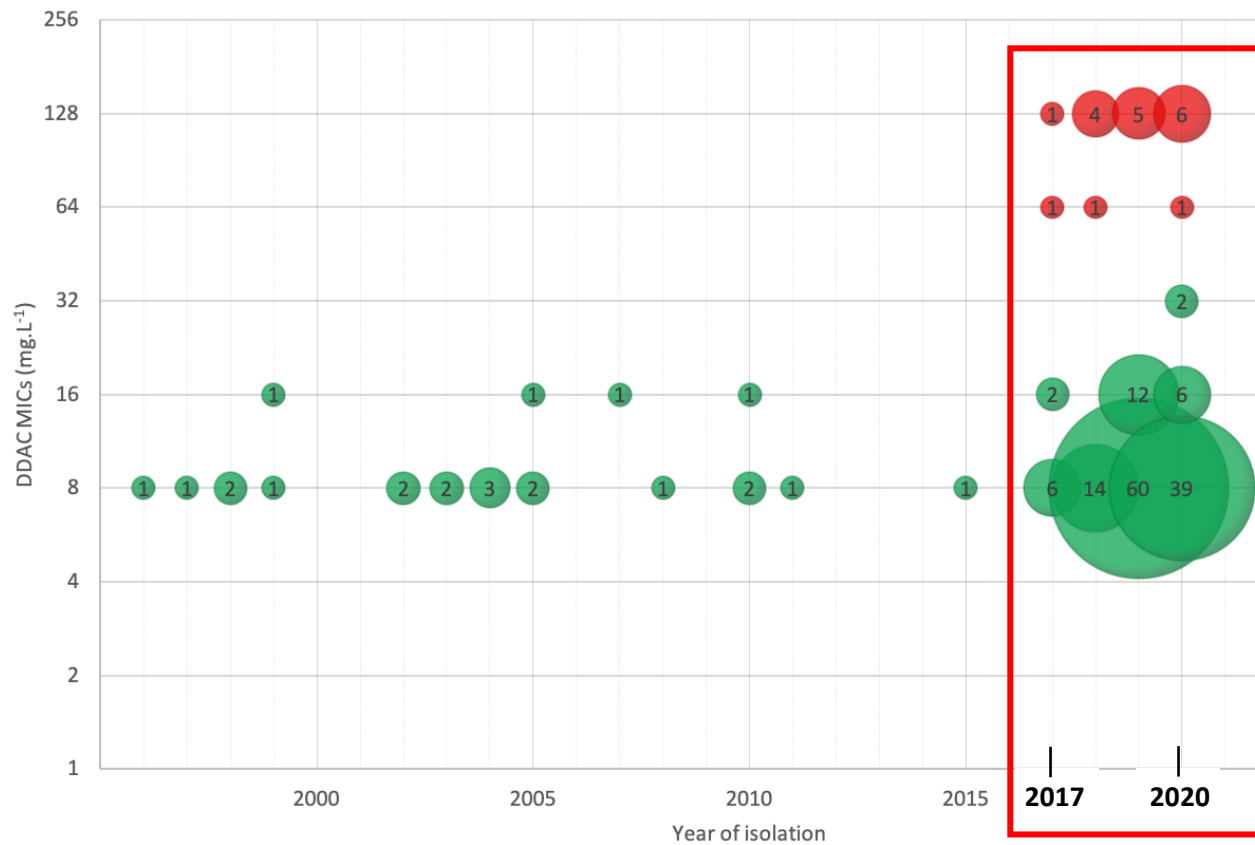
DS to DDAC was:

Not found before 2017

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

# Results

## Decrease susceptibility over time



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

DS to DDAC was:

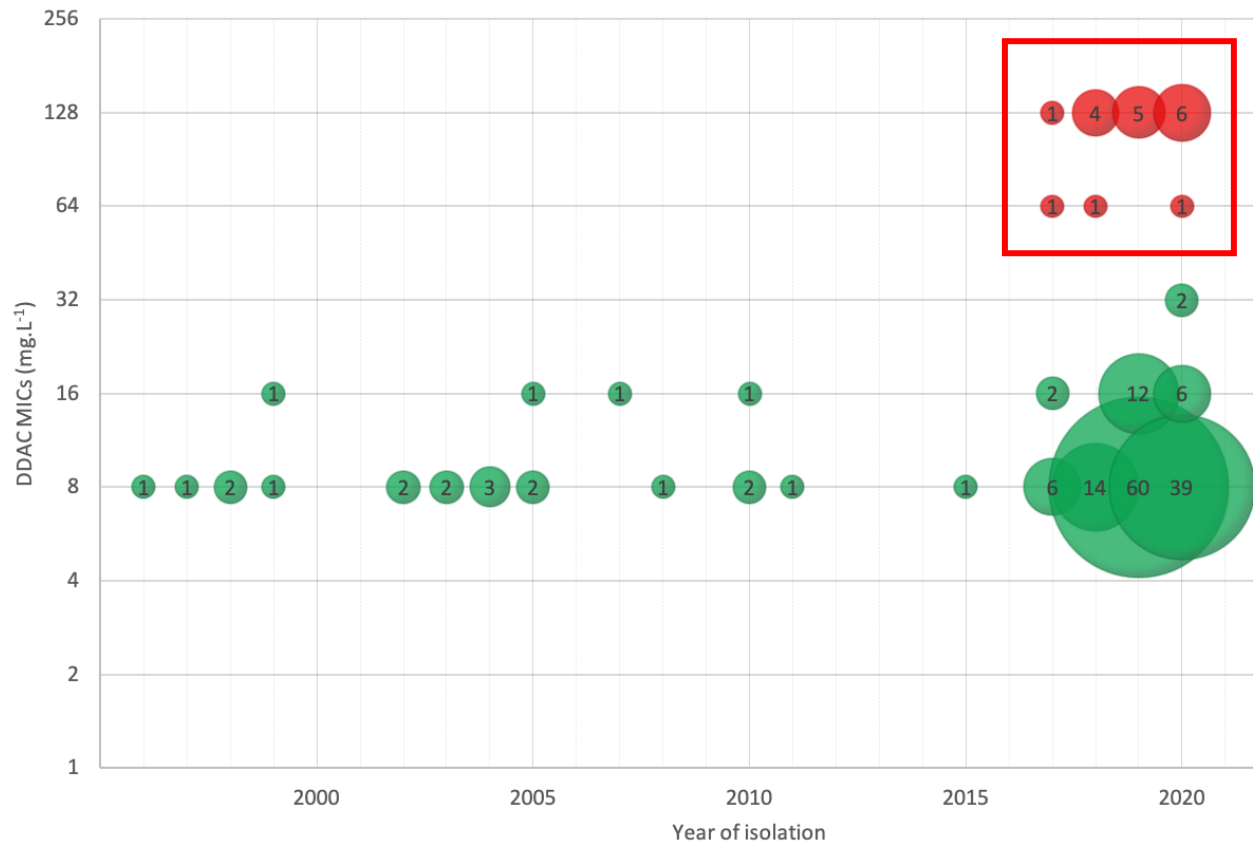
Not found before 2017

Only found between 2017 - 2020

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

# Results

## Decrease susceptibility over time



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

DS to DDAC was:

Not found before 2017

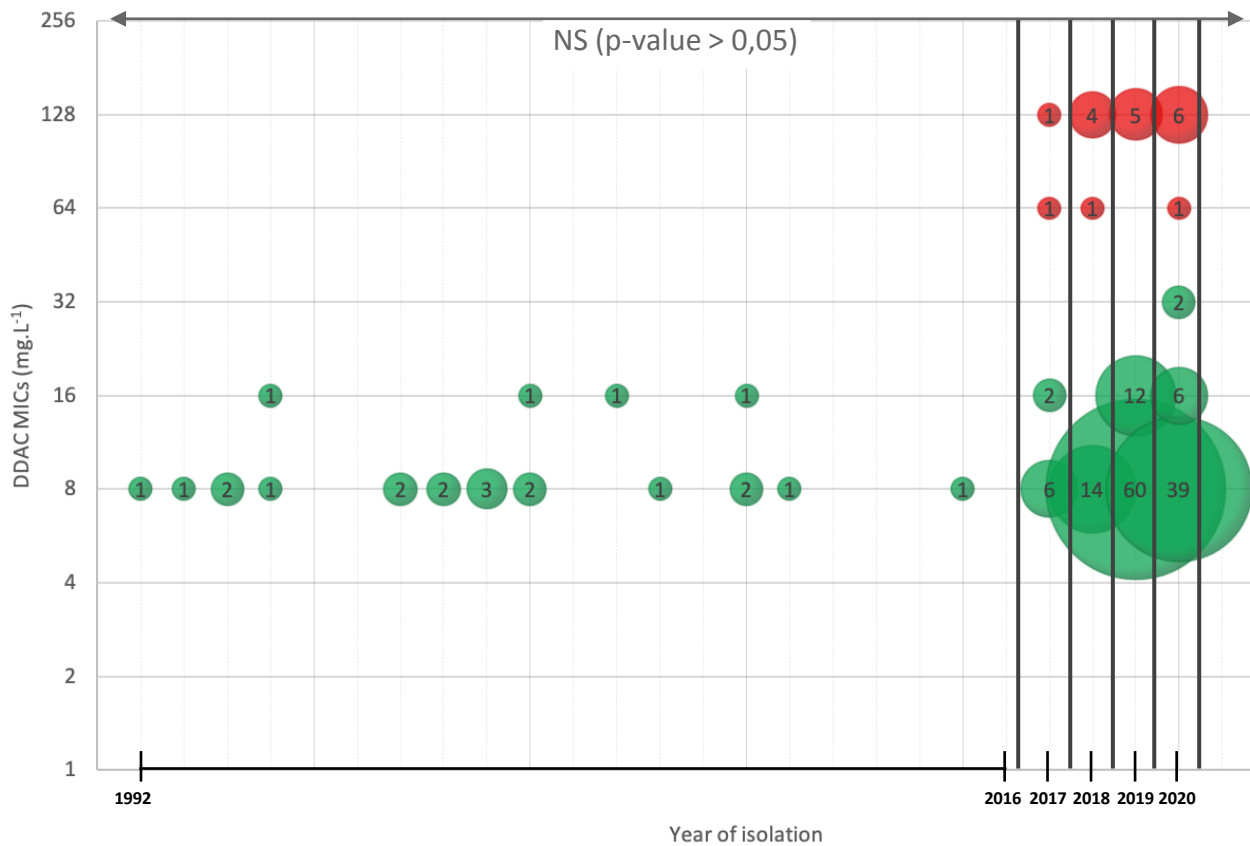
Only found between 2017 - 2020

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

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

# Results

## Decrease susceptibility over time



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

DS to DDAC was:

Not found before 2017

Only found between 2017 - 2020

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

Kruskal-Wallis test :

**No significant variation** of mean MIC between

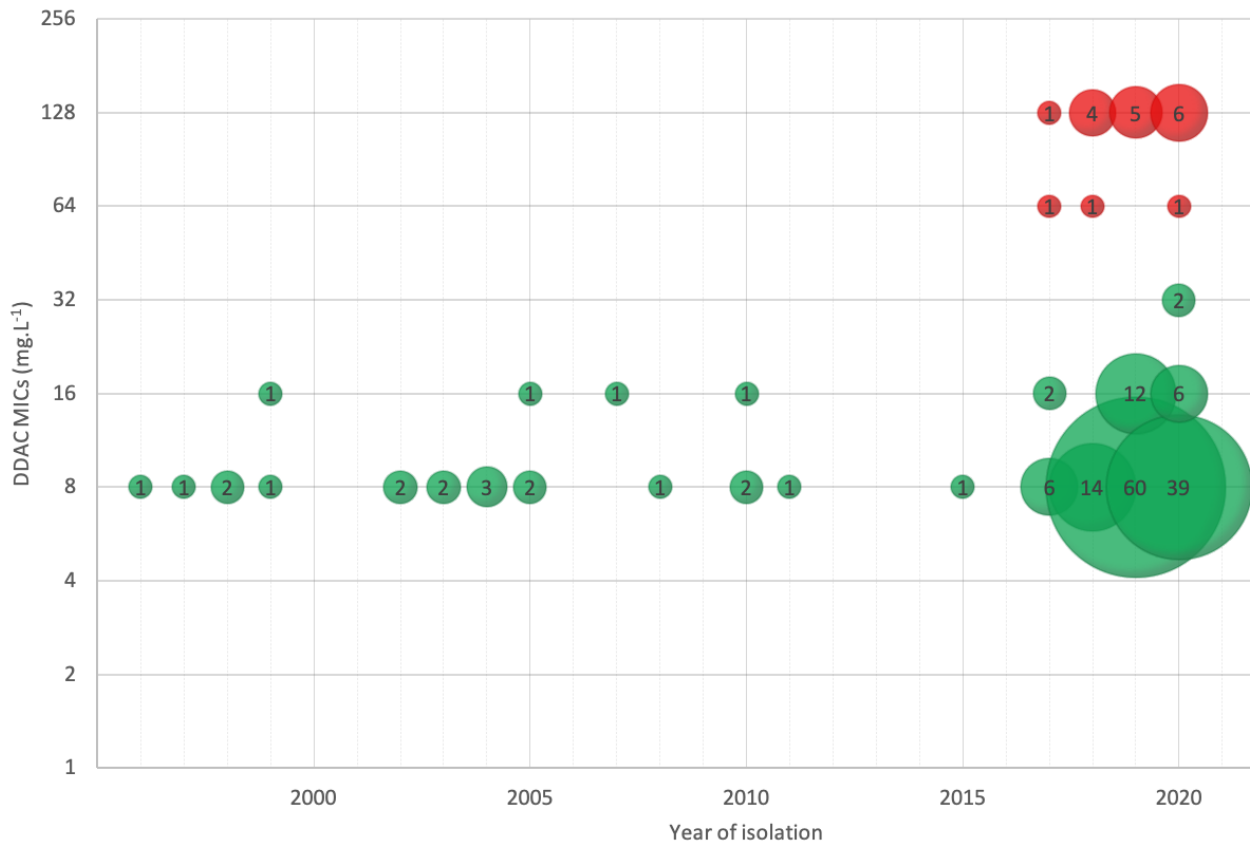
1996-2016 / 2017 / 2018 / 2019 / 2020

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



# Results

## Decrease susceptibility over time



➔ Based on our study, DS to DDAC exists since 2017 in veterinary medicine.

➔ This represents 10% of the tested isolates.

➔ No significant MICs variation over time was found.

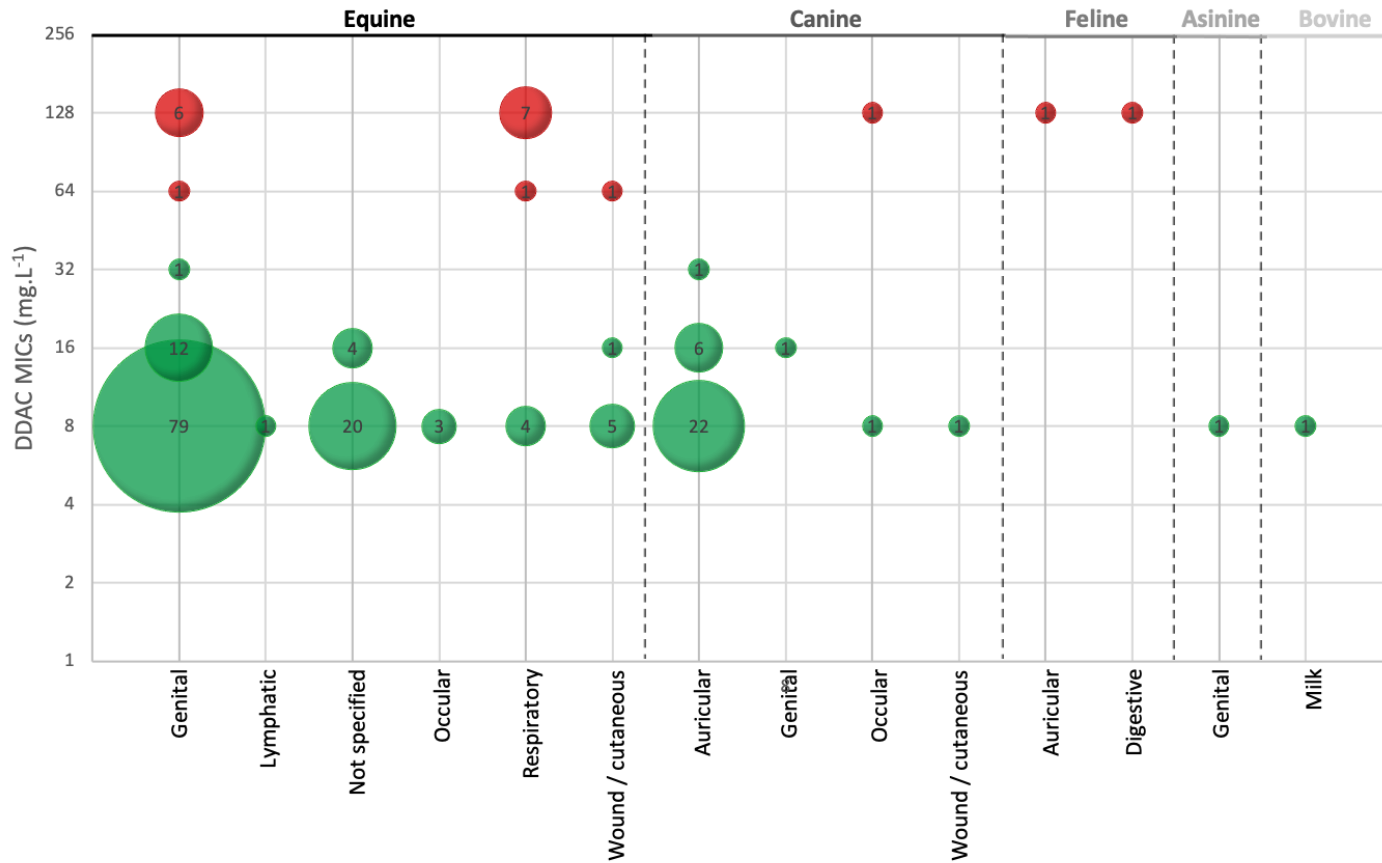
**Figure I: Temporal evolution of DDAC MICs for PSAE strains isolated from animals (n=183)**

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested



# Results

## Decrease susceptibility according to isolate origin



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

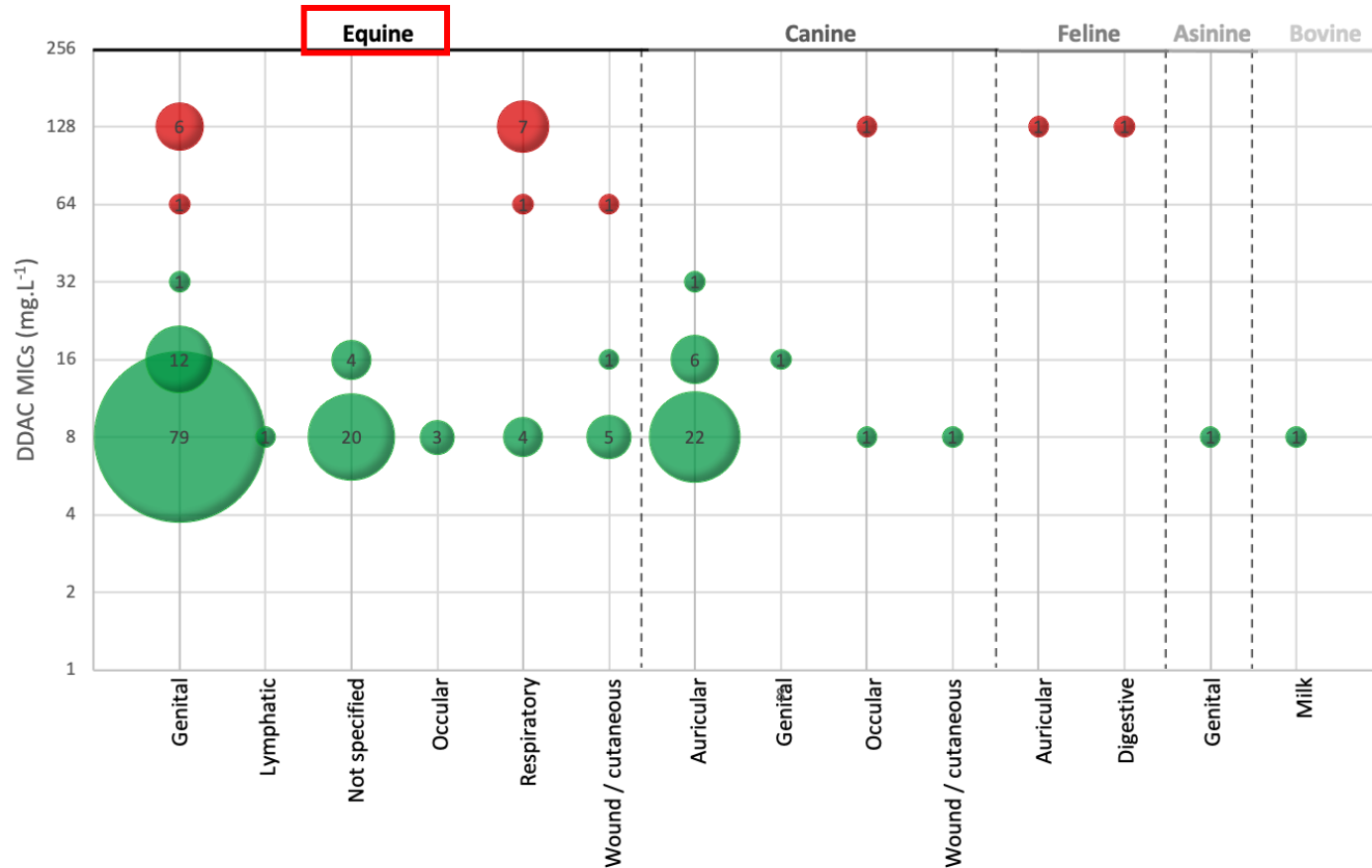
DS to DDAC was found on:

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



Green dot = susceptible isolate  
 Red dot = DS to DDAC  
 Dot size → sample size

DS to DDAC was found on:

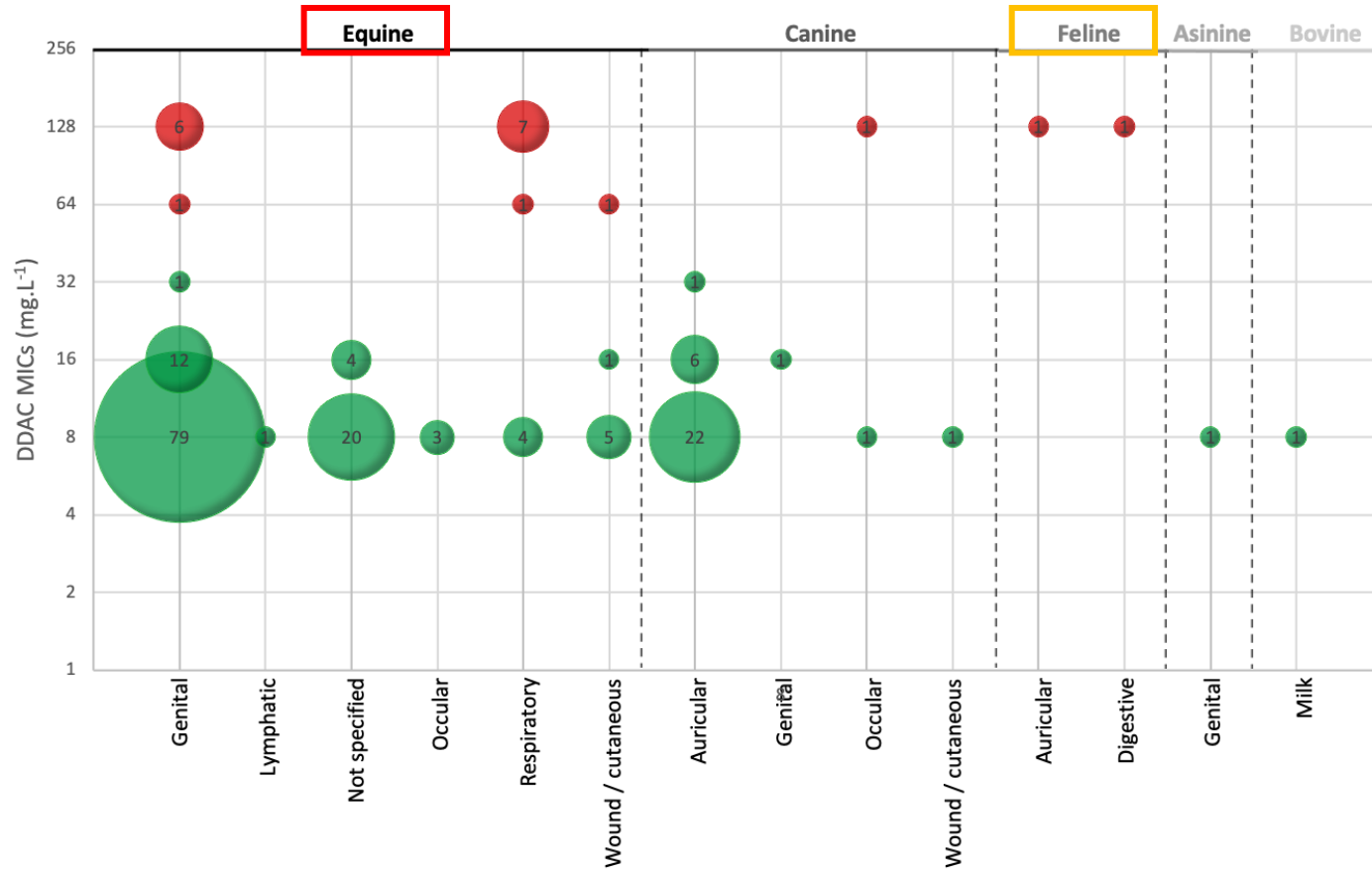
- Equine n=16/146 → 11%

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



Green dot = susceptible isolate  
Red dot = DS to DDAC  
Dot size → sample size

DS to DDAC was found on:

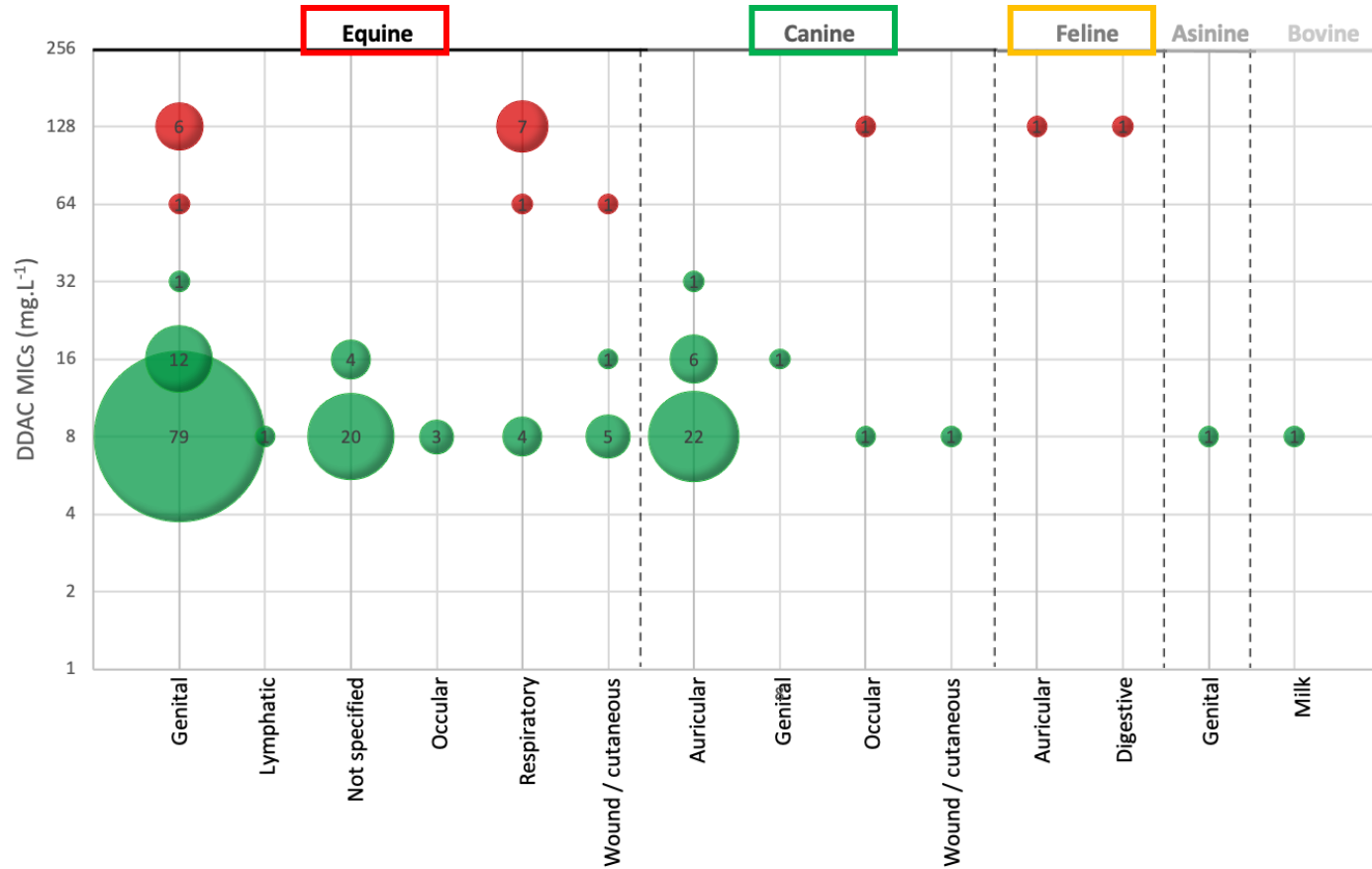
- Equine n=16/146 → 11%
- Feline n=2/2

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



Green dot = susceptible isolate

Red dot = DS to DDAC

Dot size → sample size

DS to DDAC was found on:

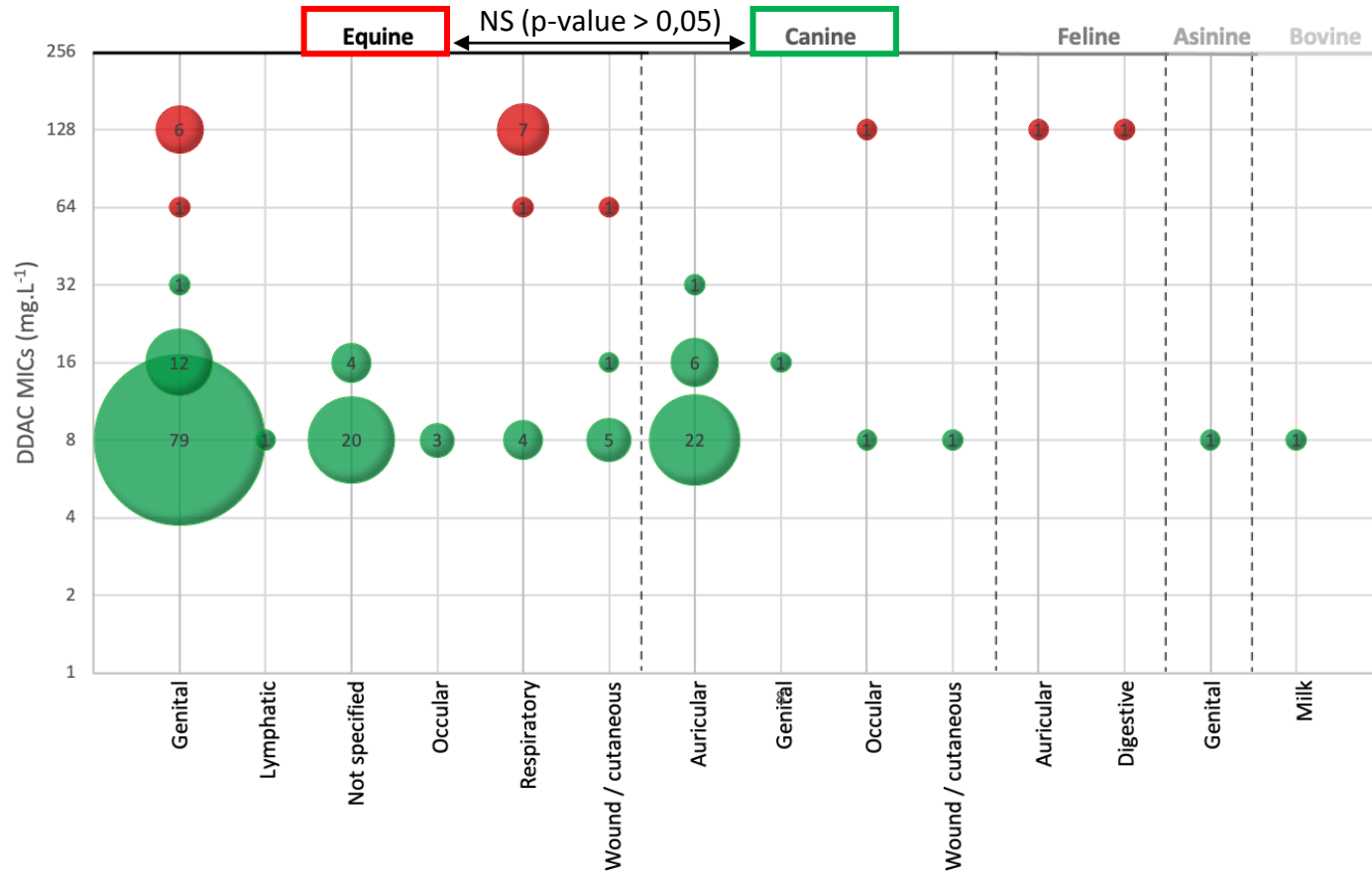
- Equine n=16/146 → 11%
- Feline n=2/2
- Canine n=1/33

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



Green dot = susceptible isolate  
Red dot = DS to DDAC  
Dot size → sample size

### DS to DDAC was found on:

- Equine n=16/146 → 11%
- Feline n=2/2
- Canine n=1/33

### Mann-Whitney test:

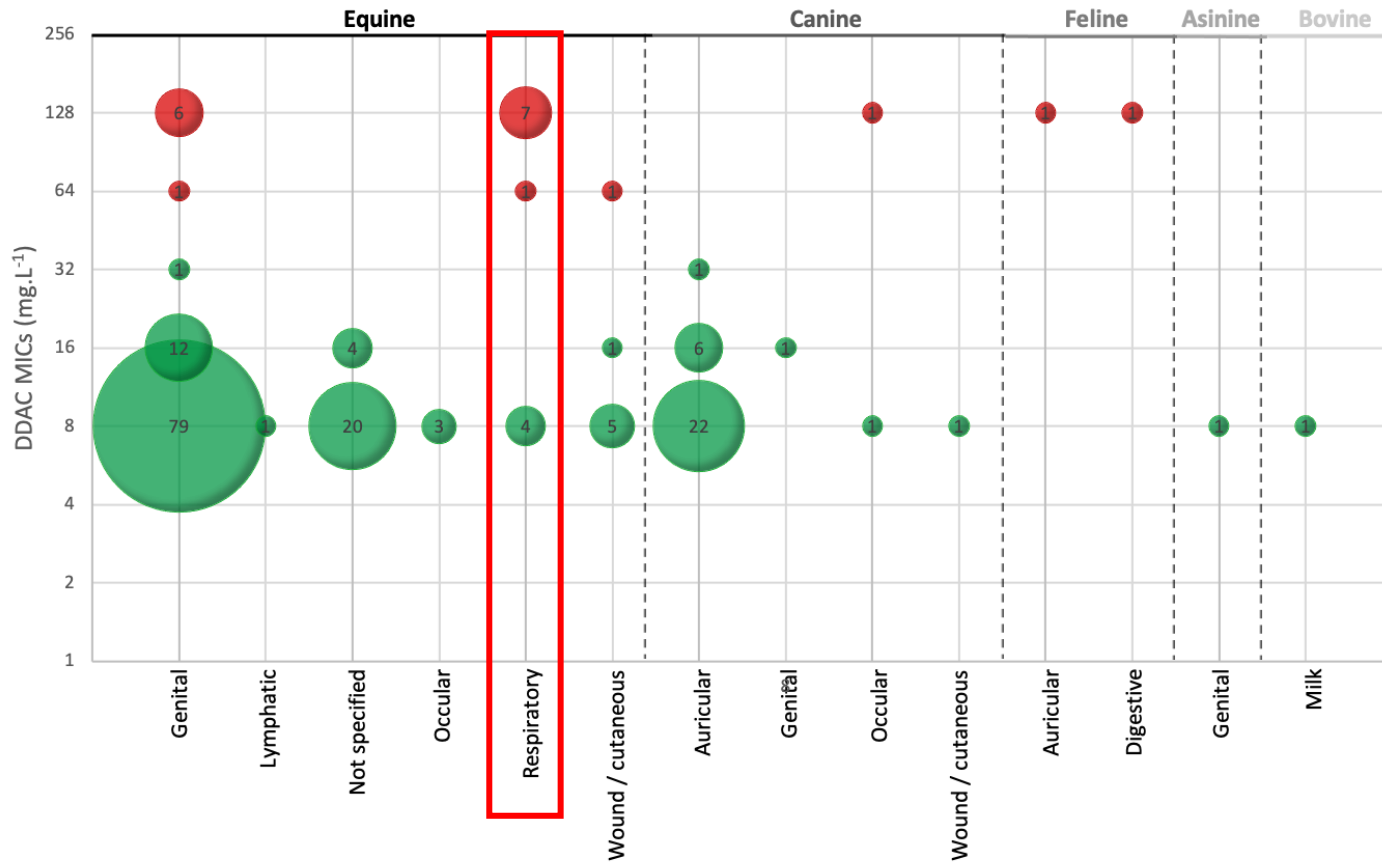
- No significant variation of mean MIC between equine and canine isolates
- For the others, the n is too small

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



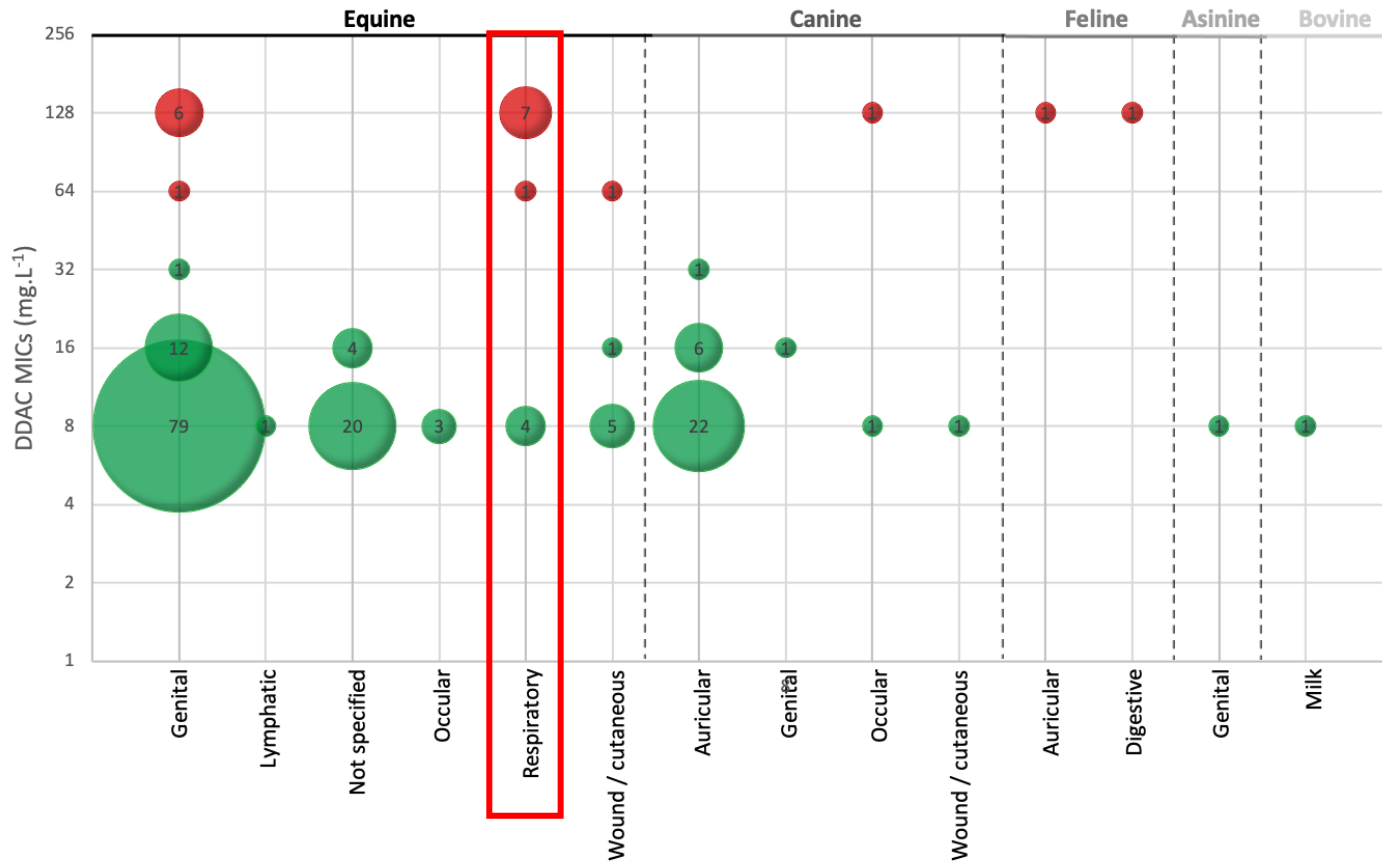
Which type of sampling?

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

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

# Results

## Decrease susceptibility according to isolate origin



### Which type of sampling?

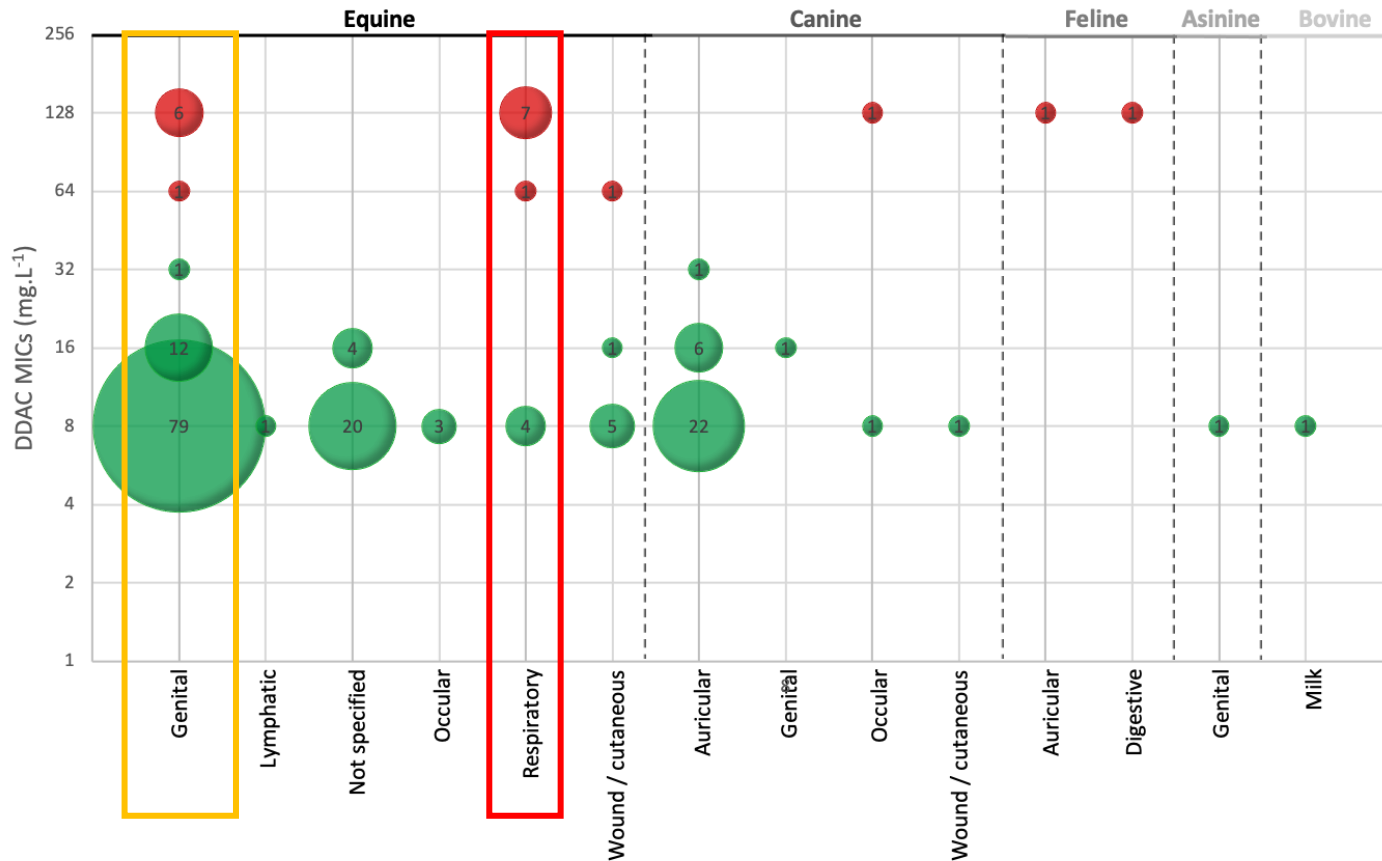
- **Respiratory** n=8/12 → 67%  
→ *Significant variation of mean MIC,*  
*p-value<sub>Kruskal-Wallis</sub> < 0,001.*  
→ *Sample types with n ≤ 5 are excluded from statistical analysis*

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

*Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested*

# Results

## Decrease susceptibility according to isolate origin



### Which type of sampling?

- **Respiratory** n=8/12 → 67%  
→ *Significant variation of mean MIC,*  
*p-value<sub>Kruskal-Wallis</sub> < 0,001.*  
→ *Sample types with n ≤ 5 are excluded from statistical analysis*
- **Genital** n=7/101 → 7%

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

*Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested*



# Results

## Decrease susceptibility according to isolate origin

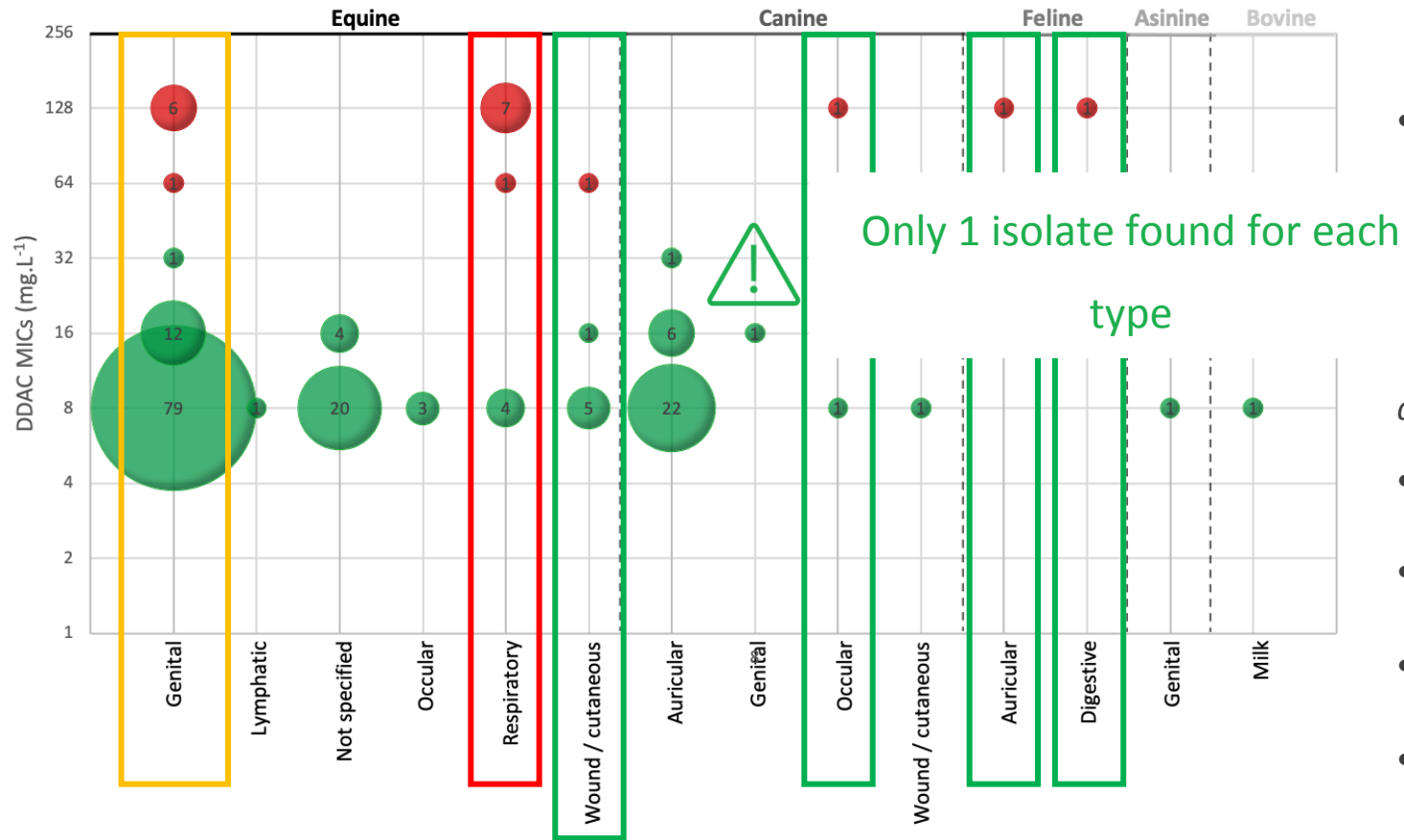


Figure II: Dispersion of DDAC MICs as a function of sample origin (n=183)

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

### Which type of sampling?

- **Respiratory** n=8/12 → 67%  
→ Significant variation of mean MIC,  
 $p\text{-value}_{Kruskal-Wallis} < 0,001$ .  
→ Sample types with  $n \leq 5$  are excluded from statistical analysis
- **Genital** n=7/101 → 7%
- Auricular n=1/30
- Cutaneous samples n=1/8
- Ocular n=1/5
- Digestive n=1/1

# Results

## Decrease susceptibility according to isolate origin

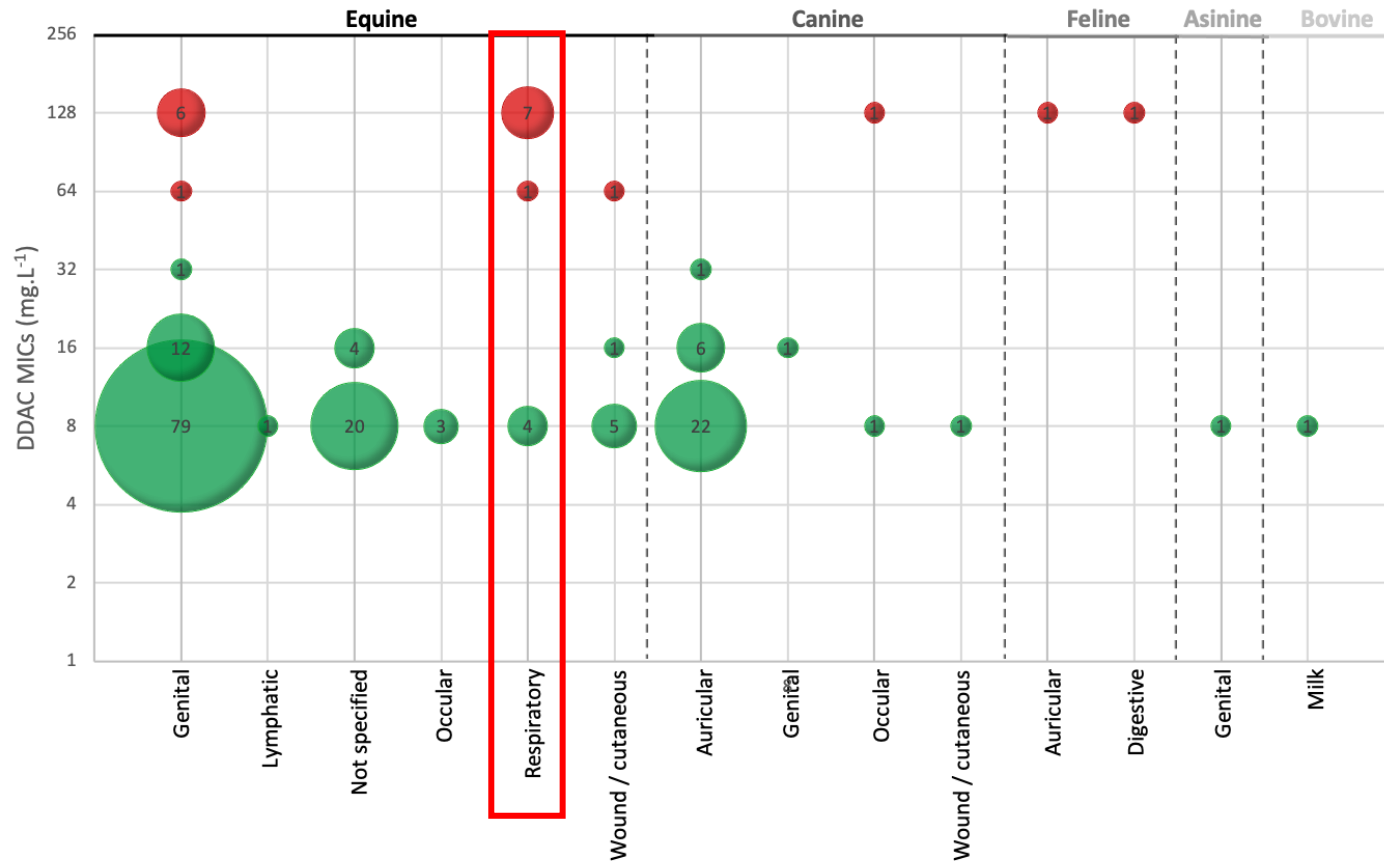


Figure II: Dispersion of DDAC MICs as a function of sample origin (n=183)

Red: sample with DDAC decreased susceptibility; Green: DDAC susceptible isolate; inside the bubbles: number of isolates tested

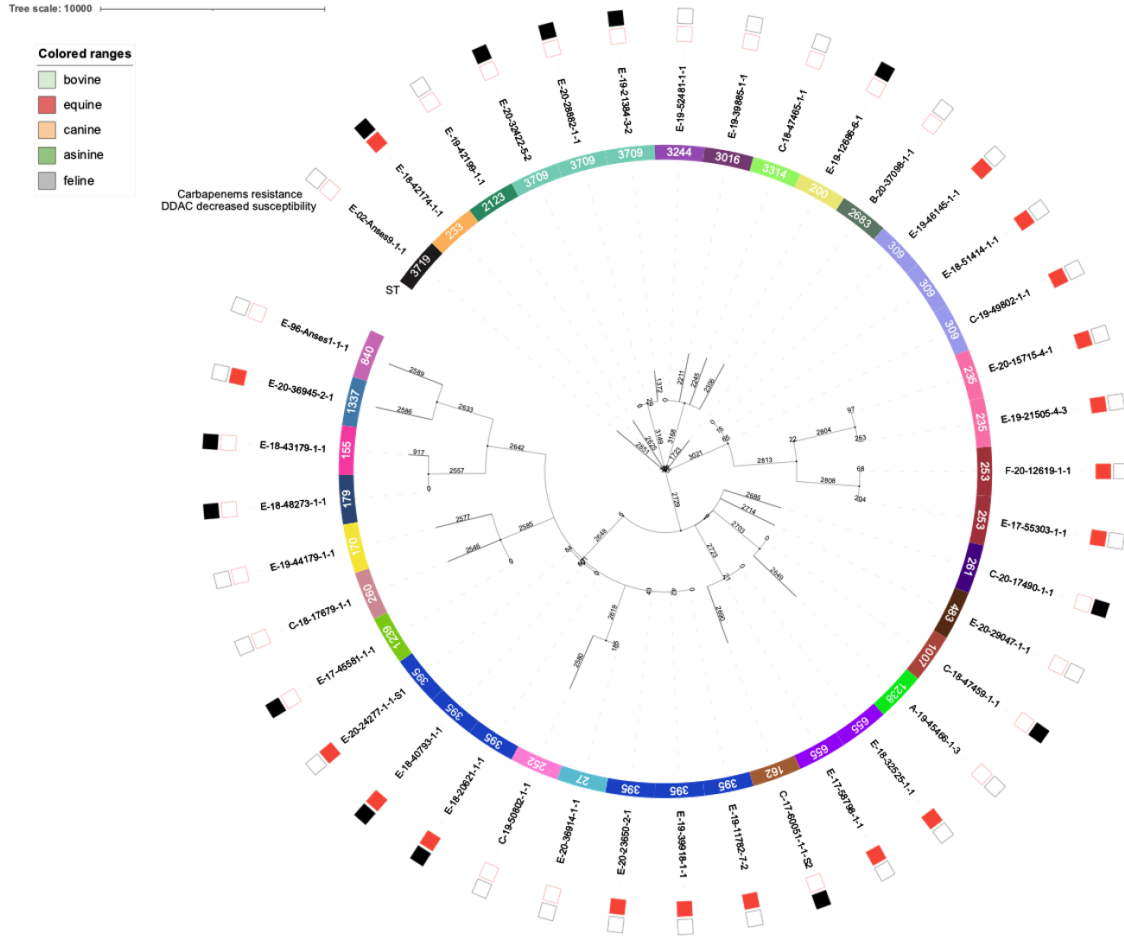
### Which type of sampling?

- **Respiratory** n=8/12 → 67%  
→ *Significant variation of mean MIC,*  
*p-value<sub>Kruskal-Wallis</sub> < 0,001.*  
→ *Sample types with n ≤ 5 are excluded from statistical analysis*
- **Genital** n=7/101 → 7%
- Auricular n=1/30
- Cutaneous samples n=1/8
- Ocular n=1/5
- Digestive n=1/1

➔ **DS to DDAC is mainly found in respiratory equine samples.**

# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST

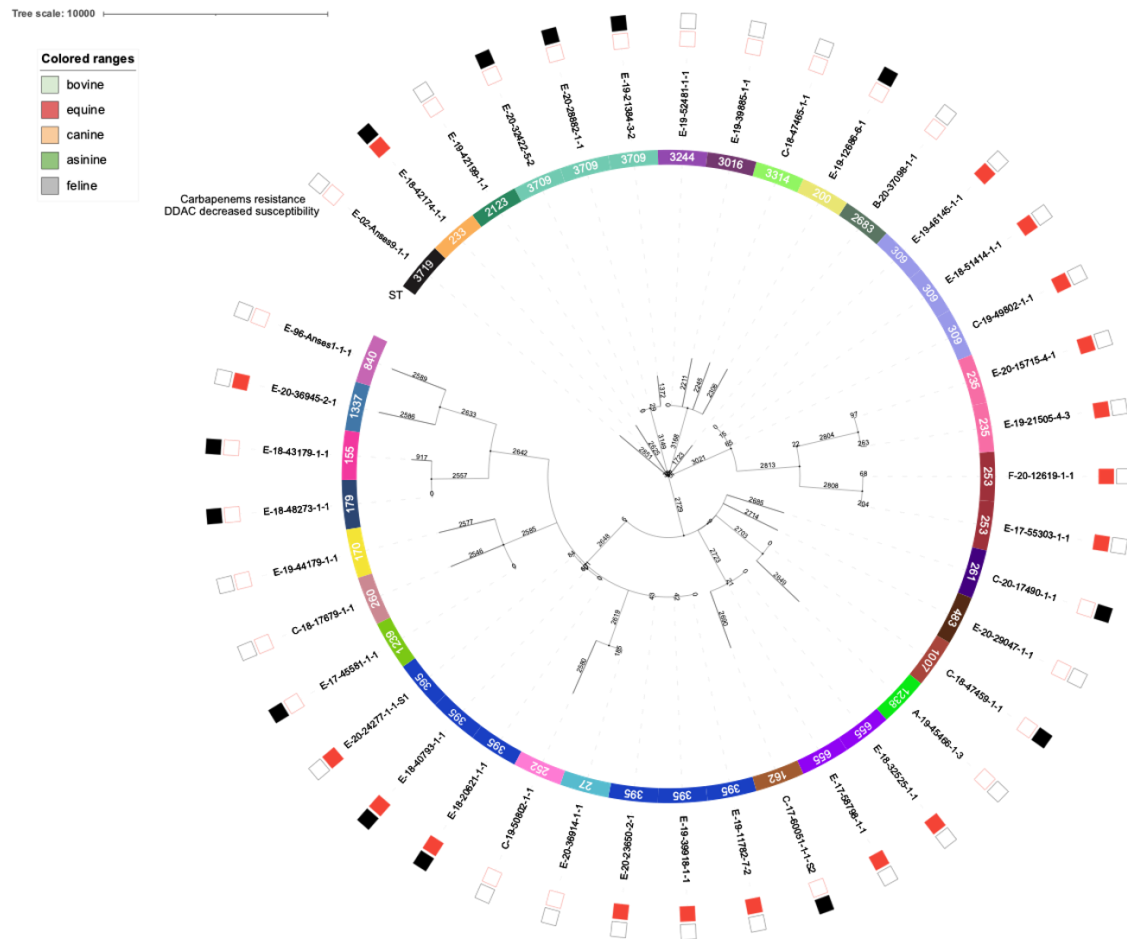


Which type of MLST profil?

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.

# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



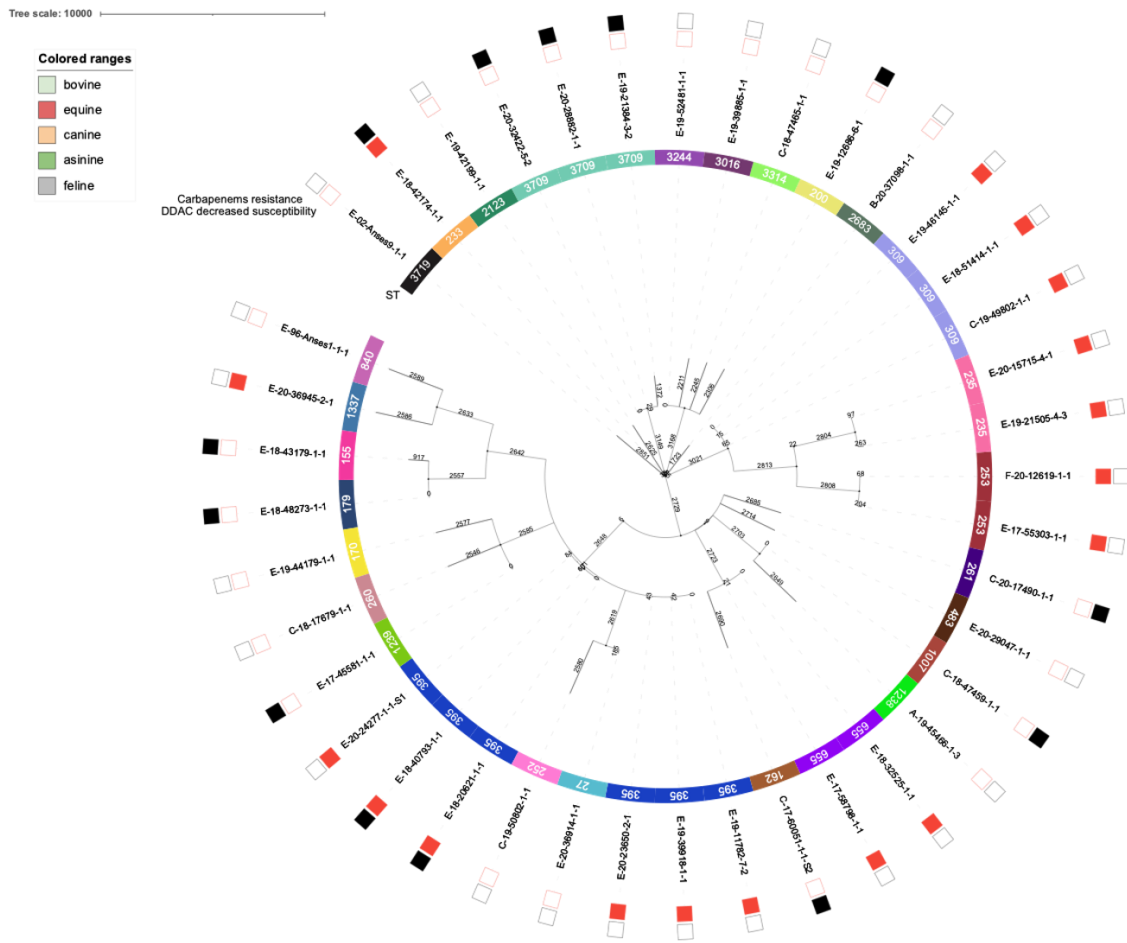
Which type of MLST profil?

28 STs found

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.

# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



Which type of MLST profil?

28 STs found



1<sup>st</sup>) ST 395 (n=6)

2<sup>st</sup>) ST 309 (n=3)

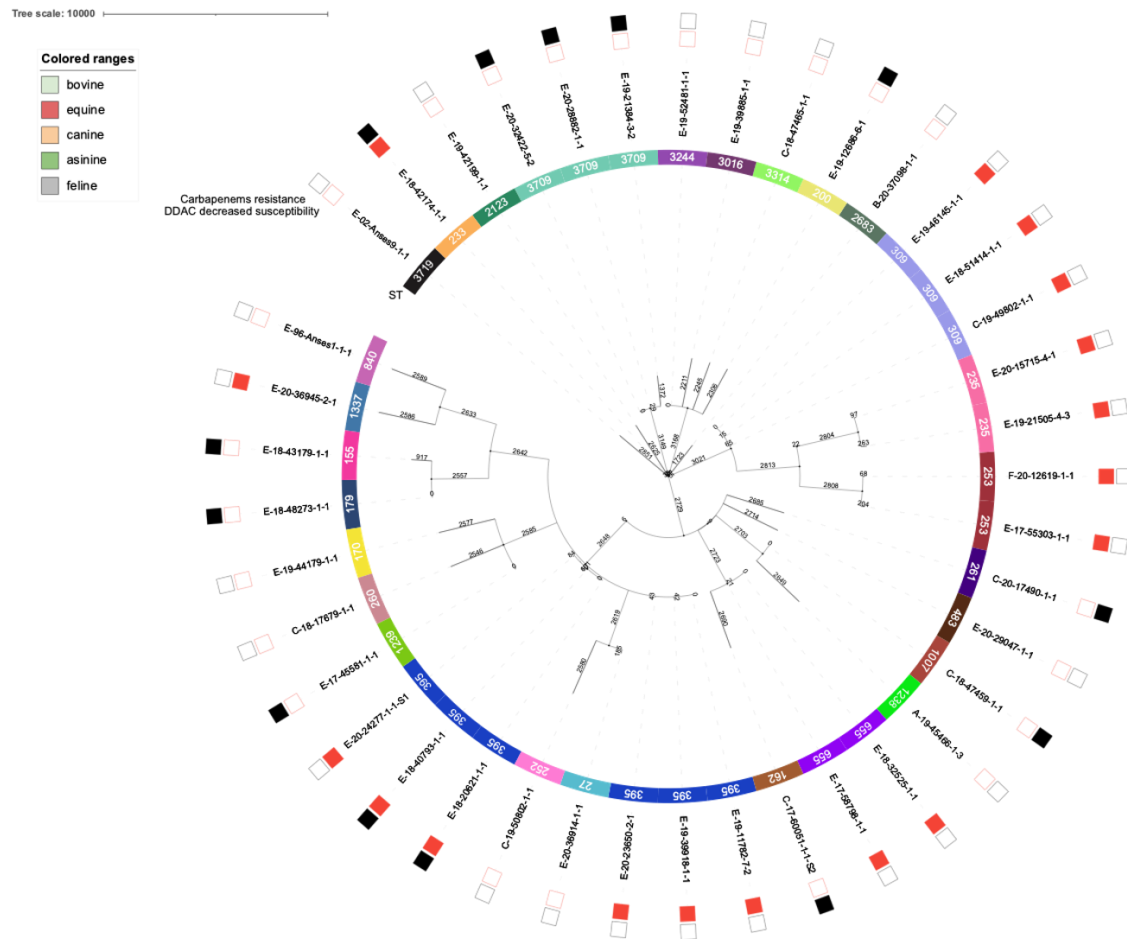
3<sup>rd</sup>) ST 3709 (n=3) → new ST

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.



# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



Which type of MLST profil?

28 STs found



1<sup>st</sup> ST 395 (n=6)

2<sup>st</sup> ST 309 (n=3)

3<sup>rd</sup> ST 3709 (n=3) → new ST

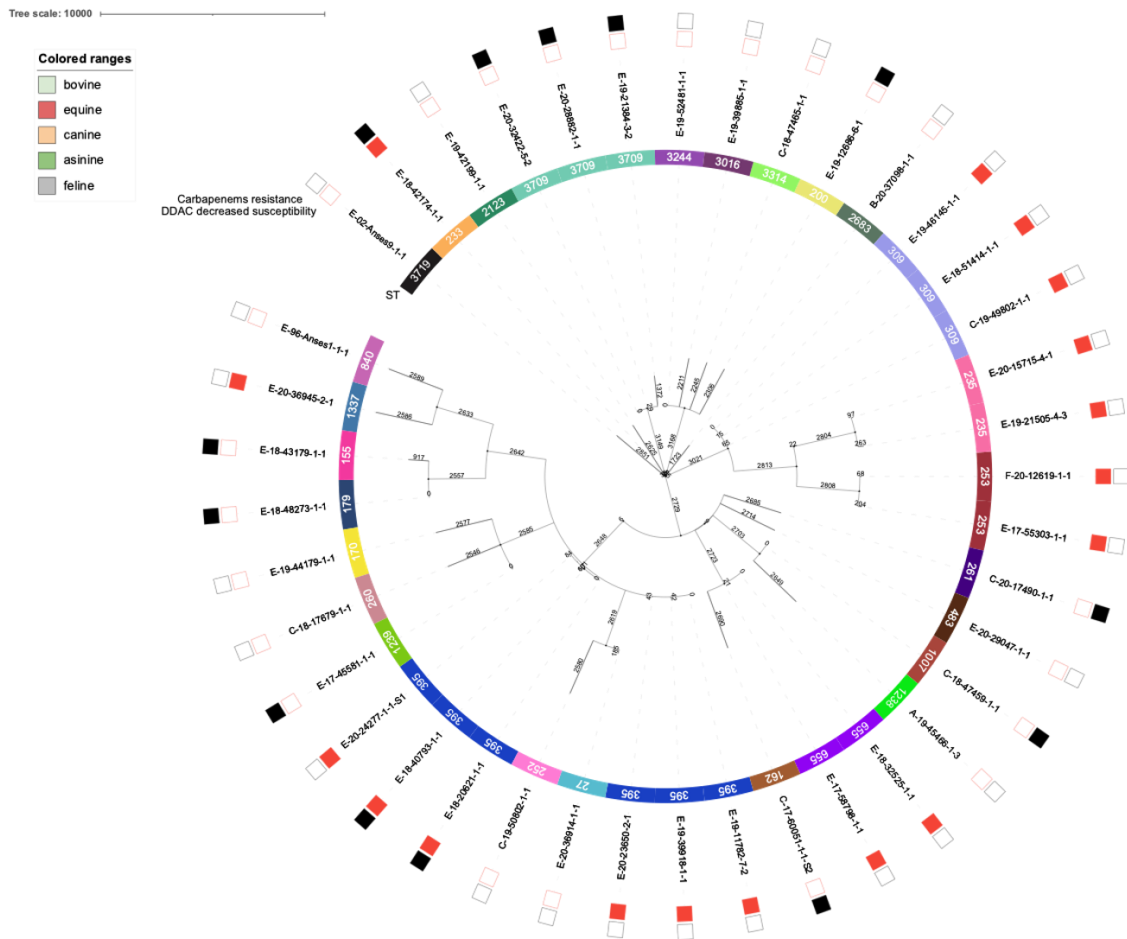
\* *Can infect humans and animals, and are known as high-risk clones in human medicine (Morales-Espinosa et al., 2017; Haenni et al., 2017)*

→ Some strains come from clones that are problematic in human medicine

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.

# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



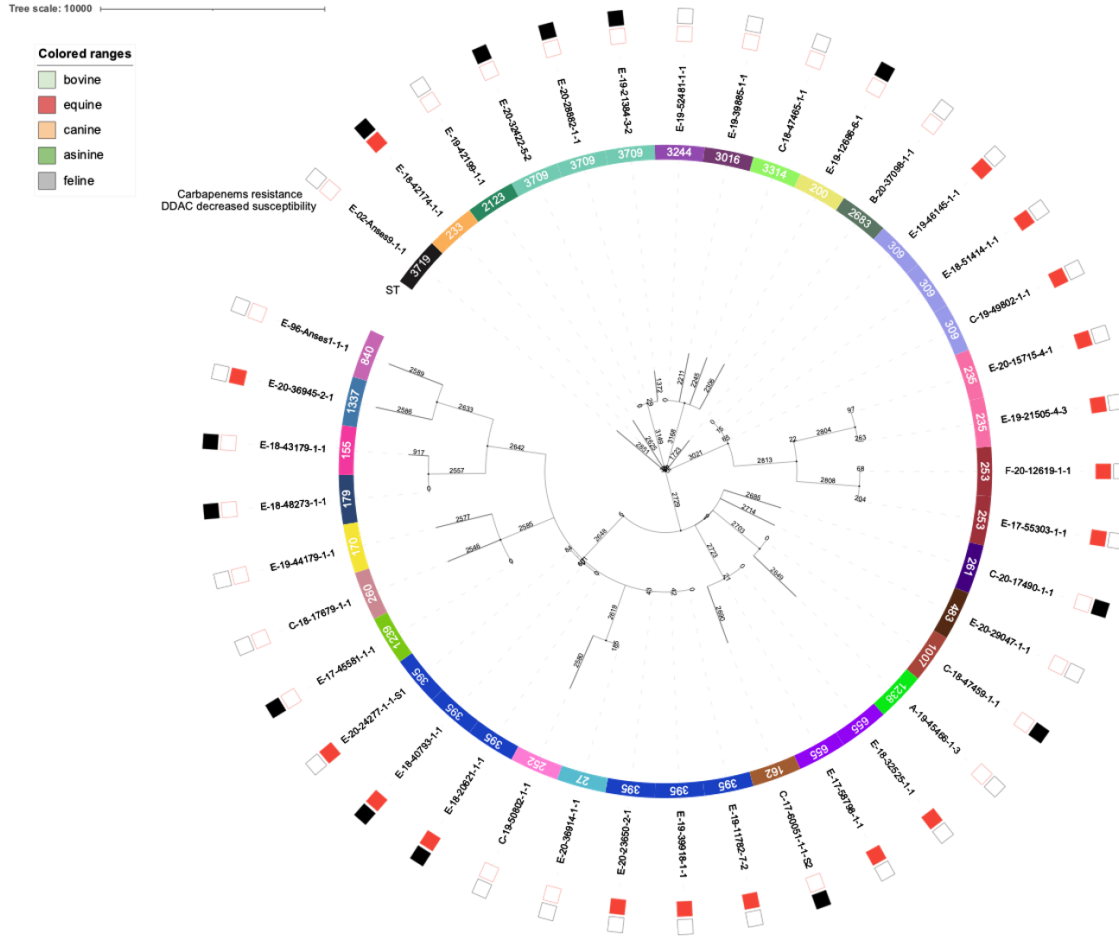
What about cgMLST?

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.



# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



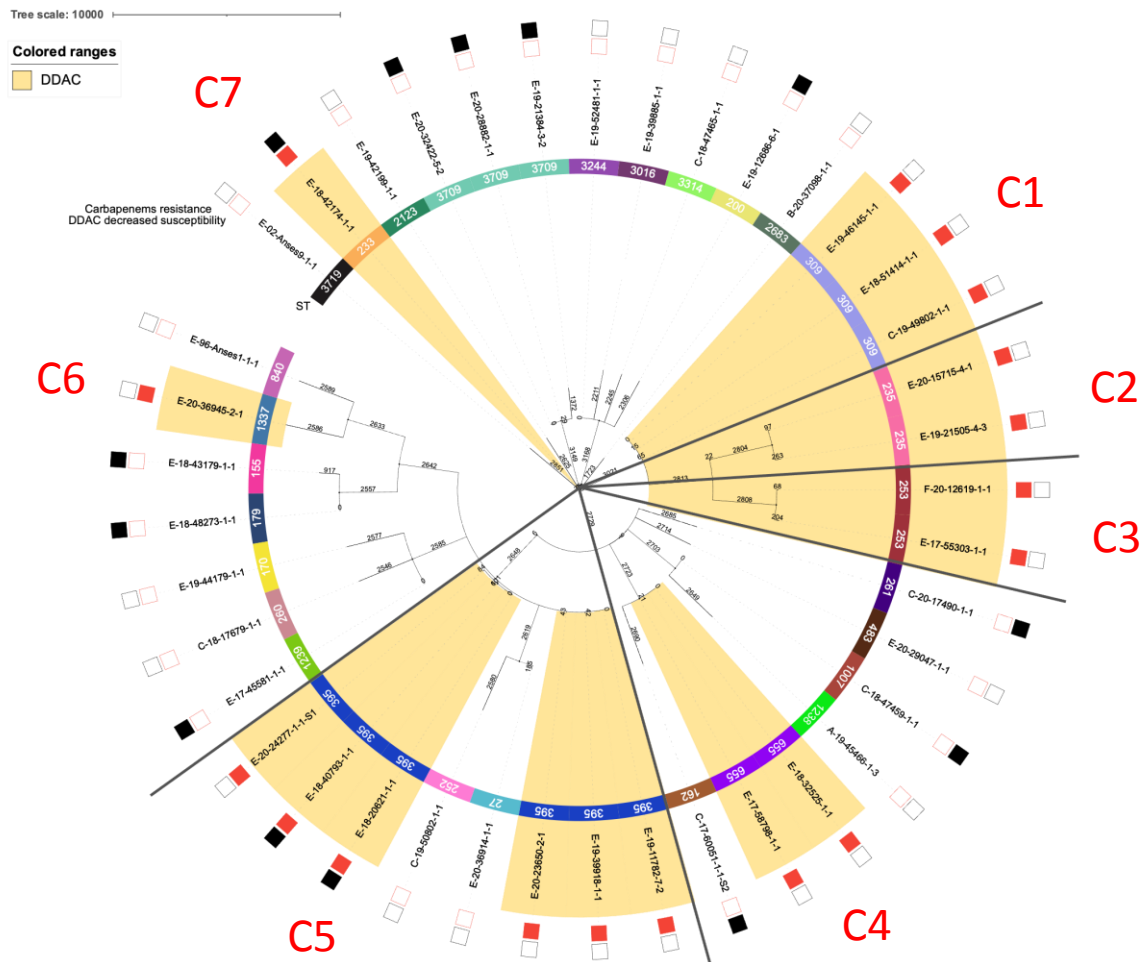
What about cgMLST?

➔ Important genetic diversity of strains depending on the **year of isolation**, the **origin** of the strains and the **type of sampling**

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus.** Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-:canine isolate, E-:equine isolate, ST: Sequence Type.

# Results

## Multilocus Sequence Typing (MLST) and Core Genome MLST



### Decreased susceptibility to DDAC:

- Only 7 various HCluster250

➔ Existence of close clonal populations expressing DS to DDAC

**Figure III: Minimum spanning tree of 40 PSAE animals isolates based on cgMLST of 3265 locus. Decreased susceptibility to DDAC is represents by red squares and carbapenems resistance (imipenem and / or meropenem) is represent by dark squares. A-: asinine isolate, B-: bovine isolate, C-: canine isolate, E-: equine isolate, ST: Sequence Type.**

# Conclusion

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

→ **Is there a decreased susceptibility to other biocides?**

→ **Is biofilm formation implicated in this phenotype?**

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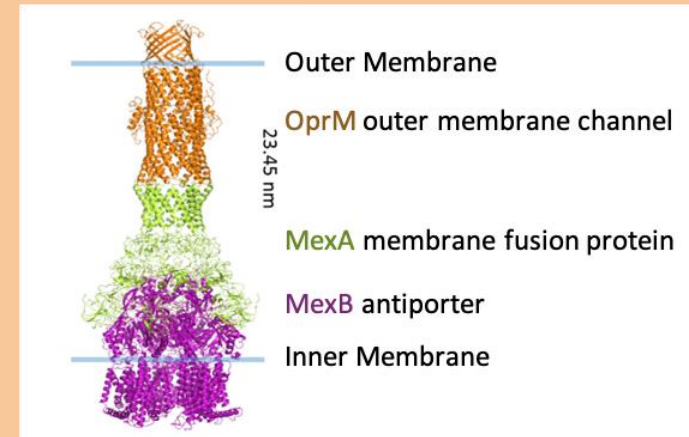
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*(Master results on humans samples)*



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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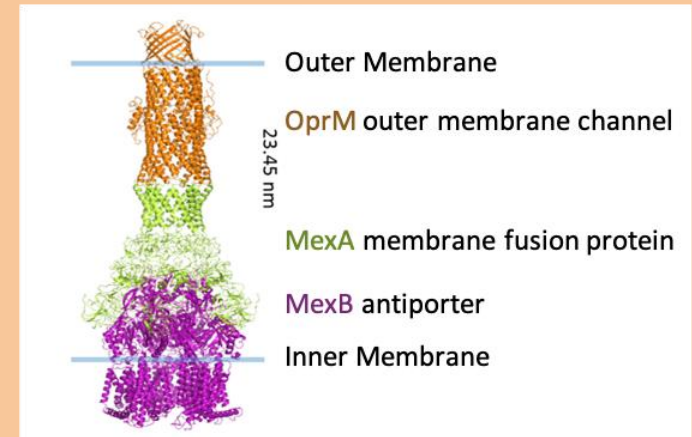
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**Is DS to DDAC associated with antibiotic resistance?**



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# Thank for your e-attention



Contact:  
[marine.pottier@laboratoire-labeo.fr](mailto:marine.pottier@laboratoire-labeo.fr)

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