RESULTS OF THE SURVEY ON THE WILLINGNESS TO PARTICIPATE IN A VOLUNTARY LINEZOLID SELECTIVE MONITORING (OWN FUNDING)

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Does your country wish to participate in a voluntary linezolid selective monitoring (own funding)?

9 said YES ☺

Reasons to say “no”:
short staffed this year/ workload at this moment

Who said yes?
not anonymous but identification bug

→ Help us to identify you to help us to construct this partnership
(you will be contacted by e-mail)

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On which bacterial species would you like to focus on?

- A) "**E. faecium, E. faecalis and S. aureus**" **(N=5)**
  - Wish to add Staphylococcus epidermidis (n=1)
- B) "**All Enterococcus spp. and Staphylococcus spp**. that would be detected": N=1
- C) "**All gram-positive bacteria** that would be detected": None
- D) Only E. faecium and E. faecalis
- E) Only Enterococcus spp: None
- F) Only S. aureus OR G) Only Staphylococcus spp: **N=1**

- A or D (i.e. E. faecium + E. faecalis +/- S. aureus): **N=1**
- A, B or C, open for discussion, depending on the chosen isolation method **(N=1)**

Consensus/preferred choice: E. faecium + E. faecalis + S. aureus (7/9)

- One country would only participate to the S. aureus monitoring (option F/G checked)
- One country would restrict his preferred scope to this (option B checked)

Able to start from the samples itself or from derived agars plates?

- **All options are possible** (N=5 + N=1 preferred from agar plates but all options possible)
- Others:
  - I could start from **Azid-Glucose broth** derived from faecal samples and from nasal swabs (N=1)
  - Based on what is in the **scope of the monitoring** of CD1729/2020 (N=1)
  - N=1:
    - Nasal samples: could only start from **Mueller Hinton broth** OR from **brilliance blue MRSA agar**
    - Faecal samples: could only start from **BPW** derived from faecal samples

- Kind of available samples **seems not a limitation (i.e. compatible with all options of isolation method)**
- The **azid-glucose broth** should be discussed:
  - broth only used in a single country?
  - azid is not allowed in some countries

Are you doing voluntary MRSA monitoring in your lab or do you collect nasal samples from animals for other purposes?

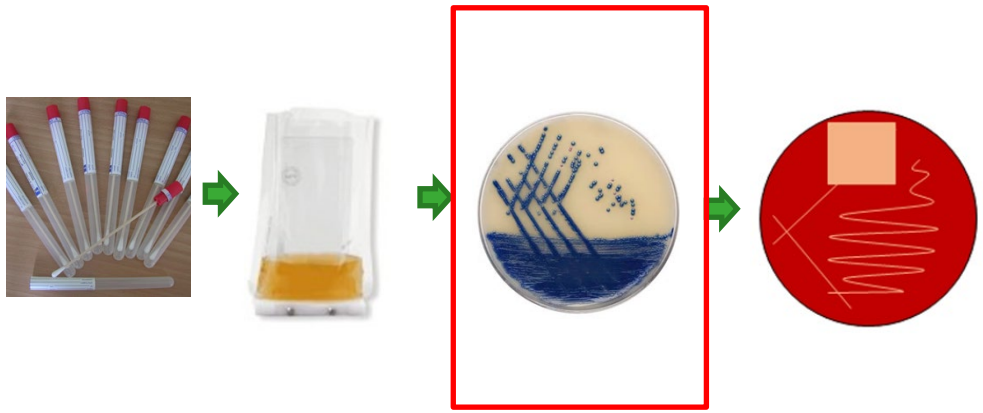
- 6/9 volunteers are doing either
  - MRSA monitoring

  OR
  - collecting animal nasal samples for other purposes

- In some countries the MRSA monitoring is done on other matrices than nasal swabs:
  - fresh meat samples (n=1)
  - nasal swabs, dust or faecal samples (n=1)

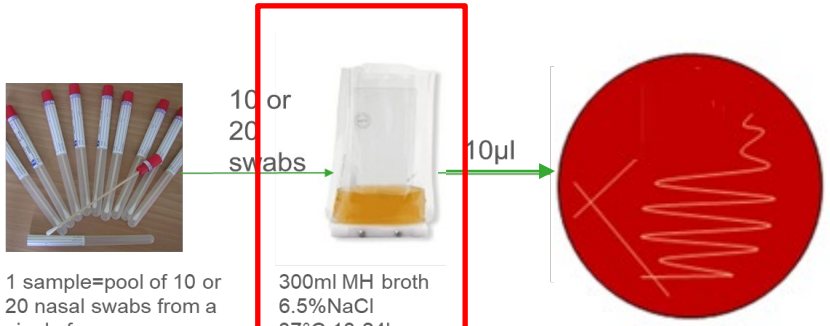
Isolation method from nasal samples among 9 volunteer countries

A)



Spreading all bacteria on CSB+LZD 4mg/L 37°C 44-48h

B)



A loop of 10µl on CSB+LZD 4mg/L 37°C 44-48h

Option A:

- Yes: N=3

A but B worthwhile trying: n=1

B>A but both possible:n=1

Option B:

- Yes: N=3

This methodology has not yet been tested and it is possible that a lot of background flora would grow on this medium.

No opinion: N=1

Should be discussed:

- **No clear consensus (half/half)**
- **Background flora of option B** should be first checked

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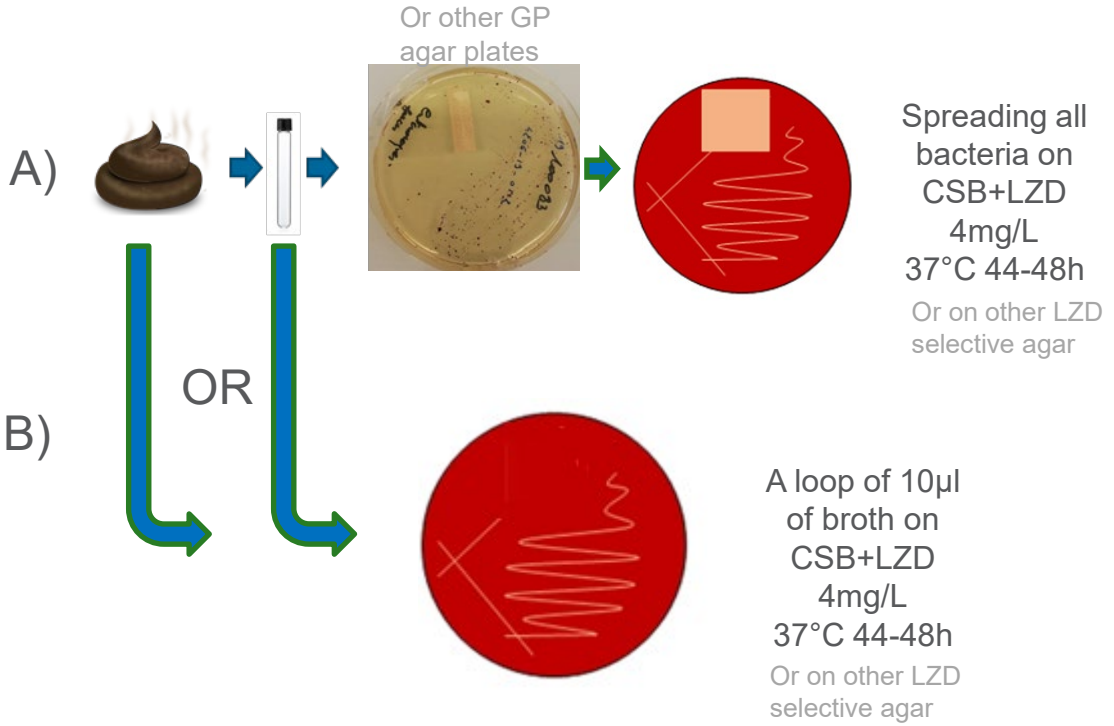
Isolation method from faecal samples



Not applicable: N=1 (not doing enterococci monitoring)

Option A: N=2

Option B>A but both possible: N=1

Option B: N=4

- N=2 with Slanetz&Bartley agar suppl with 4 mg/L linezolid rather than CSB-LZD
- N=1 not using S&B therefore chosing for option B
- N=1: no comment

Option C "others":N=1: → close to option B

starting from the Azid-Glucose broth with faecal sample (1:10) incubated 37°C /22-26h, spread a 10-µl loopful of the broth on Slanetz & Bartley agar supplemented with linezolid (4mg/L) and incubated 44±1°C /40-48h, than typical colonies of Enterococcus spread on CSB and incubated 37°C/44-48h

→ the same is proposed starting from **nasal swabs** for Enterococcus screening

Proposed consensus: **modified option B using S&B-LZD 4mg/L**

Acceptable to use alternatively modified option A using S&B-LZD rather than CSD LZD?

Which LZD selective medium? Enterococcus, Staphylococcus, both

	A (CSB-LZD 4mg/L)	B (CHROMagarTM LIN-R)	C (Enterococcosel® Agar, 2mg/L)	D (SuperLinezolid medium)	E (S&B-LZD 4mg/L)	F (others)	G (no preference)	H (no experience)	Open for discussion
1	X								X
2	X				X				
3	X				X			X	
4		X							
5			X						X
6					X				X
7							X	X	
8								X	
9								X	

Comments:

- Due to financial constraints, we do not prefer ready-made plates (n=1)
- Among “no experience“:
  - N=1: “We have a lot of experience with both S&B and Brilliance MRSA 2 agar, but not for the selective isolation of linezolid resistant bacteria.”

Preferred options:

- For enterococci: **S&B-LZD 4mg/L** or CSB-LZD 4mg/L
- For staphylococci: **CSB-LZD 4mg/L** but require a specific isolation method upstream→ **to be discussed**

→ These preferred options could satisfy everybody
→ participant #4: only volunteer for staphylococci (agar plates to be discussed)

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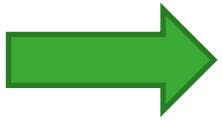
WGS of LZD-R bacteria?

- **WGS already done?** Yes for **N=6**: 5 volunteers + another country
  - Comments:
    - a few cfr-positive MRSA isolates
    - 1 *optrA* enterococcus from clinical sensititre screening
    - Only some that were suspicious
- **Do you intend to WGS new isolates?** Yes for **N=10**
  - Yes for all 9 volunteers
  - Yes for another country that could not participate (lack of staff) but detected a few LZD-R isolates in 2022 through clinical sensititre screening (1 staph, 2 enterococci)

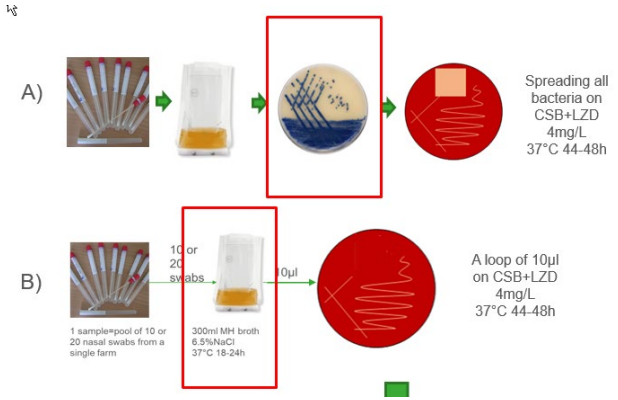


These WGS data will enable to **compare the genetic background** of these isolates with the huge dataset of isolates from 2019 in Belgium

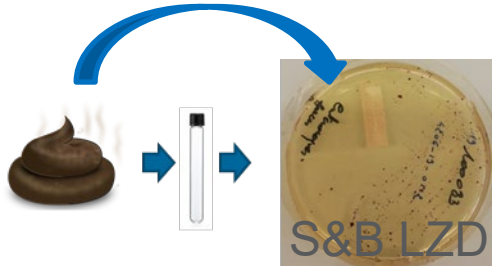
Proposed focus based on preferred options

- Bacteria: **E. faecium + E. faecalis + S. aureus**

- Isolation method:
  - **From nasal swabs:** No clear consensus (half/half); **Background flora of option B** (without brilliance blue) should be first checked or other selective approach should be investigated



  - **From faecal samples:** Proposed consensus: **modified option B: using S&B-LZD 4mg/L** from broth or from faecal samples



Discussions

What's the aim?

- Assess the **LZD-R occurrence** in different European countries through an **harmonized** (i.e. **comparable**) methodology
- Gather isolates to conduct later WGS and **compare the genetic background of the LZD-R population**

Discussions

Questions raised/limitations?

- Timeline??2024 or 2025?
  - 2024 for E. faecium and E. faecalis from faeces and a pilot study for S. aureus for the countries who can?
  - 2025 for MRSA and enterococci from nasal swabs with the baseline survey of MRSA?
  - Need some time to implement the protocol
- Budgetary constraint (own funding without EC reimbursement) → minimum number of samples to participate?→ to be discussed
- Not all animal categories sampled in every country the same year
  - My opinion: more important to have an harmonized protocol than to have the same categories on the same year in every country (if only 1 year of difference)

Conclusions/suggestions of next actions

- There is a **willingness** from several countries
- Some others will maybe join afterwards (feel free to contact us)

- Together we can **harmonize** a method to enable future comparisons and better assess the global situation in different European countries

- **Future joint publication** (if agreement)

- But **let's first draft the harmonized protocol based on the conclusions of the survey**
  - **Working group**
  - Who wants to participate to this WG? You will soon receive an invitation by e-mail

- Future funding opportunities?

Contact

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