

Survey on MIC methods, panels and rare/unusual phenotypes



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Background

- Minimum inhibitory concentration (MIC) testing is golden standard
- Methodology has been adopted in most MS for harmonization purposes and to improve the quality of susceptibility testing in general.
- However, there are different brands and plate designs available, different methodologies and equipment which can be used in the performance of such tests
- Rare phenotypes have been mentioned in discussions, but not clear what is performed at NRL level

Objectives

- To collect information from the NRL's and to share experiences about:
 - MIC Methods/resources available in each individual laboratory
 - Possibilities for acquisition of plates
 - Exchange of knowledge and possibilities of collaboration or help in setting up methods
 - Possibility to use survey data to evaluate different methods, media among labs
 - Collect information on the reactions to rare/unusual phenotypes at NRL level

Preparation of Questionnaire

- A questionnaire was performed on a internet platform (Survey Monkey®) containing:
 - Identification sheet (respondent /lab ID)
 - MIC methods and alternative methods used (3 questions)
 - Media preparation/acquisition (5 questions)
 - Procedures for microbroth dilution (4 questions)
 - MIC panels used per species and additional panels (6 questions+ additional comments)
 - Observation of rare/unusual phenotypes (4 questions)

Description of the survey

- An e-mail with the invitation to participate and the link to the questionnaire was sent to the participants on the 24 February 2012
- Data were collected and database was closed by the 16th of March
- Data from the survey was downloaded from the Survey monkey server in Excel format

Evaluation of data

- At the time of closure of the database there were 40 responses.
- After communication with the respondents 4 responses were eliminated due to duplication (4) and lack of identification (1) and 35 were considered valid for further analysis.

Data analysis- Results

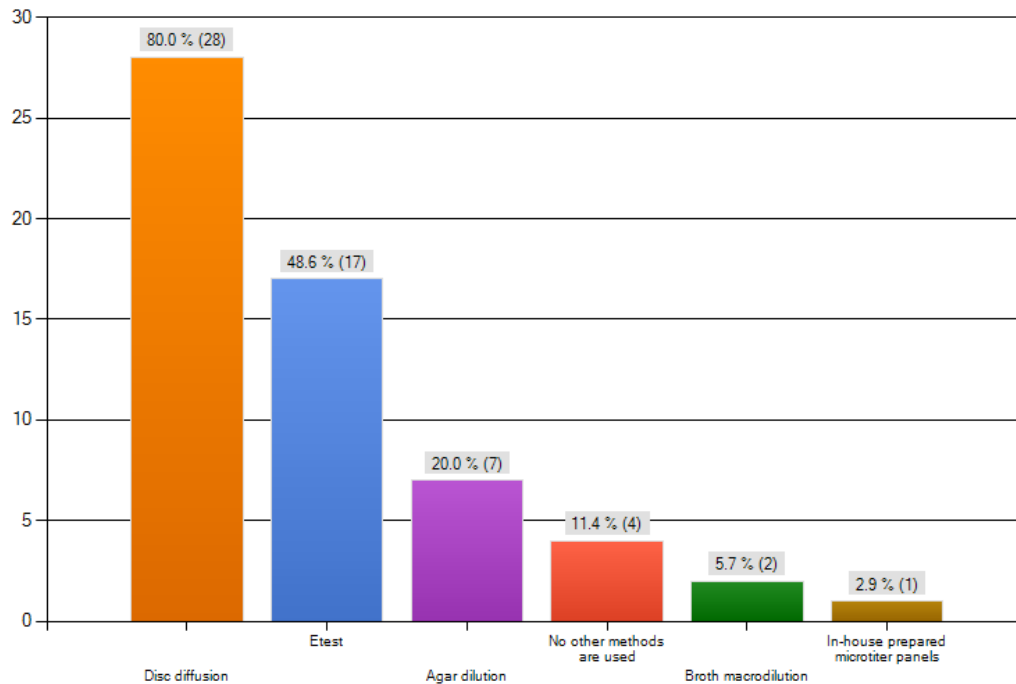
- **Question 1- Respondent ID**

–We have obtained 35 responses from laboratories performing MIC testing in 26 countries including:

- Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Norway, Poland, Portugal, Romania, Spain, Slovakia, Sweden, Switzerland, Turkey, The Netherlands and UK

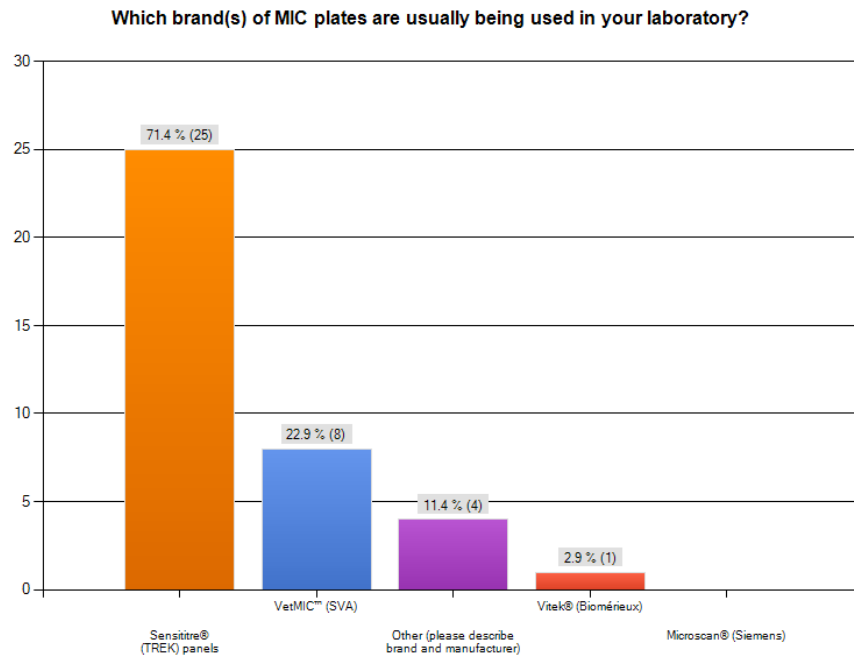
Question 2 –Alternative methods used

Besides MIC testing using commercial microtiter panels, which other methods are used in your laboratory?



- Most used alternative method is disk diffusion

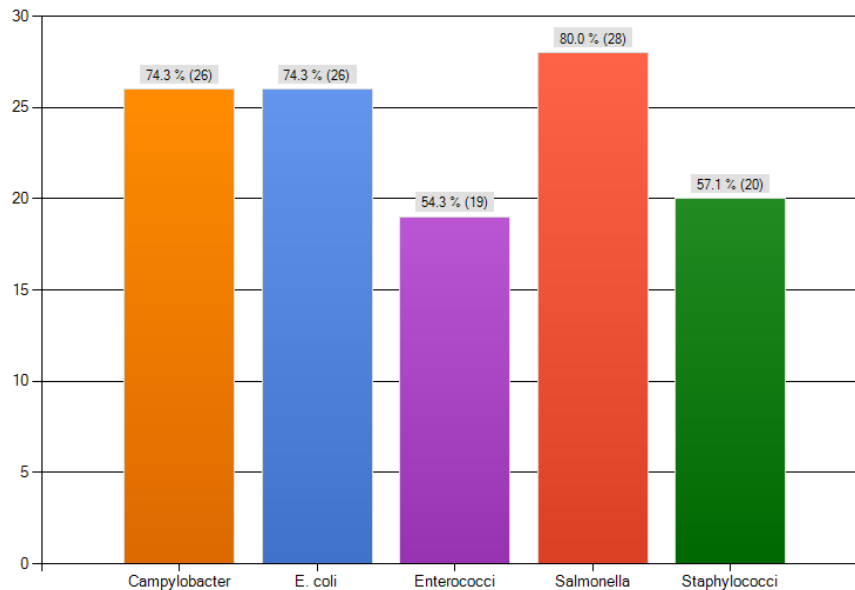
Question 3 MIC plate systems



- Sensititre system from TREK is mostly used and followed by Vet MIC
- Among other alternatives of MIC testing panels, the labs indicated Wider plates (Siemens)
- Additionally, 3 labs indicated agar dilution or e-tests as MIC determination methods

Question 4- species tested using MIC

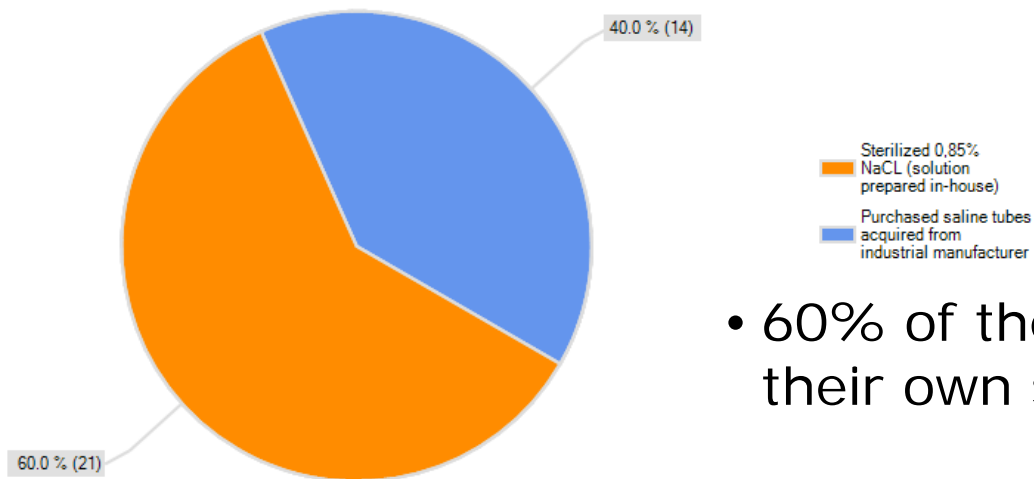
Which bacterial species are usually tested using MIC determination with microdilution plates, in your laboratory?



- The surveyed labs use mostly Microbroth dilution for testing *Salmonella*, *Campylobacter* and *E. coli* isolates

Question 5- saline for AST

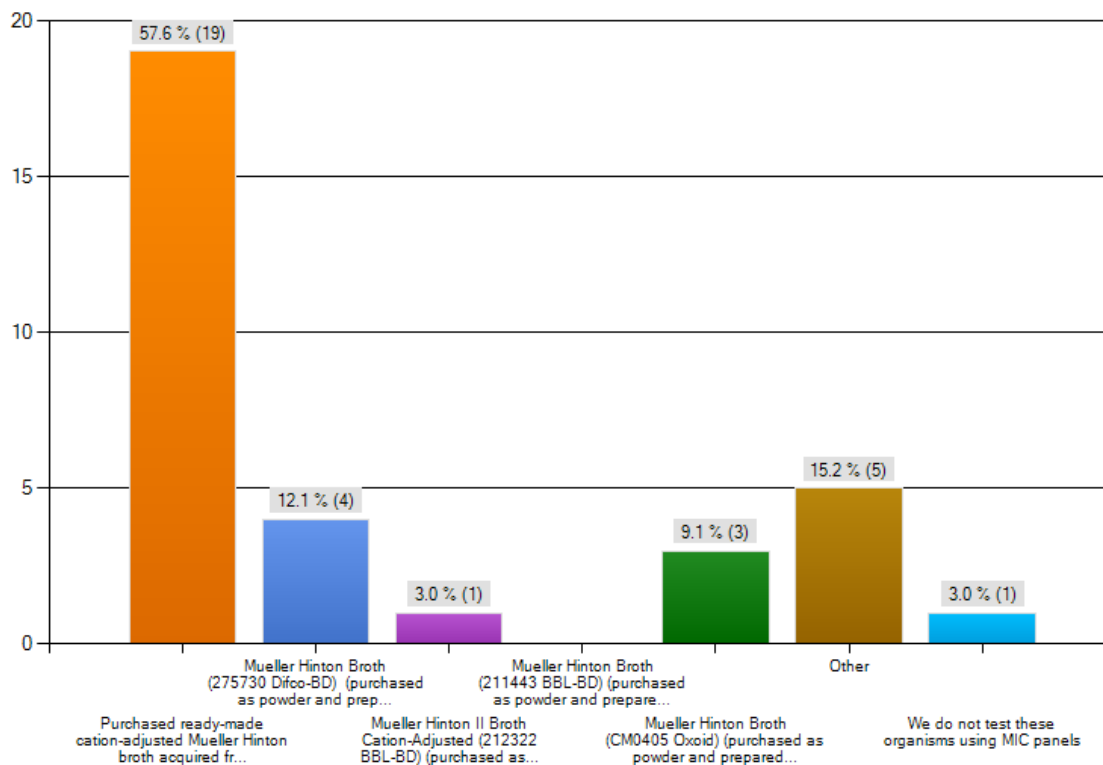
What is the origin of the saline solution for bacterial suspension used in your laboratory?



- 60% of the labs prepare their own saline

Question 6- media for *Salmonella* and *E. Coli* AST

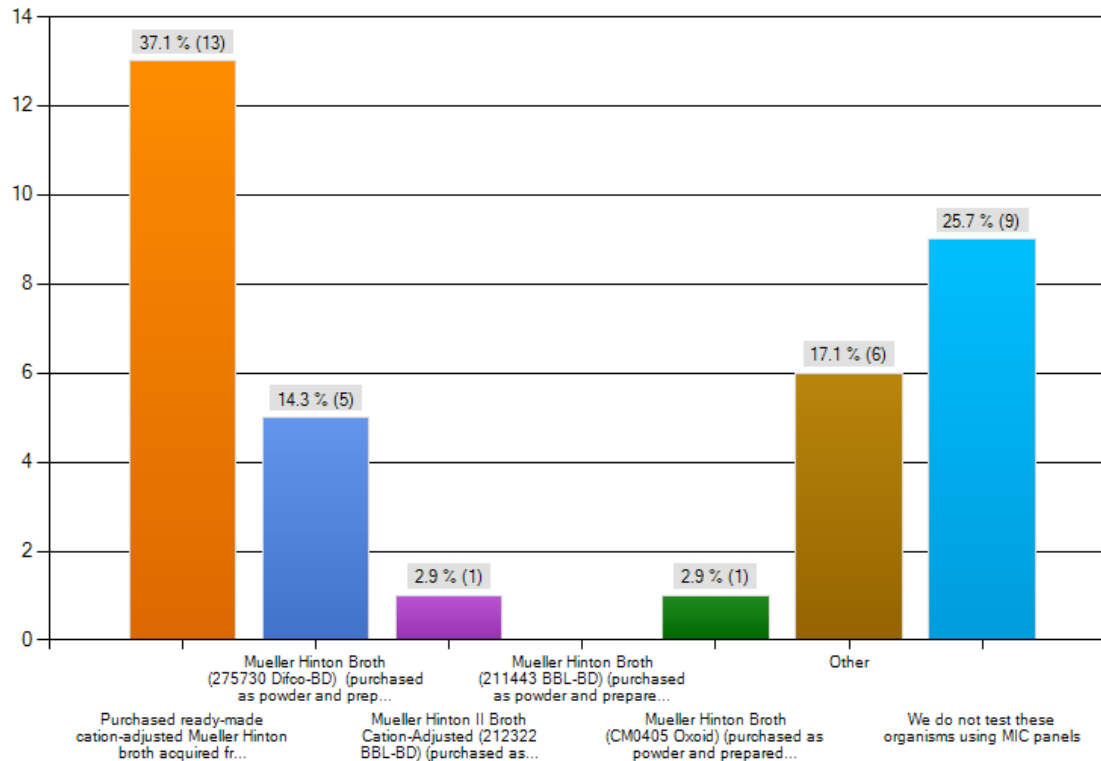
Which brand(s) of Mueller Hinton media do you use for MIC testing for antimicrobial susceptibility testing of *Salmonella* spp. and *Escherichia coli* in your laboratory?



- Purchased broth is used mostly for testing *Salmonella* and *E. coli*
- Other media include: Mueller Hinton Broth from Biorad, TREK and SIFIN

Question 7- media for Staphylococci and Enterococci AST

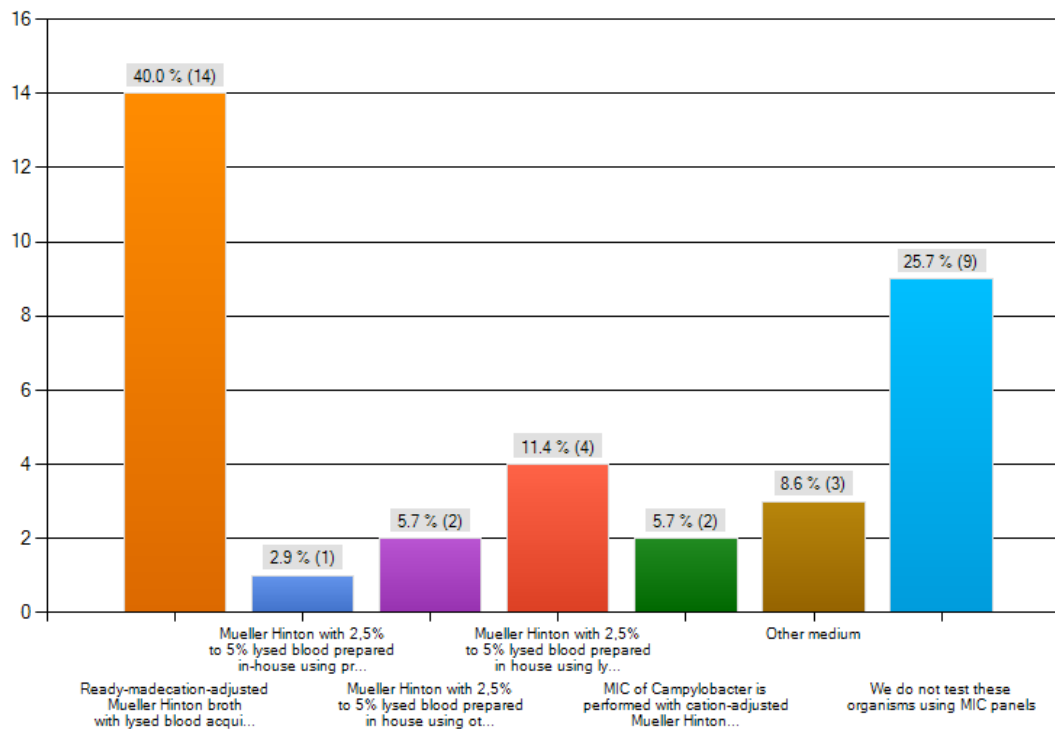
Which brand(s) of Mueller Hinton media do you use for MIC testing for antimicrobial susceptibility testing of *Staphylococcus aureus* and *Enterococcus* spp. in your laboratory?



- Purchased broth is used mostly for testing *Staphylococci* and *Enterococci*
- *Other media include: Mueller Hinton from Biorad and TREK*

Question 8- media for *Campylobacter* AST

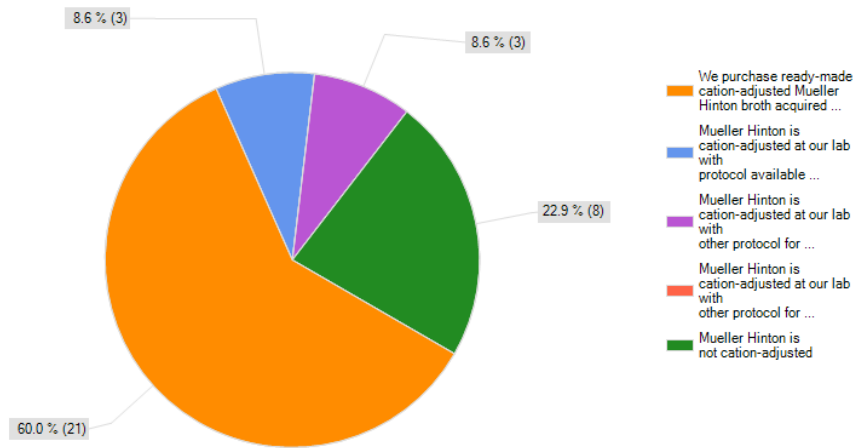
Which media do you use for antimicrobial susceptibility testing of *Campylobacter* spp. in your laboratory?



- Purchased broth is used mostly for testing *Campylobacter* spp.
- *Other media include:* Mueller Hinton Broth with foetal calf serum

Question 9- cation adjustment

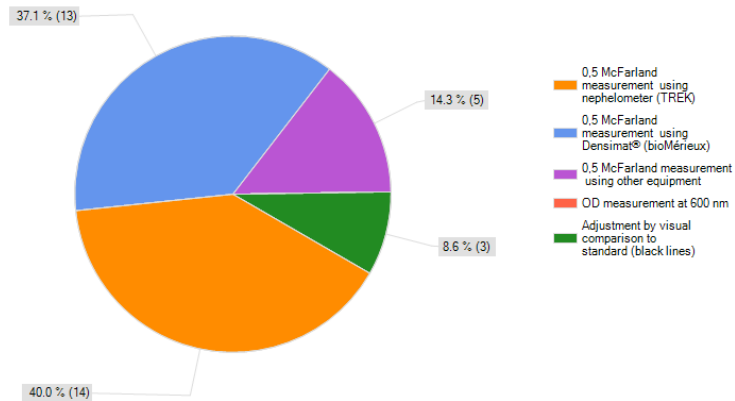
Which method do you use for adjusting the cations in the Mueller Hinton Broth used at your laboratory for antimicrobial susceptibility testing?



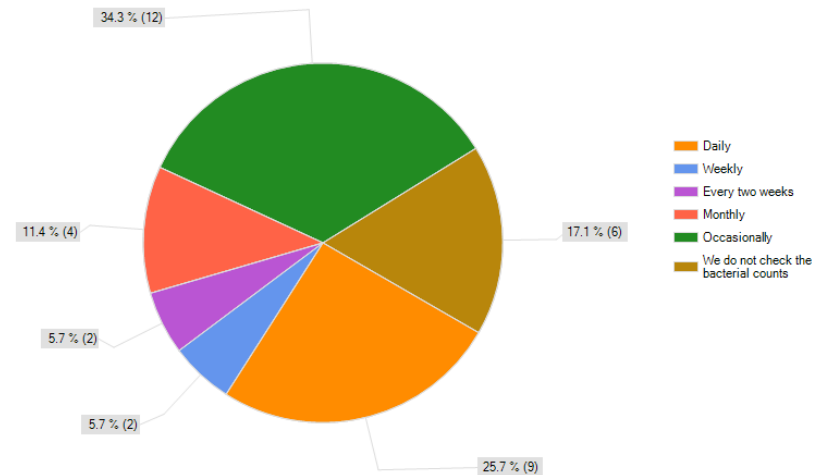
- Mostly cation adjusted medium used, but 23% still refer they do not perform cation adjustment

Question 10 and 11- inoculum preparation

Which method do you use for adjusting the bacterial suspension to Mc Farland 0,5 at your laboratory?



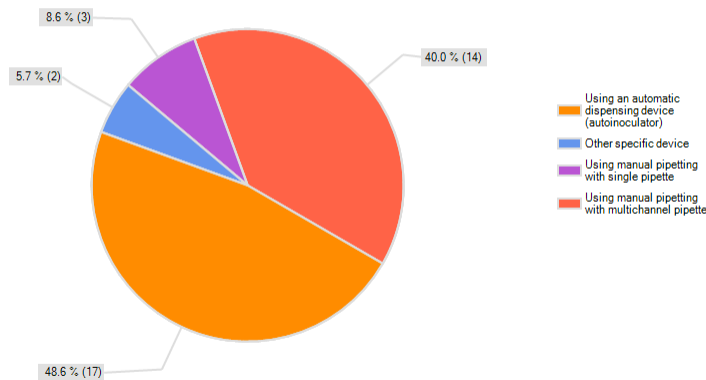
How frequently do you check the final inoculum bacterial counts in the panel ?



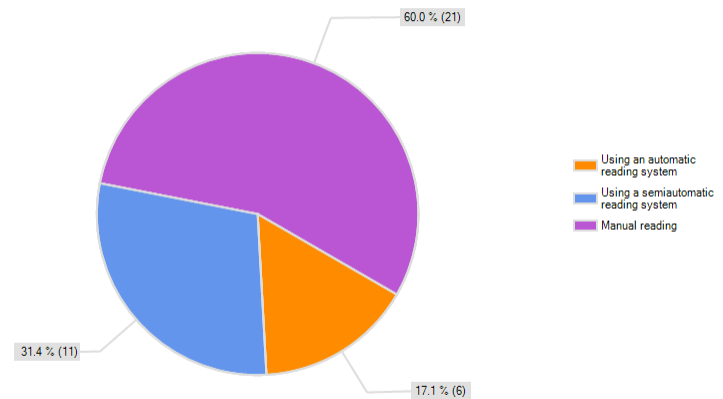
Inoculum is in general adjusted using devices and checked more or less frequently, but 17% refer it is not checked

Question 12 and 13- plate inoculation and reading

Plate inoculation: How do you perform the inoculation of MIC panels?

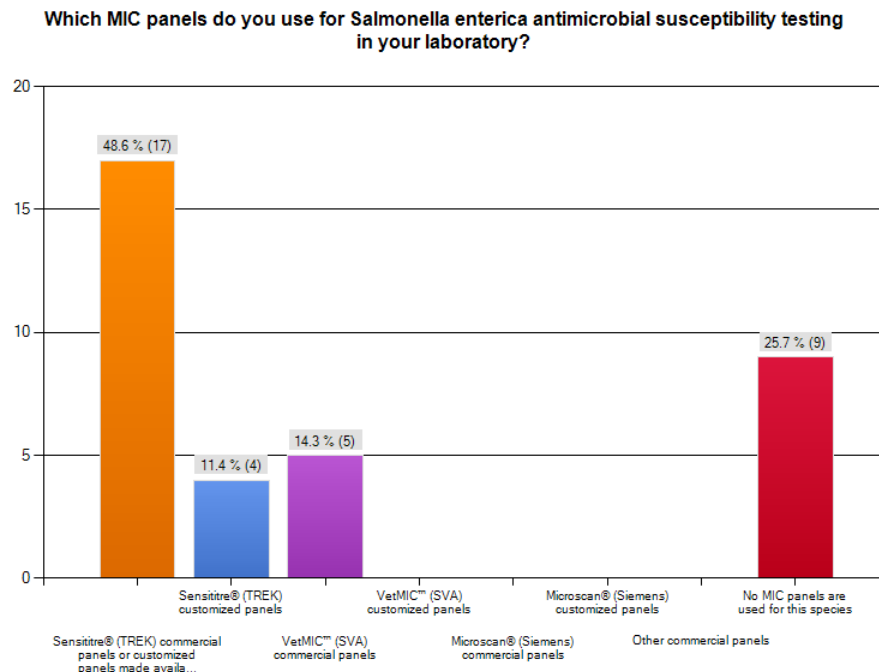


How do you perform the reading of MIC panels?



Manual pipetting with single or multichannel pipettes is used in circa half of the labs and the remaining use autoinoculation devices. MIC plate reading is mostly performed by manual reading vs automatic or semi automatic systems. Some issues with reading system mentioned.

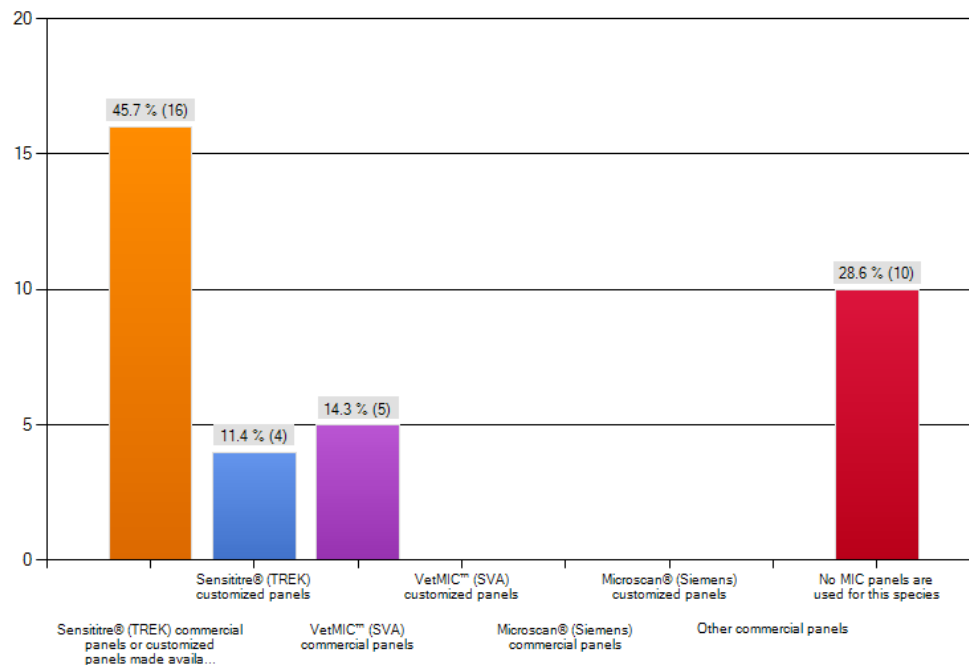
Question 14 – panels for testing *Salmonella*



- Most laboratories use commercial panels available from TREK (Sensititre) or VetMIC
 - EUMVS2 -8 labs
 - VetMIC *Salmonella* (Version 1) E395116- 2 labs
- Four respondents indicate use of customized panels

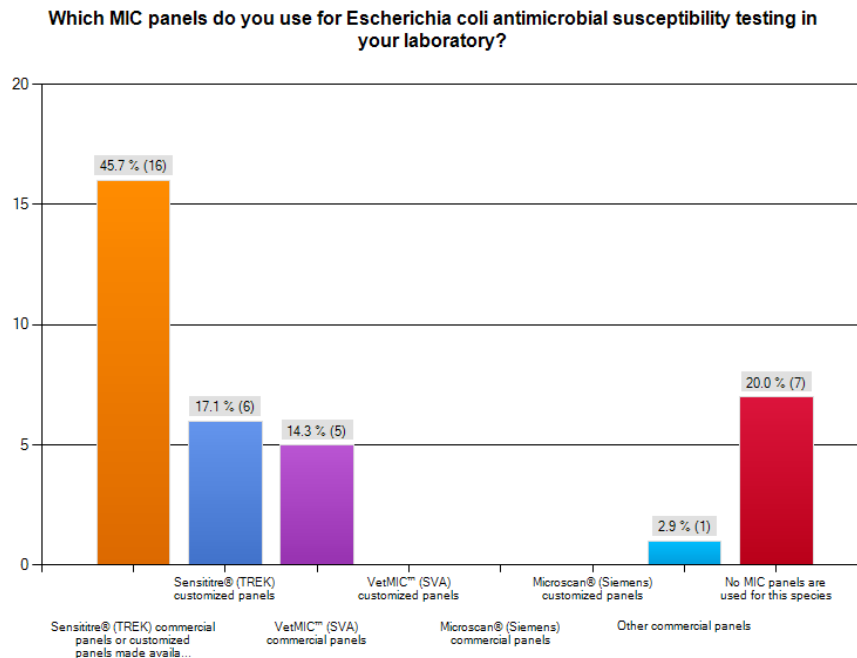
Question 15- panels for testing *Campylobacter*

Which MIC panels do you use for *Campylobacter* spp. antimicrobial susceptibility testing in your laboratory?



- Most laboratories use commercial panels available from TREK (Sensititre) or VetMIC
 - EUCAMP -6 labs
 - Vet MIC Camp(version 2) E395101- 2 labs
 - NLV64 – 1 lab
- Four respondents indicate use of customized panels

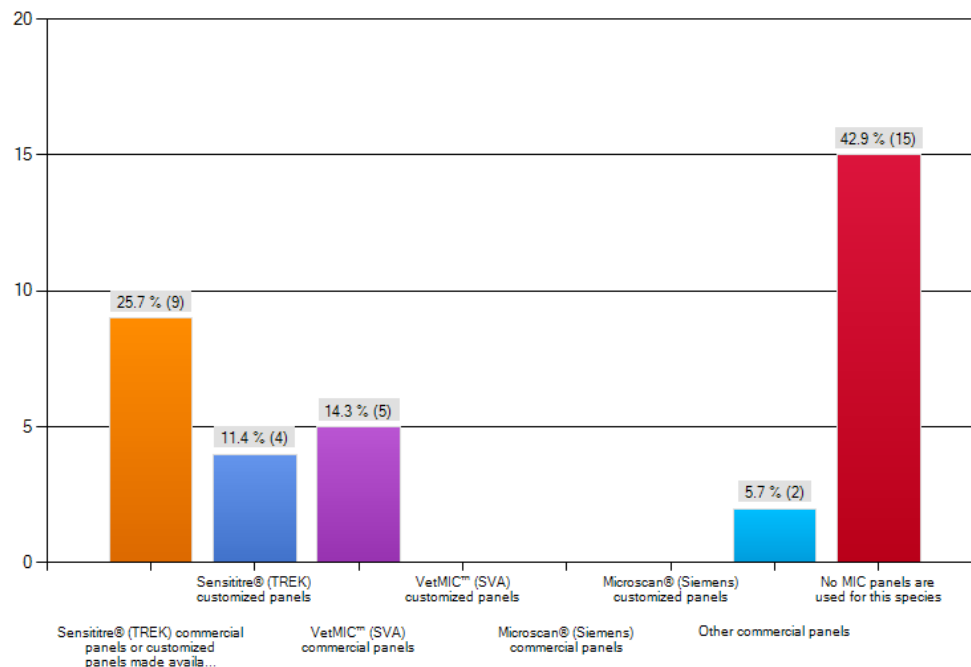
Question 16 – panels for testing *E. coli*



- Most laboratories use commercial panels available from TREK (Sensititre) or VetMIC
 - EUMVS2- 5 labs
 - CMV1AGNF- 1 lab
 - CMV2AGNF- 1 lab
 - VetMIC E395100- 1 lab (Enterococci plate)??
 - VetMIC GN-mo (version 4) E395103
- One respondent indicated use of other brands

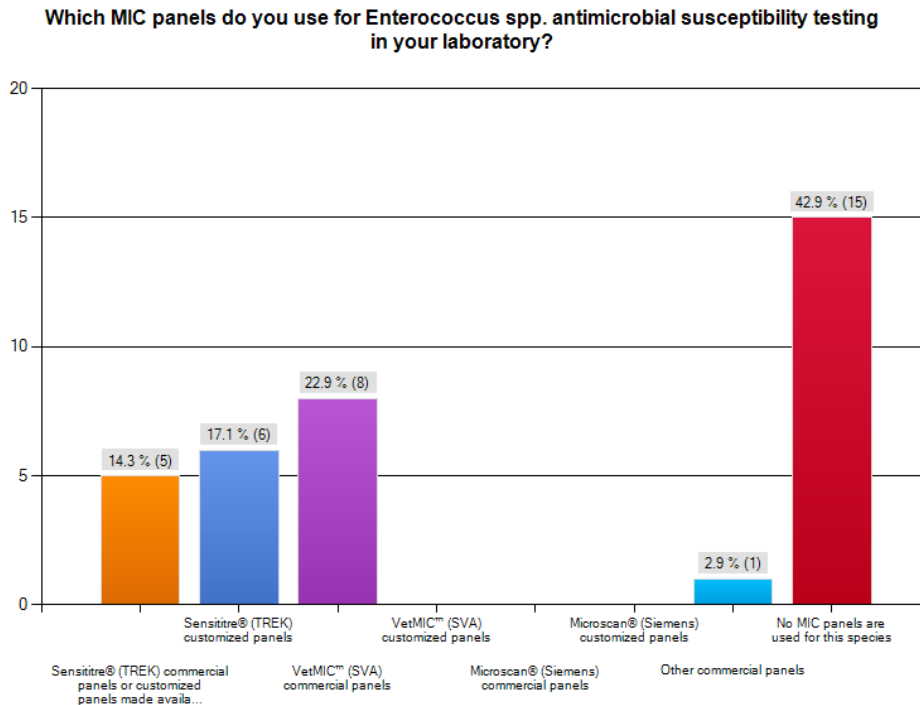
Question 17- panels for testing Staphylococci

Which MIC panels do you use for *Staphylococcus aureus* antimicrobial susceptibility testing in your laboratory?



- Most laboratories use commercial panels available from TREK (Sensititre) or VetMIC
 - EUST- 4 labs
 - NL EUST- 1 lab
 - VetMIC GP-mo (version 2) Art no 395102
- Four respondents indicate use of customized panels
- Two respondents indicated use of other brands

Question 18- panels for testing Enterococci



- Most laboratories use commercial panels available from TREK (Sensititre) or VetMIC
 - VetMIC E-cocci (version3) E395100- 2 labs
 - VetMIC E395103- one lab
 - GPALL1F- one lab
 - CMV3AGPF- one lab
 - NLV76- one lab
 - NLV79- one lab
- One respondent indicated use of other brands

Additional panels include:

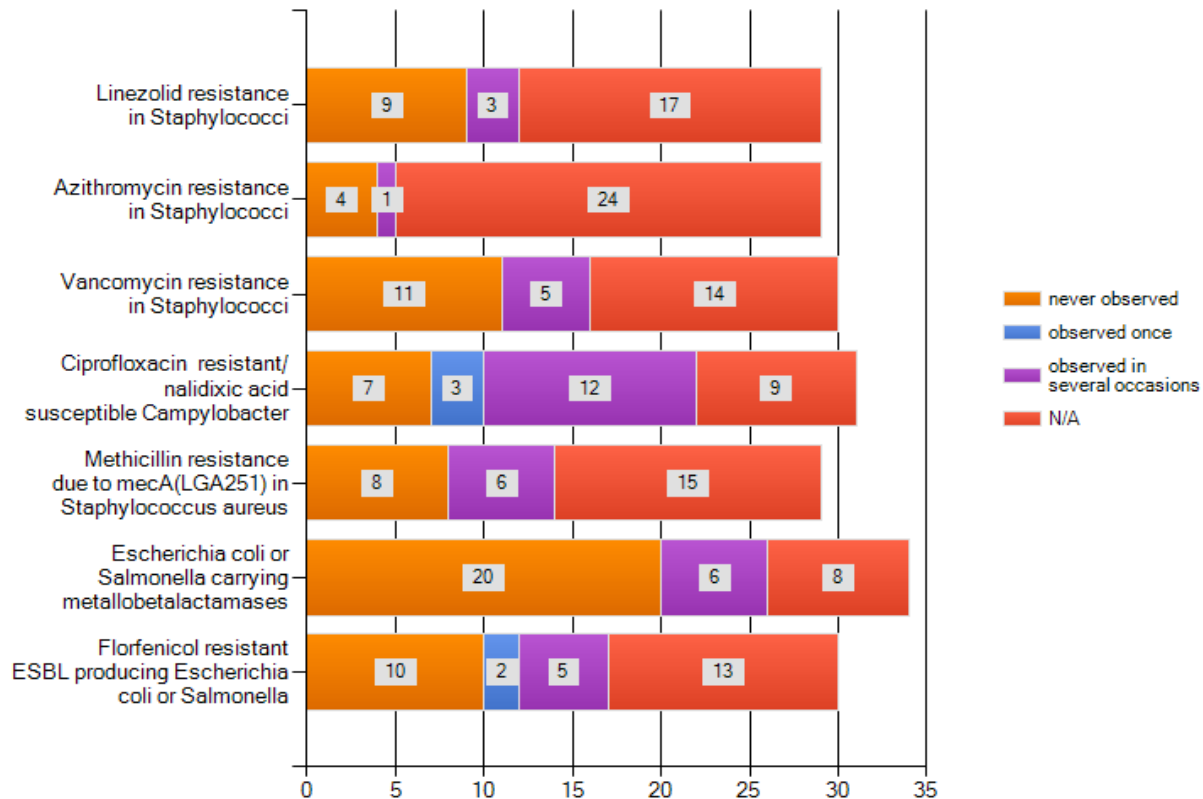
- **ESBL-** TREKESB1F; CMVAGNF; MIC-Strip ESBL (www.bagmed.cz); Vitek AST-GN38
- **MRSA-** VetMIC MRS Panel; Staph/MRSA common plate for Europe; WIDER PANEL MIC/ID CO94-31/W/REV.2 For testing MRSA
- **Veterinary antimicrobials-** TREK BOPO6F

Comments to MIC

- In general, laboratories perform MIC using mostly commercial panels, but some refer customized options
- Media are mostly purchased, except for saline which is mostly prepared in house
- Inoculum is prepared using devices for adjustment to 0,5 Mc Farland and checked in most laboratories
- Inoculation is done either using pipetting or autoinoculation devices
- Reading is performed using both manual, semiautomatic and automatic reading
- Panels are mostly purchased among those commercial available from Sensititre or VetMIC systems for the diverse organisms
- Comments regarding MIC include:
 - Need for harmonized panels: *E. coli* and Enterococci
 - Difficulties in automatic reading

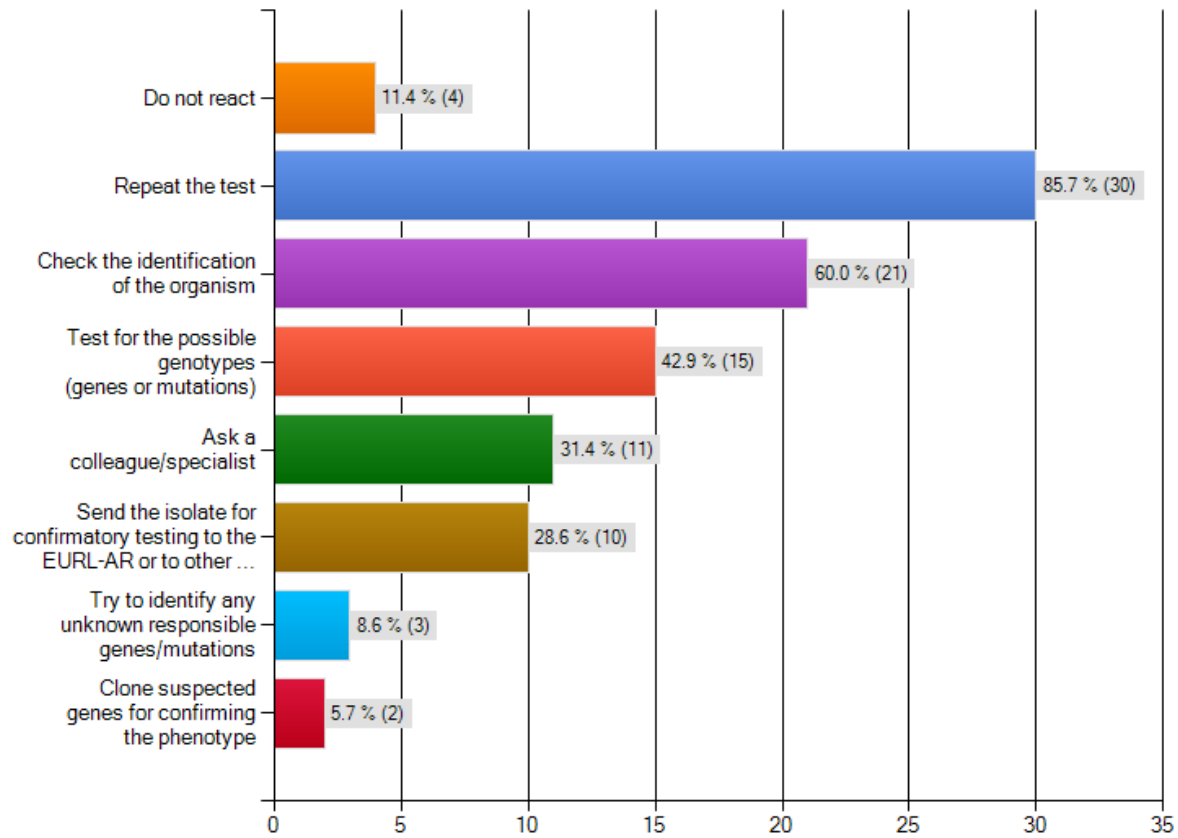
Unusual /rare phenotypes- Question 21

When performing susceptibility testing. How frequently do you observe rare/unusual phenotypes or combinations of resistances?



Unusual phenotypes- Question 22

How do you react when finding rare/unusual phenotypes?



Conclusions

- A great deal is done in direction to harmonization of methods
- MIC is performed routinely and methods are no major issues are referred in relation to the current systems
- Alternative to MIC is mostly disk diffusion, followed by e-test and agar dilution
- Rare and unusual phenotypes are observed occasionally and follow up is generally done, or referred to other NRL or EURL

Conclusions and perspectives

- More information of methods used is expected to be useful for:
 - Observing needs for further harmonization
 - Interaction between NRL's regarding setting up methods
 - Organization between Labs for ordering of plates
 - Interaction with the EURL regarding methods and results observed
 - Evaluation of methods or media used in relation to results obtained for certain drug/pathogen combinations
- More alert for observation of rare phenotypes
- Possibility of focused projects within network

