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Workpackage 5

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Contributing partners:

GENERAL INFORMATION

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Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☐ Communication Team ☐ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☐ ECDC ☐ Other international stakeholder(s): …… Social Media: ..... **Other recipient(s):** .....

OHEJP IMPART FINAL MEETING, NOTES

Purpose of the meeting:
Present the outcomes of the OHEJP IMPART project. All slides from the meeting will be made available to the participants.

1.1. Introduction of the program (Kees)

Agenda presented, 4 hour meeting from 12-16.

1.2. Round the table (all)

Short presentation of the 22 participants in this meeting covering their role at their own institute and within the project.

1.3. Introduction of the project (Kees)

IMPART: **IM**proving **P**henotypic **A**ntimicrobial **R**esistance **T**esting. In total thirteen partners from nine countries. The total funding was **1.6 M€** over the years **2018-2020** (including 1 year extension, 2 x 6 months budget neutral extension). The project covered in total five work packages (WP):
WP1: culturing of colistin resistant Enterobacteriaceae (Sophie Granier/ Agnès Perrin-Guyomard)
WP2: culturing of carbapenem-resistant Enterobacteriaceae (Jannice Schau Slettemeås)
WP3: establishing new epidemiological cut-off values (ECOFFs) (Kees Veldman)
WP4: optimising a disks diffusion method for Clostridium difficile (Sven Maurischat)
WP5: dissemination of knowledge (Sophie Granier)

Links to deliverables within the project:
https://onehealthejp.eu/groups/impart/
https://zenodo.org/search?page=2&size=20&q=IMPART#

1.4. WP1 presentation + discussion (Agnès)

Objectives of WP1 – selective isolation and detection of colistin-resistant Enterobacteriaceae. Evaluate and standardize methodologies to: i) selectively isolate acquired colistin-resistant in relevant Gram negative bacteria, ii) Phenotypically characterize colistin resistance, and iii) Confirm the presence of transferable colistin resistance mechanisms. WP1 presented the performed in the pre- and final ring trial.
For pre-ring trial: several culture conditions were tested among four labs (Anses Fougères, NVI, RIVM and WBVR). Meat and caeca samples from pig and turkey were spiked with 10^2 CFU/g colistin resistant bacteria, enriched in BPW with and without colistin and cultured in the following plates: MacConkey with 2 mg/L colistin, CHROMID® Colistin R, SuperPolymyxin and CHROMagar™ COL-*APSE*.
Outcome: good to excellent sensitivity for MacConkey w 2mg/L colistin and CHROMID® Colistin R. No significant difference in performance between selective or non-selective enrichment. No evident influence of matrices. Too many false positive (presumptive positive colonies verified negative). Non-chromogenic agar not suitable for mixed flora.

For final ring trial: focus on positive samples, not negative (exclude negative by PCR of DNA extracted from enrichment broth), include a resuscitation step, decrease matrix effect by diluting samples before selective enrichment overnight, and include chromogenic agars to better discriminate the mixed flora. Eleven labs (Anses Lyon, APHA, BfR, DTU, IZSLT, NVI, PIWET, RIVM, SSI, SVA and WBVR). Same matrices, and plates: CHROMID® Colistin R, SuperPolymyxin and CHROMagar™ COL-*APSE*. Prize of agar plates important!
Planning for a publication in Letters in Applied Microbiology (open access), draft planned, submit in April.

1.4.1. Q&A:

Marisa (Anses) – why was the final ring trial results from one lab discarded?

> This lab put the samples in the freezer and the results were discarded because the answers were different compared to the other labs. The frozen step they included limited the growth of the spiked strain in samples. Same experience with spiked samples for the regional labs in first PT trials of the French NRL. Some spiked strains cannot be grown after frozen step in sample.

Cindy (RIVM) – colonies of switched samples. General comment from all labs. Relative low number to spike samples.
Stefan (SVA) – same experience as Cindy and in WP1 ring trial – lower detection rate after freezing samples. They have tested both fresh and frozen samples from the same animal, got one or two log reduction.
Dariusz (PULAWY) – should the results have been excluded from the 11th laboratory?

> They were excluded from the evaluation of the performance of the selective agar plating in the final ring trial. It is an outcome of WP1 and the results will be mentioned in the paper/article.

1.5. WP2 presentation + discussion (Jannice)

Objectives of WP2 – selective isolation and detection of colistin-resistant Enterobacteriaceae. Present a harmonized method for selective isolation and detection of carbapenemase producing Enterobacteriaceae (CPE), i) selectively isolate carbapenemase-producing Enterobacteriaceae, ii) phenotypically characterize carbapenemase production, and iii) confirm the presence of transferable carbapenem resistance mechanisms.
For pre-ring trial: Performed in November 2018. Several culture conditions were tested among four labs (Anses Fougères, NVI, RIVM and WBVR). Meat and caeca samples from pig and turkey were spiked with 10^2 CFU/g carbapenem resistant bacteria, enriched in BPW overnight at 37°C and cultured on several in-house and ready-to-use plates in parallel to test two different incubation temperatures, 35-37°C and 44°C. Also tested PCR on DNA extracted from the overnight BPW enrichment. General outcome: do not recommend to use 44°C to incubate agar plates, no significant difference between in-house and ready-to-use agar plates.
For final ring trial: Performed in September 2019. Enrichment in BPW overnight at 37°C, include a voluntary PCR step to detect CP determinants from the overnight BPW enrichment, include only ready-to-use agar to minimize the possible differences preparing in-house; CHROMID® OXA-48, Chromatic™ OXA-48, Brilliance™ CRE Agar, CHROMagar™ mSuperCARBA™, Chromatic™ CRE, and CHROMID® CARBA. Eleven labs (Anses Lyon, APHA, BfR, DTU, IZSLT, NVI, PIWET, RIVM, SSI, SVA and WBVR). Same matrices as the pre-ring trial, but included a strain with low meropenem MIC (*E. coli* R1180 VIM-1) known to be problematic to detect using the EURL-AR protocol for detection of CP *E. coli*.

Outcome in general: All agar plates 100% specificity. When all samples were included, the sensitivity was best for CHROMID® OXA-48 (100%) and Chromatic™ CRE (76%). Two samples were excluded due to low meropenem MIC (0.25) in one and the strain in the other sample was either contaminated or a conjugation had taken place (was spiked with Salmonella Kentucky NDM-1, several labs reported detection of *E. coli* and/or *Enterobacter* spp. carrying NDM). When excluding these; the following plates performed with 100% sensitivity; CHROMID® OXA-48, Chromatic™ CRE, and CHROMID® CARBA. The CHROMagar™ mSuperCARBA™ had 96%. The Brilliance™ CRE agar performed with 75% sensitivity. With this plate there was an issue during the validation: the E. coli 16874 OXA-48 and E. coli TZ 3638 GES-5 strain did not grow on this plate from ten and nine labs, respectively, that used these two strains for validation (strains distributed by the EURL-AR to validate the selective agar plates used in the European surveillance of CP E. coli from caecal and meat samples). Conclusion to WP2: i) all agars except Chromatic™ OXA-48 performed well, ii) Brilliance™ CRE Agar did not detect the control strains (unreliable results in spiked samples?), iii) direct PCR worked well for meat samples, but should be improved for caecal samples, and iv) *E. coli* bla_{VIM-1} in one of the spiked samples was not detected using the BPW enrichment and CPE selective agars included, but it was detected from an in-house MacConkey agar with cefotaxime and meropenem→ should this agar have been added to the ring trial?
We were not able to fully validate different enrichment methods due to the limitation to a low number of samples which made it difficult to conclude on the best method.

1.5.1. Q&A

Jette (DTU, EURL-AR) comment to GES-5 strain which was an issue in the validation of the plates. It has been problems with this strain earlier. The EURL-AR are think of replacing it.

1.6. WP3 presentation + discussion (Kees)

Setting epidemiological cut-off values (ECOFFs). Objective: generate data to allow to set ECOFFs for animal pathogens. Nine partners (Anses, APHA, BfR, IZSLT, NVI, PULAWY, SVA, UU, and WBVR). Priority list of species to test agreed on the kick off meeting. Three antimicrobial panels (GN, GP and macrolide plate) covering 10-12 different antimicrobials. Nineteen different pathogens were included. Decided to use the CLSI method to test more or less according to ISO standards. *Pasteurella multocida* was a problem regarding test medium; should not have CAMHB with lysed horse blood (LHB). Most of the *P. multocida* did not grow in regular CAMHB - ended up to test all in CAMHB + LHB (same for streptococci). For Enterobacteriaceae, Staphylococci and *Mannheimia haemolytica*, only CAMHB was used as test medium, no feedback of difficulty to grow for the latter. *Actinobacillus pleuropneumoniae;* everyone used VFM according to CLSI.
Approximately 3000 strains were tested with up to 26 antimicrobials. A total of 51696 MIC values were produced and 1352 MIC distributions. Need at least five distributions to set an ECOFF – missed some distributions for some bacteria.
There was some delay in plate delivery from Sensititre. MIC values were uploaded to the EUCAST web site at the end of summer 2020. In September 2020, there was a meeting with EUCAST and VETCAST to discuss the results of IMPART.
New tentative ECOFFs were presented for *S. pseudintermedius* and *S. hyicus*.

There was an issue with the acceptance of MIC distributions by EUCAST regarding the methods used (test medium) for some of the bacterial species. The criteria for accepting the

MIC data have become more stringent over the past years. Have to use EUCAST developed methods. Streptococci should be tested in Mueller Hinton Fastidious broth (MH-F broth, in-house EUCAST media) and not CAMHB + LHB. Bridging studies for *P. multocida* and streptococci have been performed on small scale during December 2020 to prove that the MIC valued produced are equal using both methods. Some media effects were observed when testing *P. multocida*, but with streptococci there was a high correlation between MIC results. Proposed but not decided was to repeat the trial with the same *P. multocida* and streptococci isolates in 3 laboratory (EUCAST, University of Bern and WBVR) to evaluate the outcomes of the pilot. More bridging studies are planned within VetCAST (*M. haemolytica* and APP).

1.6.1. Q&A:

Antonio (IZSLT): descriptive analysis between the broths – any statistical test applied? Skewed towards a certain MIC? Not only descriptive - comparison is interesting.

> EUCAST strict on method – complicated discussion, veterinary diagnostics labs use CLSI guidelines not EUCAST. Goal to have IMPART streptococci MIC values accepted at least after the bridging studies. All MIC values have been uploaded to the EUCAST database.

Marisa (Anses): What about zone diameters? EUCAST happy with zone diameters, but VetCAST has not started with working on diameters yet. This is something to consider.

1.7. WP4 presentation + discussion (Sven)

The objective of WP4 is to: Develop and standardize a disk diffusion method for susceptibility testing of *Clostridioides (Clostridium) difficile*. A strain collection of *C. difficile* strains from the participants was created, a disk diffusion method was developed and used in a ring trial performed in the summer 2020. Inhibition zone diameters (IZD) were produced.
Antimicrobial resistance in *C. difficile* occur, but at low rates for therapeutic antimicrobials. Some antimicrobials have high resistance rates, but it differs between countries. Antimicrobial resistance in *C. difficile* can lead to global spread of special virulent *C. difficile* lineages with resistance to for instance quinolones. The antimicrobials of interest are also those used in treatment.
Established a disk diffusion method based on CLSI and EUCAST guidelines for anaerobes (only agar dilution) and published methods for anaerobes, but this covered only a few antimicrobials. Tested different conditions; inoculum densities (McFarland), solid media, liquid media and anaerobic conditions. Anaerobic conditions played the most important role. Used i) anaerobic work bench (fully anaerobic + pre-reduction of media), ii) semi-anaerobic conditions (pre-reduction of media), and iii) aerobic conditions (no pre-reduction). i) and ii) gave similar results. Tested repeatability of the antimicrobial with few *C. diff* strains – four repeats.
Performed ring trial in June 2020 with seven participants, who tested the sensitivity of eight *C. diff* strains by disk diffusion to eight antimicrobials. Performance was good between the labs. Metronidazole was the most difficult antimicrobial to test for. No vancomycin nor imipenem resistant strains included. High reproducibility between labs.
Anaerobic jars vs chamber – gave different results. Jars gave bigger IZD compared to chambers. Total value different, but also Standard Deviation was higher in labs using anaerobic jars.

Used strain collection to produce IZD distributions. Could propose cut offs for six of the eight antimicrobials included. Low amount of non-wild type strains for some antimicrobials.

Summary: identified anaerobic conditions as the factor with highest impact. Could determine cut off values for resistant phenotypes. The optimized protocol ensures: reliable determination of non-wild type vs wild type – suitable for most antimicrobials, high repeatability and reproducibility. Low cost method.

1.7.1. Q&A:

Kees (WBVR): Do not need resistant (non-wild type) isolates to produce ECOFFs.
The IZD distribution could not distinguish between the non-wild type and wild type.
Cindy (RIVM): Commented on the resistance mechanisms, if the resistance mechanism to imipenem is known in *C. diff*?
No known publication on the mechanism of imipenem in *C. diff*.

1.8. Gap analysis and self-evaluation (Kees, all)

OHEJP Committee asked us to perform a gap analysis covering: i) What were the goals, ii) what was achieved, iii) what were the gaps, and iv) how to fill the gaps.
WP1 and WP2 – did not achieve the initial goal, but got to test different media next to one another.
WP3 – no accepted ECOFFs yet, but some proposed ECOFFs are ready.
WP4 – miss imipenem and vancomycin cut offs, limited number of vancomycin resistant strains in the C. diff strain collection.
WP5 – no extranet or videos

How to fill the gaps:
WP1 – more trials (many strains with MIC around the breakpoint)
WP2 – consider selective pre-enrichment, commercial media for detection Salmonella
WP3 – ECOFFs published, continue work on this in VetCAST supported by COST action ENOVAT. More ECOFFs will follow!
WP4 – include more vancomycin and metronidazole resistant strains
WP5 – finish publications in WP1, WP2 and WP4. WP3 – publish ECOFFs.
No perfect method yet for WP1 and WP2.

1.8.1. Q&A

1.8.1.1. WP2 –

Jette (DTU, EURL-AR) - consider to add step to the EURL-AR protocol. Will perform a survey of what people do, what they use the BPW enrichment for. Complicated step.
Stefan (SVA) – consider enrichment broth for CPE and ESBLs – conjugation might occur in the enrichment step. Have to evaluate the conjugation in the enrichment broth.
Alex (BfR) – Natalie Pauly did a lot of work on improving the isolation method. Best plates were MacConkey with meropenem and cefotaxime. Included enrichment step; incubation in microaerophilic conditions could reduce background flora. This also improved realtime PCR results. Helps with second enrichment in microaerophilic conditions overnight. To be published!

1.8.1.2. WP3 –

EUCAST advice VetCAST to come up with more ECOFFs. "After" IMPART the activities will be ongoing.

1.8.1.3. WP4 –

Concentration in the disks. Clindamycin IZD of susceptible strains were close to the resistant strains, diameters low for susceptible strains. Difficult to discriminate between susceptible and resistant strains. Imipenem – not possible to determine resistance by disk diffusion – might be it does not work for this antimicrobial. No standardized method by EUCAST. Disk diffusion works for 6 of the 8 antimicrobials!! For a new antimicrobial – test if the disk diffusion method is working. Regarding imipenem as choice for carbapenem; imipenem chosen for scientific reasons not because it is the treatment option. Could be an epidemiological driver. Could consider choosing another carbapenem like meropenem.

1.9. Things to finish/ publication plans (WPL's)

Writing of publications and establishing ECOFFs, and other administrative parts of the IMPART project. Still a bit of work in 2021 after the project has ended.

1.10. Possibilities for follow-up of the project? (all)

WP1 – do not know if they will continue working on the colistin method. Is it a goal for surveillance, or not? Not a mandatory part of the AMR monitoring at the moment. Publish work and see if anyone interested.
WP2 – follow up the EURL-AR survey.
WP3 – the work is continued within VetCAST supported by COST action ENOVAT
WP4 – followed up in OHEJP FED-AMR – application of the disk diffusion protocol. Could be a side project to test the disk diffusion and strain collection for other antimicrobials. More interesting is probably to establish microdilution methods – does not exist at the moment – there are some publications. Could consider to contact EUCAST lab in Växsjö to see if they are interested to look at development of a microdilution method. Etest are mostly used by labs – but are quite expensive and commercial.
Cindy (RIVM) - there is an ESCMID group working on *C. difficile* – could see or contact this working / study group.
Dariusz (PULAWY) – consider showing results at the OHEJP ASM conference in Copenhagen in June 2021? Present gap analysis, what to fulfil, might be a good idea to present some output from IMPART. Abstract deadline in 15 March.
Cindy (RIVM) – future things – small projects can use MedVetNet and apply for money/funding, like for student projects working on colistin resistance or ECOFFs. They have a call for workshops at the moments.

1.11. Summary and closure of the meeting (Kees)

Thank you for attending and input.