Deliverable D-JRP1-2.8

Workpackage 2

Responsible Partner: NVI

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PROPOSAL(S) FOR EPIDEMIOLOGICAL STUDY TO MONITOR RESISTANCE TO CARBAPENEMS

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The WP2 task 2.8 deliverable is to make proposal(s) for epidemiological study to monitor resistance to carbapenems.

For the multicenter study performed in WP2, a non-selective enrichment of samples 1:9 in BPW-ISO (i.e. EURL-protocol) was used followed by streaking upon selective commercial agars. The evaluation showed that the investigated agars were not suitable for detection of non-chromogenic carbapenemase-producing *Enterobacterales* (CPE) strains (like Salmonella) nor for the detection of CPE with low meropenem MICs (like bla_{VIM}).

A PCR step was added to the protocol on DNA extracted from the overnight broth. This could be an effective step, although it is crucial that the protocol for DNA extraction and PCR are optimized to overcome inhibitors especially in the caecal samples. It is also crucial to define markers (i.e. primers/probes) for detection of specific carbapenem resistance genes of interest since you only detect what you are searching for. It should also be highlighted that relying on a PCR step may result in that uncommon, emerging and novel genes may be overlooked and their occurrence underestimated.

The study investigated growth from samples of caecal or meat material spiked with 100 CFU/g of *Escherichia coli*, *Klebsiella pneumoniae* or *Salmonella* Kentucky carrying the most common carbapenem resistance genes detected in animals; bla_{VIM-1}, bla_{NDM-1}, bla_{OXA-48}, bla_{IMP} and bla_{KPC-2}. The selective agar plates included were; *Brilliance*™ CRE Agar, Chromatic™ CRE, Chromatic™ OXA-48, CHROMagar™ mSuperCARBA™, CHROMID® CARBA and CHROMID® OXA-48.
All plates included showed 100% specificity, and when including all eight samples the Chromatic™ CRE and the CHROMagar™ mSuperCARBA™ showed the greatest sensitivity, 76% and 69% respectively, while the CHROMID® CARBA performed less well with 54% sensitivity. However, when excluding the meat sample with bla_{NDM-1} carrying *Salmonella* Kentucky (non-chromogenic) and the caecal sample with bla_{VIM-1} *E. coli* (low level producing carbapenemase), the CHROMID® CARBA, Chromatic™ CRE and CHROMagar™ mSuperCARBA™ showed 96-100% sensitivity. For the OXA-48 agars, the CHROMID® OXA-48 agar performed with 100% sensitivity compared to the Chromatic™ OXA-48 at 47%.

The results emphasize that when choosing a selective agar one should always consider which carbapenemase and which bacterial species to detect.

The results of the study show the need for further development of the pre-enrichment step in the current EURL-AR protocol for detection of CPE's. However, the consequence of such a change would likely be that the protocol would only be applicable for the specific monitoring of CP *E. coli*, instead of as now when used for several screenings (like extended spectrum cephalosporin resistance). One should be aware that the reasoning behind choosing the current EURL-AR protocol included other criteria than sensitivity and specificity. For example, the protocol needed to be cheap and easy to implement in all member states, so it was preferable that the protocol could be combined with already

utilized protocols, such as the isolation of *Salmonella*, and not requiring additional sample collection for the national reference laboratories.

Proposing an epidemiological study for the monitoring of resistance to carbapenems is challenging. There are several resistance markers (genes) involved expressing resistance to carbapenems on different levels. Low level producing carbapenemase are shown to be difficult to detect using the EURL-AR protocol. There are also several bacterial species involved; and there are no selective media that is chromogenic for all species (especially Salmonella). Further evaluation of the protocol is also needed concerning samples with mixed population of genes and/or isolates with the same geno-/phenotype. As designed at the moment, the protocol is mainly detecting precence or absence of a resistance phenotype and corresponding genes, which is the main objective of the European mandated surveillance. So it does not take into consideration that the strains might be present in different concentrations, have different generation times or other competitive advantages, thus it might only detect the most abundant and prolific strain. This issues needs to be evaluted before proposing an epidemiological study.

The current EURL-AR protocol for detecting CPE in faecal and meat samples is suitable for the detection of most CPEs. However, low level producing carbapenemase have shown to be difficult to detect using the EURL-AR protocol. Furthermore, the choice of the selective agar is not specified in this protocol. However, the results of our multicenter study show that the sensitivity of the method is very dependent on the choice of the agar. Considering the potential differences in the sensitivity of the method combined with the very low prevalence of CPE in livestock and meat in Europe (no CPE found in most countries) makes the design of an epidemiological study for the monitoring of resistance to carbapenems very difficult. The study provides additional guidance for choosing a protocol, but further efforts for harmonisation and optimization of a screening protocol is needed if it is to be used for an epidemiological study. It is therefore the joint conclusion of the partners/authors that it is currently unsuitable (scientifically imprudent) at this point to recommend/outline an epidemiological study.