Annex 1

Interim report

RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats (Year 3 – beef and pork)

Report on the presence of colistin resistant and *mcr-1* plasmid mediated colistin resistant *E. coli* and *Klebsiella* on five beef knuckle samples

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Summary of results

A beef steak sample (expensive steak) was found to be positive for *mcr-1* plasmid mediated colistin resistant *E. coli*. This sample was submitted to APHA Weybridge for testing on 16th May 2017 as part of RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in *E. coli* from Retail Meats (Year 3 – beef and pork). APHA performed whole genome sequencing on three *mcr-1* positive isolates from this original sample, and results have previously been reported to the FSA.

At the request of the FSA, five additional beef knuckle samples (samples from two different slaughter dates, with different lot numbers to the original sample) were subsequently sent to APHA and tested on the 14th July 2017 for *mcr-1* plasmid mediated colistin resistant *E. coli* and *Klebsiella.* These samples were processed to include enumeration for both colistin sensitive and resistant *E. coli* and *Klebsiella* following both swabbing and homogenisation of meat samples and also detection of colistin sensitive and resistant *E. coli* and *Klebsiella* after enrichment of samples.

Most meat samples had low (~ 1 to 3 logs) counts of *E. coli* per gram of meat sample, but only two meat samples gave rise to low (~ 2 log) counts of *Klebsiella* from a 100 cm^2 swab. Post enrichment, all of the meat homogenates were positive for *E. coli* and *Klebsiella* on agar without colistin and several samples were also positive for *E. coli* and *Klebsiella* on selective agars with colistin.

A total of 179 isolates from the different agars, from both swabs and homogenates, were identified by MALDI ToF to the bacterial species level. Most isolates from the *E. coli* selective agars were confirmed by MALDI ToF to be *E. coli.* Results from the Klebsiella selective agar were more variable, and whilst many isolates were *Klebsiella pneumoniae* or *Klebsiella oxytoca,* many isolates were also *Raoultella ornithinolytica,* which was formerly *Klebsiella ornithinolytica* and is closely related to *Klebsiella*, and some isolates were *E. coli.*

Of the 179 isolates identified by MALDI ToF, 164 were tested for the presence of plasmid mediated colistin resistance genes *mcr-1* and *mcr-2,* and all were negative. The results of this study show that the meat samples were positive for low numbers of *E. coli* and *Klebsiella* species, and included some samples positive for isolates that grew on the agars

containing 2 mg/L colistin. However, none of the isolates were positive for *mcr-1* or *mcr-2* plasmid mediated colistin resistance genes.

Whilst the original meat sample was positive for *mcr-1 E. coli*, none of the subsequent five beef knuckle samples (deemed related, but with different lot numbers) tested at a later date were positive. There are many possible explanations for this. It could be that the original positive sample was cross contaminated during processing by a food handler or due to contact with other meats, rather than the original animal being positive for *mcr-1 E. coli*? Alternatively, it could be that the first sample was from a batch of beef cattle positive for *mcr-1* plasmid mediated resistant *E. coli,* whilst the five subsequent beef knuckle samples were all from *mcr-1* negative animals? Although all meat samples were non-UK and from the same source, it is unknown how the meat samples related to each other, given they had different lot numbers. For the additional meat samples tested, both surface swabbing and sampling of 25 grams of meat was performed to detect *mcr-1 E. coli,* whilst for the original meat sample only sampling of 25 grams of meat was employed. As such, the sensitivity of the detection technique should be the same or possible improved for the five subsequent beef knuckle samples tested. However, for the five subsequent beef knuckle samples, microbiological testing was performed about 4 months after slaughter of the animals. Whilst this was before or only shortly after the expiry date of the meat samples, it could be that die off of *mcr-1 E. coli* on the meat sample had occurred during storage, reducing sensitivity of detection. However, most meat samples had low counts of *E. coli.* As such the results are likely to be true negatives within the overall bounds of sensitivity of the test, as compared to the results for the initial positive meat sample.