Evaluation of methods for enrichment of carbapenemase-producing E. coli in pork meat and cecal samples of porcine and bovine origin

Hasman, Henrik; Agersø, Yvonne; Cavaco, Lina; Svendsen, Christina Aaby; Nielsen, H.; San Jose, M.; Fisher, J.; Schmoger, S.; Rosa, P.; Guerra, B.

Publication date: 2015

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Ev0266

EPoster Viewing

Antimicrobials: resistance surveillance

Evaluation of methods for enrichment of carbapenemase-producing E. coli in pork meat and cecal samples of porcine and bovine origin

H. Hasman1, Y. Agersø1, L. Cavaco1, C. Aaby Svendsen1, H. Nielsen1, M. San José2, J. Fischer2, S. Schmoger2, P. Rosa3, B. Guerra2

1DTU - National Food institute, Kgs. Lyngby, Denmark
2Federal Institute for Risk Assessment- BfR, Berlin, Germany
3DG Health and Consumers- European Commission, Brussels, Belgium

Objectives.
The European Commission (EC) legislation (2013/652/EU) has decided to initiate a voluntary pan-European specific monitoring of carbapenemase producing Escherichia coli in meat and cecal samples of both porcine and bovine origin. The isolation method to be used should ensure high detection levels of E. coli producing carbapenemases previously found in animal production. Based on our experience of validating a wide range of methods covering different brands of commercial and non-commercial selective and indicative agars to detect these isolates in beef meat (ECCMID 2014, eP334), a narrower selection of methods were validated to detect these isolates in pork meat and bovine and porcine cecal samples.

Methods.
E. coli isolates producing the following ESBL/AmpC/carbapenemase were included in the study: CTX-M-1 (ESBL), CMY-2 (AmpC), VIM-1 and OXA-48. Bacterial suspensions were added (spiked) to the matrices in concentrations of 0.1 (only meat samples), 1, 10, 100 and 1000 CFU/gram matrix sample. Two different methods for pre-enrichment were considered: 1) MacConkey broth and 2) Peptone Buffered Water (PBW), both without addition of any antibiotic. Pre-enrichment in MacConkey was done by incubating overnight at 44°C and in PBW at 37°C followed by semi-quantitative plating and overnight incubation at 37°C on ChromID CARBA, ChromID OXA agar plates and ChromID-SMART (BioMerieux).

Results.
E. coli producing CXT-M-1 or CMY-2 did not grow on neither the CARBA nor the OXA or the SMART plates, regardless of the chosen pre-enrichment method. E. coli producing VIM-1 were detected on CARBA and SMART plates (but not on OXA plates) by both pre-enrichment methods except when porcine cecal samples were incubated in MacConkey broth (method 1). E. coli producing OXA-48 was not detected when spiked to porcine cecal samples and incubated in MacConkey broth (method 1) but was detected on OXA plates and SMART plates after pre-enrichment in PBW (method 2). In general, detection limits were between 100-1 CFU/gram, being higher for CARBA and OXA-plates than for SMART plates.

Conclusions.
Protocols to specifically select for carbapenemase producers in pork meat and cecal samples from pigs and cattle were developed. These include a pre-enrichment step without antibiotics in PBW and subsequent plating on selective ChromID CARBA and ChromID OXA agar plates (higher detection limit), followed by incubation at 37°C. This method was the only of the tested methods, which was able to detect production of VIM-1 (on CARBA plates) and production of OXA-48 (on OXA plates) from all tested matrices.