Project Title: Method Development to Enable Surveillance of Rare Resistance Phenotypes of Public-Health Concern

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Background

In cattle production, resistance to third generation cephalosporins (3GC) is not uncommon. Until recently, this resistance was mainly carried by the blaCMY-2 gene, however the emergence of extended-spectrum β-lactamases (ESBLs) conferring resistance to 3GC has been observed and associated with the presence of the blaCTX-M gene family, and more precisely with two groups of this family, blaCTX-M1 and blaCTX-M9. At the opposite of strains carrying blaCMY-2, strains carrying blaCTX-M or other families of genes such as blaTEM conferring the resistance to 3GC, are below the limit of detection of routine surveillance methods. Furthermore, the emergence of carbapenemase-producing bacteria has also been shown, raising more public health concern regarding treatment in case of infections caused by such multi-drug resistant microorganisms. To improve surveillance of this threatening multi-drug resistant bacteria, better detection, isolation and characterization methods are needed.

This study was developed around 2 main objectives: (1) development of a more sensitive isolation method for carbapenemase-producing bacteria from bovine fecal samples, and (2) development of a molecular method to detect and identify ESBL and carbapenemase genes (blaCTX-M, blaCMY, blaTEM, blaNDM, blaKPC, and blaOXA-48) from fecal samples.

Methodology

Method for the detection and isolation of carbapenemases producing bacteria: Three grams of feces enriched in 27 mL of BPW were incubated at 33±1°C for 18-22 hours. Ten µl of enrichment were streaked to SC II agar plate for screening of carbapenem resistant bacteria. Five colonies were selected and streaked onto SC for isolation and GSP for differentiation of *Pseudomonas* spp, and incubated for 18-22 hours at 35±1°C and 48 hours at 28±1°C, respectively. All isolates inhibited or morphologically yellow in color on GSP were selected for further investigation. Isolates of interest from the SC plate were streaked on MHA plate and incubated for 18 to 22 hours at 35±1°C. CIM test was performed on each isolate to determine the production of carbapenemases, using meropenem disk.

The whole genome of potential carbapenemase bacteria was sequenced. The sequences were analyzed to determine the genus and species of the isolated bacteria and to look for already-characterized antimicrobial resistance genes.

Targeted amplicon sequencing: Four grams of sterilized feces was enriched with 36mL of NB supplemented either with 2µg/mL of cefotaxime or 0.25µg/mL of ertapenem, spiked with up to 12 strains at defined concentration and incubated for 18h at 37±1°C. One mL of enrichment was used for DNA extraction by boiling and then used as template for PCR using

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the developed primers targeting the blaCTX-M, blaCMY, blaTEM, blaNDM, blaKPC, and blaOXA-48 genes. After migration on agarose gel, the positive amplification reactions were pooled for each sample and amplicons were purified. After quantification, and library preparation, the pooled amplicons were sequenced. The identity of the amplified variants was assessed using a home-developed pipeline based on the ResFinder database and a clustered version of this database.

Findings

The novel more sensitive isolation method of carbapenemase-producing bacteria was validated on spiked samples with reference strain. However, none of the tested isolates displayed the production of carbapenemase based on the CIM test.

The primers developed for the amplification of the blaCTX-M, blaCMY, blaTEM, blaNDM, blaKPC, and blaOXA-48 genes display an important sensibility and specificity. The identification of the variants from the targeted amplicon sequences is challenging as the position of the primers at the extremities of the encoding sequence of each gene does not allow the precise determination of the first nucleotides at each extremity. For this reason, the variants were clustered to provide the identity of a subset of variants. Using this strategy, we can properly classify all the investigated variant amplicons. While this method is not suitable for the identification of multiple variants within a sample, this method allows the proper identification of the resistance genes of a strain spiked at an initial concentration as low as 10 CFU/g of feces.

Implications

This research project led to the development of (1) a phenotypic method for the isolation of carbapenemase- producing bacteria and (2) a molecular method for the detection and identification of β-lactamases genes, which represent an important public health concern and should be better monitored.

Figure 1. Percentage of proper identification of the *bla* genes

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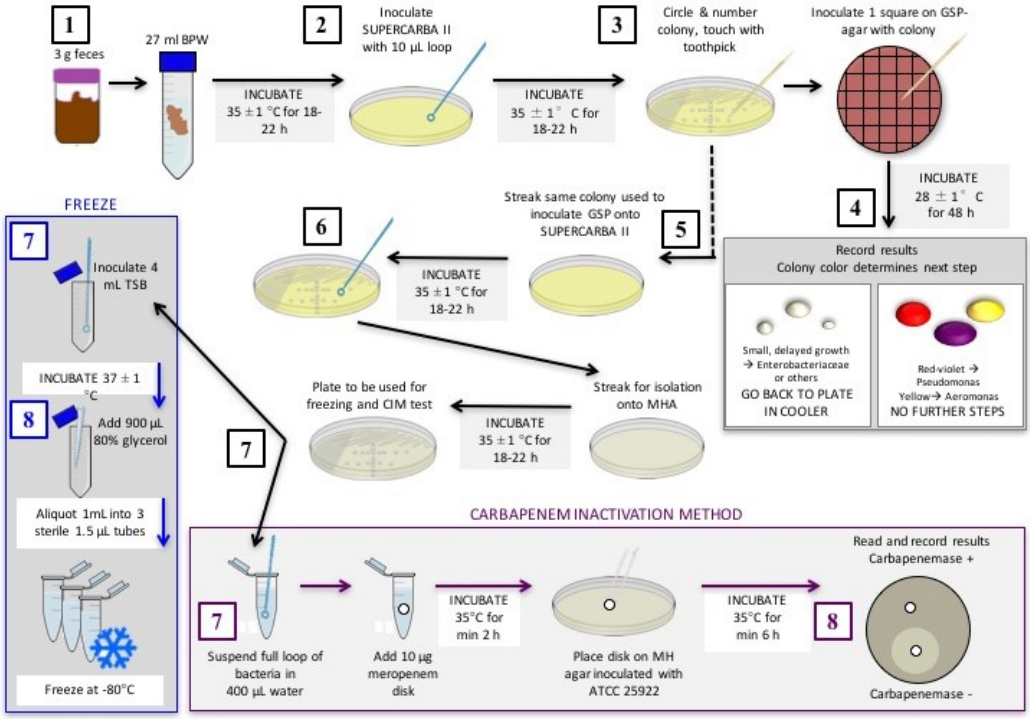

Figure 2. Detection and isolation of carbapenemase-producing bacteria.

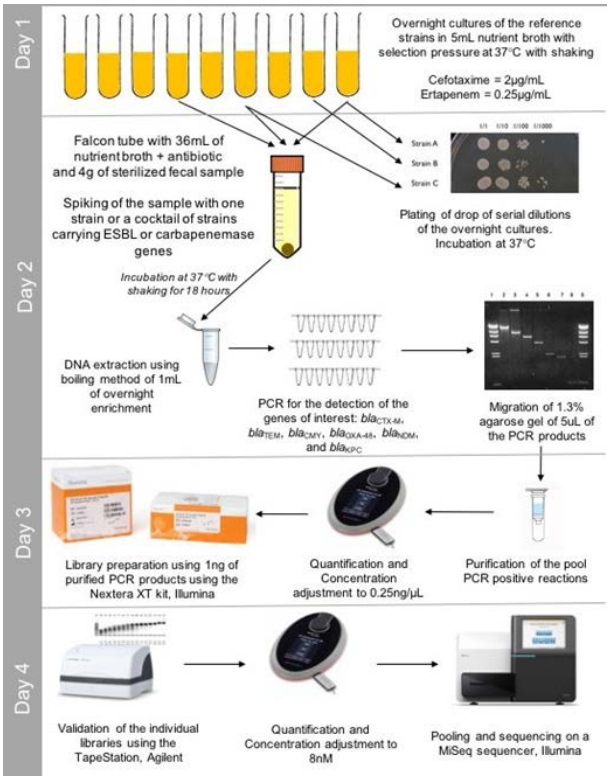

Figure 3. Targeted amplicon sequencing series.

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