β-lactamase-encoding gene identification by micro-arrays in phenotypically resistant pathogenic *Escherichia coli* from young calves in Wallonia, Belgium.

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Objectives

Since 2012, a decrease of β-lactam resistance of pathogenic *Escherichia* (*E.*) *coli* from calves has been observed at ARSIA, the Regional Veterinary Diagnostics laboratory in Wallonia, Belgium. This may be a consequence of the recommendation for a prior antibiotic sensitivity test of bovine pathogenic *E. coli* for human critical antibiotics, like 3rd and 4th generation cefalosporins. The most frequent β-lactam resistance mechanism is the production of a β-lactamase enzyme that inactivates the antibiotic. Actual classification(s) of β-lactamases (BLA) is highly complex, but four groups can be summarily described: classical BLA (BLA_C), extended cefalosporinases (BLA_{AmpC}), extended spectrum BLA (BLA_{ESBL}) and carbapenemases (BLA_{CPE}). The aim of this study was to identify the resistance genes coding for β-lactamases in pathogenic *E. coli* from calves with a resistance phenotype at the disk diffusion assay.

Materials and methods

From October 2017 to March 2018, pathogenic *E. coli* with BLA resistance phenotypes will be collected at ARSIA from calves with clinical and/or necropsy diagnosis of enteritis or septicemia. After an initial growth on Columbia blood and Gassner agar plates, three colonies from diarrheic feces or intestinal contents are transferred onto Minca and Ehly agar plates. One isolate positive at the agglutination for the F5, F17 or CS31A surface antigens and/or one isolate producing an enterohaemolysin are subsequently tested by the disk diffusion assay. The samples from blood and internal organs are also inoculated onto Columbia blood and Gassner agar plates. When a pure culture is obtained one colony is tested by the disk diffusion assay. The disk diffusion assay is performed with 16 antibiotics including 8 β-lactams (amoxicillin, amoxicillin + clavulanic acid, cefoxitin, cefotaxime, cefotaxime + clavulanic acid, ceftofiur, cefquinome and meropenem). The results are read with a Sirscan (I2A). A total of 500 colonies with a β-lactam resistance profile will be stored at -80°C in peptone broth with 40% glycerol and 120 of them will be chosen for the micro-array assay (Check-MDR ICT 103XL, Check-points).

Results

Right now (December 2017), 61 *E.coli* with a BLA_C, 2 with a BLA_{AmpC}, 12 with a BLA_{ESBL}, but none with a BLA_{CPE} resistance profile have already been collected. One additional isolate simultaneously presents a BLA_{AmpC} and a BLA_{ESBL} profile. So far, *E. coli* with an intermediate result for some of the 8 β-lactams tested have been included in the resistant isolates. The choice of the 120 isolates for the micro-array assay will of course be based on their resistance profiles. However they will include a higher proportion of BLA_{AmpC} and BLA_{ESBL}, than BLA_C, since 3rd and 4th generation cefalosporins are critical antibiotics in human medicine.

Conclusion

According to the results of the micro-array assay, specific PCR will be designed to test the whole *E. coli* collection. The comparison between the results of the disk diffusion assay and the resistance gene identification will link the β-lactam resistance phenotypes to the prevalence of the different β-lactamase-encoding gene families. Moreover their evolution in time will be followed since a second study is already planned in 2018-2019.