AUSTRALIAN PORK LIMITED

Surveillance for antimicrobial resistance in enteric commensals and pathogens in Australian pigs

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Background

The Australian Government has been actively progressing the development of a coordinated plan for the management of antimicrobial resistance (AMR) and antimicrobial use (AMU) in humans and animals. Broad support for the development of the “National Antimicrobial Resistance Strategy” was obtained from key stakeholders across the medical, health, veterinary, agricultural and pharmaceutical communities at the “Australian One Health Antimicrobial Resistance Colloquium” in 2013. A review of the national surveillance programs in place for monitoring AMR and AMU in animals around the world was then conducted, with a view to defining a program suitable for Australia and combining this with roundtable discussions with key stakeholders in the agriculture and veterinary sectors. The review “Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia” identified one of the major components of surveillance being the assessment of AMR in commensal bacteria and pathogens present in the gut of food animals at slaughter. In March 2015, the “Antimicrobial Resistance Surveillance Task Group”, established by the Department of Agriculture, reviewed the recommendations from the surveillance report and provided advice from technical and industry perspectives for developing an AMR surveillance component based on the collection of faecal samples from food animals at slaughter. A surveillance model for use in the Australian pig industry was proposed to examine issues including feasibility, cost, timing, methodology and logistics, which could also be applied to other major food animal industries in the future. The key outcomes of this proof of concept study and recommendations for future surveillance strategies are highlighted below.

Methodology

A total of 200 caecal specimens were collected from Australian finisher pigs (across 31 farms) at slaughter to estimate the prevalence of resistance against specified antimicrobials amongst E. coli, Salmonella spp., Enterococcus spp., and Campylobacter spp. Commensal E. coli were isolated from diluted caecal material; and Salmonella spp., Enterococcus spp. and Campylobacter spp. from faecal samples collected at each abattoir. Isolates were identified using standard procedures, confirmed using matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF) and tested for antimicrobial susceptibility. The minimum inhibitory concentrations (MIC) were interpreted according to veterinary CLSI VET01S or the European Committee for Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values (ECOFFs). Where no EUCAST or CLSI interpretative criteria were available, breakpoints were harmonised with those of the National Antimicrobial Resistance Monitoring System (NARMS), USA. Resistance profiles to the antimicrobial classes were generated to examine co-resistance for E. coli, Salmonella spp., E. faecium and C. coli. An isolate was considered non-susceptible to an antimicrobial agent when it tested resistant, intermediate or non-susceptible when using clinical breakpoints as interpretative criteria, and not ECOFFs, provided by EUCAST or CLSI. Only acquired (and not intrinsic) resistance was taken into consideration when defining an isolate as exhibiting multidrug resistance (MDR). MDR was defined as a profile comprising non-susceptibility to at least one agent in three or more associated antimicrobial classes.

Key outcomes

E. coli was isolated from samples from all farms. No Salmonella spp. were recovered from pigs originating from ten (32%) farms. Enterococci were isolated from pigs originating from all but one farm. Eight enterococcal species were recovered, three of which contributed to 93.8% of all species (E. faecium, 57.5%; E. hirae, 24.7%; E. faecalis, 11.6%). Campylobacter spp. was recovered from all farms; C. coli (91.8%) was the dominant species, followed by C. hyointestinalis (7.0%). One campylobacter isolate could only be identified to the genus level by MALDI-TOF.

High rates of resistance to antimicrobials with a lower importance rating were found for E. coli - these outcomes were more similar to those in North America than Europe. Non-susceptibility (i.e. isolates classified as either intermediate or resistant according to clinical breakpoints) to tetracycline, ampicillin and streptomycin in both E. coli and Salmonella spp. was high (range 55–77%). None of the isolates showed non-susceptibility to extended-spectrum cephalosporins including cefotaxim. No resistance to colistin was observed, in line with absence of any colistin containing products currently registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use in pigs and anecdotal reports that this agent has not been used in pigs in Australia for over thirty years. Rates of resistance to florfenicol and gentamicin among E. coli and Salmonella spp. was low - less than 10% and 2%, respectively. These outcomes provide evidence of historic use patterns of antimicrobials (i.e. reliance on 1st and 2nd line antibiotic treatments) and it was concluded that resistance mechanisms could be located on a plasmid that is also responsible for resistance of 1st line of antimicrobials). For Salmonella, resistance was found to antimicrobials with a lower importance rating. Reduced susceptibility to the critically important antimicrobials were not observed for the majority of the isolates. However, a couple of Salmonella spp. showed reduced susceptibility to ciprofloxacin (a fluoroquinolone), but none of the Australian isolates could be regarded as
resistant according to CLSI clinical breakpoints. It was considered likely that these isolates were transported onto farm by birds, rodents or humans. It was recommended that the *Salmonella* and *E. coli* isolates showing reduced susceptibility to fluoroquinolones, the multidrug-resistant *Enterococci* and a selection of multidrug-resistant *Campylobacter* and *Enterococci* isolates be subjected to whole genome sequence analysis to further elucidate their epidemiology, likely origins and public health significance.

No fluoroquinolone resistance was observed among *Campylobacter* species. However, high rates of macrolide, lincosamide, ketolide (telithromycin) and tetracycline resistance were observed. This may be reflective of the use of first-line antimicrobials (veterinary use of antimicrobials highly important to human health-Australian Veterinary Association, April 2017) i.e. those with a lower importance rating, to treat and control respiratory and enteric infections among pigs in Australia. It was recommended that molecular characterization be undertaken of the multidrug-resistant *Campylobacter* species isolated in this study to further clarify their public health significance; this is hypothesized to be low based on the *Campylobacter* species identified.

None of the *Enterococci* isolates were resistant to vancomycin and linezolid. The observed resistance to quinupristin-dalfopristin was unexpected; virginiamycin use in pigs was banned in Australia over 13 years ago. It is still registered for use in other animal species, namely horses and feedlot cattle, however, label recommendations prohibit its use in pigs and the compound also has a Schedule 4 rating (i.e. the compound can only be dispensed on prescription by a registered veterinarian). Surveillance data from other countries have documented *Enterococcus* isolates returning to full susceptibility to quinupristin-dalfopristin quite rapidly following removal of virginiamycin. Whole genome sequencing was conducted to evaluate if the quinupristin-dalfopristin-resistant isolates carried genetic determinants encoding this resistance. The genotypic results shows that the elevated resistance observed for quinupristin-dalfopristin among *E. faecium* was not due to the carriage of any known resistance gene. It was proposed that the elevated resistance to quinupristin-dalfopristin enterococcus may be due to:

1. An inappropriate break point for both clinical and epidemiological breakpoints.
2. Presence of a new resistance mechanism.

**Conclusion**

This proof-of-concept study provides a baseline for the Australian pig industry and was a good report card for the Australian pig industry. It also provides a benchmark for the other livestock industries in Australia to establish further animal-specific proof of concept surveys, as the basis for an ongoing integrated livestock AMR surveillance program. It is recommended that the generated data are integrated into current antimicrobial stewardship programs being developed by the Australian pork industry and be monitored on an industry or farm level to reflect practice change as producers are encouraged to reduce antimicrobial use.