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Antimicrobial resistant bacteria in red meat producing animals

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Executive Summary

Where we were in 2004

Antimicrobial therapy is essential for the treatment of infectious disease in humans and animals. However, due to the development, spread and persistence of bacteria resistant to antimicrobials, the effectiveness of antimicrobial therapy is threatened. As food is a vehicle by which people can acquire antimicrobial resistant bacteria, the growing risks to human health posed by the selection of antimicrobial resistant bacteria in the food chain have been recognised.

Previous research conducted in partnership between Food Science Australia and MLA had determined that Enterobacteraceae present in cattle and cattle production environments carried a limited number of antibiotic resistance genes carried on mobile genetic element called integrons. Our research indicated that the isolates or genes could be acquired from bacteria present in the soil environment associated with cattle. No evidence for increased prevalence due to selection pressure through the use of antibiotics was observed for integron-associated antibiotic resistance genes. Similarly, the low prevalence of antibiotic resistant *E. coli* suggested that overall selection pressure for bacterial antibiotic resistance in beef cattle was low.

While data was available addressing the presence and prevalence of AMR in live cattle and cattle at slaughter, there was no substantial body of data that described the presence of AMR bacteria on red meat carcases and retail meat. The 2004/5 Microbiological Survey of Australian Red Meat was identified as an opportunity to assess the national prevalence of antimicrobial resistance in *E. coli* and *Enterococcus* isolates associated with red meat carcases and retail meat.

Where we are in June 2007

Representative isolates of *E. coli* and *Enterococcus* isolated from carcases and retail meat in the 2004/5 Microbiological Survey of Australian Red Meat were tested for AMR to a panel of antimicrobials commonly used in human medicine. The prevalence of AMR in carcase *E. coli* was low (0 – 3.2%) for representative antimicrobials such as ciprofloxacin, trimethoprim/sulfamethoxazole and cephalothin. The corresponding prevalence of AMR in retail meat *E. coli* increased to a range of 0 - 25.3% for the same panel of antimicrobials. (The highest rate of resistance was to cephalothin (25.3%). Expression of resistance to cephalothin by *E. coli* correlates poorly with susceptibility/resistance to other antimicrobials in the 1st generation cephalosporin class, suggesting that cephalothin may not be a reliable indicator of true resistance). AMR *Enterococcus* prevalences showed a similar trend to that observed for *E. coli*. The prevalence of AMR *Enterococcus* was 0 – 9% (carcases) and 0 – 12.9% (retail meat). Characterisation of the

Enterococcus species present in carcases and retail meat revealed that *E. faecium* and *E. faecalis* were dominant. Since *E. faecium* and *E. faecalis* are human commensal microorganisms, and may also be opportunistic pathogens, there is some concern regarding their increased AMR prevalence and generally increased prevalence in retail meat. In subsequent in-line sampling of butcher premises, the observations of *E. faecium* and *E. faecalis* dominance in retail meat were confirmed. In butcher premises these bacterial species were also predominantly isolated from worker and premises samples. These data and additional molecular typing data indicate that related *E. faecalis* strains can be widely distributed through retail meat premises as a consequence of operational practices within the premises.

Where to from here

Through this study Food Science Australia and MLA have established important baseline data concerning the presence and prevalence of AMR in carcase and retail meat. The probable role of retail meat worker practices in the increased prevalence of AMR *Enterococcus* in retail meat is a matter of concern. Further investigation of the role of retail meat worker practices could be achieved through the design of appropriate interventions and subsequent assessment of impact on microbiological quality. We have also identified that *E. faecalis* may resist sanitation practices in retail meat premises through the potential formation of biofilm structures. Investigation of biofilms as sources of persistence and distribution of AMR bacteria such as *E. faecalis* may also inform the implementation of avenues for improved control and microbiological quality in retail red meat.

General Project Summary

Antimicrobial resistant E. coli and Enterococcus from the national carcase survey (MS1)

Abattoir isolates of *E. coli* and *Enterococcus* spp obtained in the 2004 Microbiological Survey of Australian Red Meat were examined for antimicrobial resistance. Two hundred and twenty one (221) *E. coli* isolates were tested for resistance to 17 antimicrobials and 300 *Enterococcus* isolates were tested for resistance to 8 antimicrobials. A low level of antimicrobial resistance was found for *E. coli* isolates. The highest rate of resistance was to cephalothin (3.9%). Expression of resistance to cephalothin by *E. coli* correlates poorly with susceptibility/resistance to other antimicrobials in the 1st generation cephalosporin class, suggesting that cephalothin may not be a reliable indicator of true resistance.

Enterococcus antimicrobial resistance was detected with the following frequencies: gentamicin (1.3%), levofloxacin (1.3%), quinupristin/dalfopristin (5.0%), streptomycin (5.0%), tetracycline (7.0%) and vancomycin (9.0%). No *Enterococcus* isolates were resistant to ampicillin or linezolid. Six (6) *Enterococcus* isolates from sheep carcases were resistant to both quinupristin/dalfopristin and tetracycline; such data may indicate potential selection pressure for resistance through the use of virginiamycin and tetracycline in sheep production systems. The detection of *E. faecalis* and *E. faecium* with resistance to vancomycin indicates potential that these isolates carry acquired resistance genes. Further clarification of the genetic basis for vancomycin resistance in these *E. faecalis* and *E. faecium* isolates will provide information that may indicate if identifiable antimicrobial selection plays a role in the presence of such isolates.

Antimicrobial resistant E. coli and Enterococcus from the national retail meat survey (MS2)

Retail meat isolates of *E. coli* and *Enterococcus* spp obtained in the 2004/2005 Microbiological Survey of Australian Red Meat were examined for antimicrobial resistance (AMR). Ninety-nine *E. coli* isolates were tested for resistance to 17 antimicrobials and 241 *Enterococcus* isolates were tested for resistance to 8 antimicrobials. Although the level of resistance to the majority of antimicrobials tested remained generally low for *E. coli* isolates, there were substantial increases in resistance to some antimicrobials when compared with those observed in *E. coli* from the national baseline abattoir survey (Milestone 1, PRMS.061). The highest rate of resistance was to cephalothin

(25.3%). Expression of resistance to cephalothin by *E. coli* correlates poorly with susceptibility/resistance to other antimicrobials in the 1st generation cephalosporin class, suggesting that cephalothin may not be a reliable indicator of true resistance. Resistance to ampicillin (10.1%), ticarcillin / clavulanic acid (11.1%) and trimethoprim / sulfamethoxazole (13.1%) were also observed at much higher rates than *E. coli* isolates from the national baseline abattoir survey.

The majority of *Enterococcus* isolated from retail meats were *E. faecalis* (73.4%). This represents an almost 3 fold increase from the rate observed in the national baseline abattoir survey (Milestone 1, PRMS.061). This result, coupled with a noticeable drop in the 'environmental' Enterococci (*durans*, *gallinarum* and *hirae*) suggests that *E. faecalis* and *E. faecium* (13.7%) may have enhanced survival capabilities. *Enterococcus* AMR, like the *E. coli* AMRs, were observed at generally higher rates in this study than in the national baseline abattoir survey. Resistance to quinupristin / dalfopristin (9.4%), streptomycin (10.4%), and tetracycline (12.9%) were most commonly detected. Significantly, resistance to clinically significant antimicrobials such as levofloxacin (0.4%), vancomycin (2.5%) and linezolid (0.0%) were low and did not increase from the rates observed in the national baseline abattoir survey. While the genetic basis of the resistances observed have not been determined and are beyond the scope of this study, the results suggest that retail *Enterococcus* isolates may carry acquired resistance genes and have the potential to impact on human health via the food chain.

Both the AMR rates and the narrowed *Enterococcus* species distribution (compared to the results for the national baseline abattoir survey; Milestone 1, PRMS.061), raise important questions concerning the underlying epidemiological/environmental factors that contribute to the decreased AMR microbiological quality of retail meats. It is recommended that investigation of the factors contributing to AMR in retail meat be commenced.

Identification, confirmation and validation of antibiotic resistant E. coli and Enterococci isolates from the nationwide microbiological survey of Australian red meat (MS3)

Carcass and retail meat isolates of *E. coli* and Enterococci from the 2004/2005 Microbiological survey of red meat have previously been tested for resistance to a variety of antibiotics (PRMS.061 Milestones 1 & 2). The levels of antibiotic resistance identified in the isolates were generally lower

or at least comparable with figures reported from similar surveys in other countries. However, analysis of the data and project design identified 2 key areas that required further investigation. The first of these was the potential for *E. coli* isolates to demonstrate phenotypic resistance to tetracycline, chloramphenicol and florfenicol. Resistance to these antibiotics can be associated with the carriage of large multiple-resistance plasmids and as a consequence are of importance in livestock production and slaughter. Secondly, although relatively low, the levels of antibiotic resistance in retail Enterococci isolates increased notably when compared to the carcass isolates. Validation of the VITEK Junior apparatus and confirmation of previous results using the agar dilution technique was sought prior to investigating the reasons for the higher levels of resistance in retail isolates.

Two hundred and eighty-three *E. coli* isolates were tested for resistance to tetracycline, chloramphenicol and florfenicol using disk susceptibility testing. Twenty (7.1%) isolates were resistant to tetracycline. Resistance to chloramphenicol (0.01%) and florfenicol (0.004%) was minimal. Analysis of the tetracycline resistant *E. coli* determined that these isolates were unlikely to carry multiple-resistance plasmids as only 2 of 20 isolates demonstrated resistance to additional antibiotics. **The results of this study further indicate that the levels of antibiotic resistance in *E. coli* isolates from the Australian red meat survey are generally low.**

Antimicrobial resistant E. coli and Enterococcus from in-line sampling of butcher premises (MS4)

In a recent national survey of red meat from abattoir and retail sites, we studied the antibiotic resistance of *Escherichia coli* and *Enterococcus* isolates. Our results provided preliminary indications that the prevalences of antimicrobial resistant (AMR) *E. coli* and *Enterococcus* are greater in retail meat samples compared to abattoir carcasses. If such AMR indicator bacteria are better able to survive and propagate under conditions associated with retail meat preparation and storage, this may provide a plausible explanation for their increased prevalence. Alternatively, AMR strains may be introduced through contamination into the retail segments of the red meat production chain, causing an increase in prevalence of such bacteria. In the present study, the prevalence of AMR in *E. coli* and *Enterococcus* isolated from in-line sampling of retail butcher shops was examined. In-line sampling in four butcher premises was performed by an external

microbiology laboratory (Alliance Consulting). Presumptive *E. coli* and *Enterococcus* were cultured from red meat carcase, raw meat, contact surface, water and hand wash rinse water samples. The presumptive *E. coli* and *Enterococcus* cultures were then provided to Food Science Australia for isolation and antimicrobial susceptibility testing to confirm previous testing.

A total of 20 *E. coli* (n=20) and 71 *Enterococcus* (n=71) were isolated from all samples. Seventy percent (70%) of *E. coli* were isolated from raw meat or carcase swab samples. In contrast, the majority (68%) of *Enterococcus* isolates were obtained from butcher shop and worker sources including apron, bandsaw, cutting board, door, hand, knife, mincer, slicer, tongs, tray and tubs. The remaining isolates (32%) were from raw meat and carcase sources. The overall prevalence of AMR *E. coli* and *Enterococcus* isolates resistant to at least one antimicrobial was 10% and 25% respectively. The prevalence of AMR *E. coli* determined in this study is broadly consistent with the prevalence of AMR *E. coli* determined in the national retail meat survey (Barlow and Gobius, 2006). In addition, the types of AMR phenotype present in *E. coli* from butcher shops are also consistent with those observed in *E. coli* from cattle production systems, abattoirs and the previous national retail meat survey. *Enterococcus faecalis* (70%) and *E. faecium* (17%) were the most prevalent *Enterococcus* species isolated (predominantly from worker and premises samples). Resistance to gentamicin (20%) and streptomycin (17%) were the most commonly observed AMR phenotypes. Resistance to the remaining antimicrobials tested was low (tetracycline, 4%; penicillin G, 6%, and quinupristin/dalfopristin, 4%). Increased AMR *Enterococcus* prevalence in butcher shops is consistent with that observed in the national retail meat survey.

The sample sources from which most *E. coli* and *Enterococcus* (both AMR and non-AMR) were isolated differed in this study. *E. coli* were predominantly isolated from from carcase and raw meat samples, while *Enterococcus* were predominantly isolated from worker and premises samples. It was also notable that *Enterococcus* isolates were predominantly made up of *E. faecalis* and *E. faecium*, and were more likely to possess AMR. These data suggest that factors are present in retail butcher premises which promote an increased prevalence of AMR *E. faecalis* and *E. faecium.*

In considering potential explanations for the increased prevalence of AMR *E. faecalis* and *E. faecium* in retail butcher shops, it is possible that these *Enterococcus* spp possess factors conferring resistance to normal sanitising procedures, allowing their persistence and increased prevalence. If resistant to sanitisers, the sanitising processes may contribute to their physical distribution within the butcher premises and also foster co-selection of AMR expression.

At present there is no direct evidence that AMR *E. coli* or *Enterococcus* from retail red meat contribute to a known public health risk. Notwithstanding this fact, it is a matter of concern to

industry that AMR *E. coli* and *Enterococcus* appear to occur with increased prevalence in retail butcher shops, when compared to the prevalences of similar bacteria on abattoir carcases. Further directed research is required to understand the origin and potential persistence of such bacteria in butcher shops, facilitating the ability to continue to effectively manage and decrease the potential public health risks which may occur due to AMR bacteria.

Molecular typing of Enterococcus faecalis isolates from butcher premises to investigate sources of contamination (MS5)

Previous milestones for this project have investigated the prevalence of antimicrobial resistant (AMR) *E. coli* and *Enterococcus* isolates on beef and sheep carcases, retail meat and in retail butcher premises. In comparison to their prevalence on carcases, both *Enterococcus faecium* and *Enterococcus faecalis* are found with increased prevalence in retail meat and retail butcher premises. Furthermore, the prevalence of AMR in these *Enterococcus* species is similarly increased in retail meat and retail butcher premises. The reasons for the increased prevalences are not readily apparent; however, recent analysis (Barlow and Gobius 2007) suggests that practices within retail premises may promote the survival and subsequent spread of *E. faecium* and *E. faecalis* in these environments. The aim of the current study was to analyse the Pulsed Field Gel Electrophoresis (PFGE) patterns of *E. faecalis* isolates obtained from retail premises with a view to determining the relationships between *E. faecalis* isolates from different samples and providing information on the possible sources of these isolates.

Examination of 82 *E. faecalis* strains using *Sma*I-PFGE analysis defined 29 separate *E. faecalis* types. Isolates in a single type were at least 91% similar and all *E. faecalis* isolates were at least 63% similar. The variety of type groups present in retail meat premises indicate that *E. faecalis* isolates are clonally diverse. Several type groups were comprised of isolates from both butcher and undesignated retail outlets, indicating that related *E. faecalis* strains are present in different retail meat premises across time and in alternative geographic locations. Within the separate type groups, the isolate source data indicated that related isolates were recovered from a variety of different raw meat, retail premise equipment and environment sources, thus providing evidence for cross contamination and potential distribution of *E. faecalis* strains through the retail premises. In a single butcher premise 23 *E. faecalis* isolates were identified as 100% related and these were recovered from diverse sources including door, cutting board, lamb carcase, bandsaw, tongs, steel, hand rinse

water, diced lamb, tub, apron, slicer, and beef mince samples. This data indicates the extensive distribution of a common isolate within the one retail premise.

This study provides evidence that related strains of *E. faecalis* are present in separate premises and that they can also be widely distributed within these premises. Since *E. faecalis* (and *E. faecium*) strains present in red meat retail premises also have higher AMR prevalence than the same bacterial species on carcases at slaughter (Barlow and Gobius 2007), coupled with the potential of these *Enterococcus* species to be opportunistic human pathogens, it is important that their ecology in the red meat retail environment be understood. Identification of specific point sources or reservoirs of *E. faecalis* (potentially human worker/environmental biofilms or both) in retail meat premises will facilitate appropriate intervention and control strategies. These hygiene interventions will in turn provide the means for managing any public health risks due to the presence of these organisms.

References

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