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FOOD SCIENCE AUSTRALIA

A joint venture of CSIRO & the Victorian Government

Pilot survey for antimicrobial resistant (AMR) bacteria in Australian food

prepared for
the Australian Government Department of Health and Ageing

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3 June, 2008
Updated 21 November, 2008

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Executive Summary

The pilot survey for antimicrobial (AMR) resistant bacteria in Australian food is designed to provide data that can be used to estimate the prevalence of AMR bacteria in selected foods purchased at retail outlets. Four retail foods; poultry, beef, pork and lettuce along with four target organisms; *Campylobacter, Salmonella, Escherichia coli* and *Enterococcus* constitute the nine food / bacterium combinations included in the survey. The survey sampling plan was designed to allow for the recovery of 100 isolates from each food / bacterium combination. Ongoing monitoring of the prevalence of each food / bacterium combination identified *Campylobacter* in poultry, *E. coli* in pork and *E. coli* in lettuce as three combinations that were unlikely to achieve the 100 isolate goal using the initial sampling plan. An increase in the number of tests for *Campylobacter* in poultry and *E. coli* in pork were made during the survey to provide the greatest opportunity for the 100 isolate goal per food / bacterium combination to be met. These increases were offset by similar sized reductions in the collection and testing of lettuce for *E. coli* as the prevalence of this combination indicated that 100 isolates would not be achieved. At the conclusion of sampling, 7 of the nine 9 food / bacterium combinations exceeded the 100 isolate goal of the survey using the modified sampling plan. Pork / *E. coli* (92 isolates) and lettuce / *E. coli* (7 isolates) did not reach the 100 isolate goal.

The results of AMR testing indicated that resistance to the majority of antimicrobials tested is low (< 10%). However, it is notable that the data indicates trends of higher prevalences of AMR in particular food / bacterium combinations. In *E. coli* from poultry and pork the prevalence of AMR was ≥15% for ampicillin, streptomycin and tetracycline, in contrast to beef *E. coli* isolates where prevalence of resistance to these antimicrobials was ≤11%. Similarly, *E. faecalis* isolates from poultry were distinguished from beef and pork isolates by high prevalences of resistance to erythromycin (48%) and tetracycline (76%). Resistance to tetracycline (16%) was observed for *Salmonella* isolates from chicken. AMR resistance to all antimicrobials tested in *Campylobacter* from chicken was low (≤4%). Resistance to quinolones was not observed in any *E. coli* or *Campylobacter* isolates, whereas naladixic acid resistance was present in only a single *Salmonella* isolate (1%) from chicken.

The current Australian food AMR data has been compared with data from the international AMR surveys: The Danish Integrated Antimicrobial Resistance

Monitoring and Research Programme (DANMAP), Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and the United States of America National Antimicrobial Resistance Monitoring System (NARMS). Where variations in Australian and international AMR prevalences, of ≥ or ≤ 10%, occur, these have been considered notable and are indicated below:

- In retail chicken, notable differences in AMR prevalence in the bacteria *Salmonella*, *E. coli*, *Enterococcus* and *Campylobacter* are reported.
    - *Salmonella* (US and Canada) possess a greater prevalence of resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, streptomycin and tetracycline.
    - *E. coli* (US and Canada) possess a greater prevalence of resistance to amoxicillin/clavulanic acid, ceftiofur, gentamicin and streptomycin.
    - *Enterococcus* (US, Canada and Danish imported product) possess a greater prevalence of resistance to kanamycin, streptomycin and flavomycin (US only).
    - *Campylobacter* (US, Canada and Danish imported product) possess a greater prevalence of resistance to ciprofloxacin, nalidixic acid and tetracycline.
- In retail beef, notable differences in AMR prevalence in the bacteria *E. coli* and *Enterococcus* are reported.
    - *E. coli* (US) possess a greater prevalence of resistance to tetracycline.
    - *Enterococcus* (US) possess a greater prevalence of resistance to tetracycline and flavomycin.
- In retail pork, notable differences in AMR prevalence in the bacteria *E. coli* and *Enterococcus* are reported.
    - *E. coli* (Australia) possess a greater prevalence of resistance to ampicillin.
    - *Enterococcus* (US) possess a greater prevalence of resistance to tetracycline and flavomycin.

The testing of isolates collected as part of the survey for AMR provides a snapshot of the prevalence and types of AMR bacteria present in selected retail foods in

Australia. The use of Sensititre equipment and panels has generated data that is internationally equivalent and which can be compared to available overseas information. Whilst the survey data cannot be used to directly provide information about the development of antimicrobial resistance, it provides baseline data suitable for future use in the determination of antimicrobial resistance trends at the Australian retail food level. When correlated with similar Animal Isolates and Human Clinical AMR surveys this data may be useful in managing and controlling AMR development in the Australian community.

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Introduction

On behalf of the Food Regulation Standing Committee (FRSC), the Department of Health and Ageing ('the Department') has contracted Food Science Australia (FSA CSIRO) to conduct a pilot survey of antimicrobial resistant (AMR) bacteria in food which may be used by the Department to inform an ongoing surveillance program. The pilot survey is designed to provide data that can be used to estimate the prevalence of AMR bacteria in food purchased at retail outlets. It is anticipated that the results of the survey will provide statistically sound scientific data that can be used to inform future research on AMR bacteria in food and assist in developing preventative strategies and measures.

The aim of the pilot survey for AMR bacteria in Australian food has been to recover at least 100 isolates per food / bacterium combination and to test each of these isolates against a panel of antimicrobials using the Sensititre apparatus (TREK Diagnostic Systems, UK). Testing of the isolates for AMR was conducted at two timepoints; the first occurred after the 6th monthly sampling round (testing approximately 50 isolates for each food / bacterium combination) and the second has occurred after the 12th monthly sampling round (testing a further approximately 50 isolates for each food / bacterium combination). The following document is a review of the 12 month prevalences for each of the survey target organisms and a summary of completed AMR testing.

Statement of Deliverable Objectives

Fifth deliverable [Final report] – This report will include the following components:

- A contents page;
- An executive summary;
- A summary of methodologies utilised;
- Detailed description of the survey of AMR bacteria in food and the results of that survey;
- A discussion of the analysed results, including brief comment about their relationship with similar international food survey results such as the

Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, Denmark), National Antimicrobial Resistance Monitoring System (NARMS, United States) and Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS, Canada);

- Identification of any specific strengths and limitations of the survey; and
- A brief discussion of any lessons learned in relation to the methodology used to undertake the Services.

Materials and Methods

Sampling, isolation & characterisation

Sampling in each of the four capital city areas progressed as scheduled. Recommended changes to the initial sampling plan were made during the survey in an attempt to ensure at least 8 of the 9 food / bacterium combinations achieved the 100 isolate goal of the survey. Isolation and characterisation of the target organisms was conducted as per, First Deliverable – Methodology Summary (Appendix A).

Antimicrobial susceptibility testing

The antimicrobial resistance phenotype of isolates was determined using the broth micro-dilution method and the Sensititre apparatus. The susceptibility panels AUSVN, AUSVP and CAMPY were used for Gram negatives, Gram positives and *Campylobacter* respectively. AUSVN and AUSVP are custom plate formats designed for this survey. CAMPY is a standard Sensititre plate format. The susceptibility plate formats are shown in Appendix B. All susceptibility panels were prepared and read as per the manufacturer's instructions using the Sensititre Autoinoculator and Sensitouch apparatus. *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33291 were used as quality controls.

The range of antimicrobial concentrations tested and resistance breakpoints for each antimicrobial/bacterium combination are presented for *E. coli* and *Salmonella* (Table 1), *Campylobacter* (Table 2) and *Enterococcus faecalis* (Table 3). Where available, antimicrobial resistance breakpoint criteria defined by the Clinical and

Laboratory Standards Institute (CLSI) in document M100-S18 were used for *Salmonella*, *E. coli* and *Enterococcus* (1). Where CLSI breakpoints were not available (including all antimicrobials for *Campylobacter*), harmonisation with CIPARS and NARMS breakpoints was implemented (2, 3).
The susceptibility of *Campylobacter* isolates to azithromycin was determined, however since azithromycin and erythromycin are both macrolide antimicrobials, only erythromycin resistance is reported. The susceptibility of *E. faecalis* isolates to lincomycin, quinupristin/dalfopristin and virginiamycin was determined. Since *E. faecalis* in intrinsically resistant to these antimicrobials resistance data is not reported.

Detailed survey design and methodology

Documentation of the complete survey design and agreed methodology are available in the reports **'Scope and design of a pilot survey for the assessment of antimicrobial resistant (AMR) bacteria in Australian food'** and **'First Deliverable – Pilot survey for antimicrobial resistant (AMR) bacteria in Australian food – Methodology Summary.'**

Table 1. Range of antimicrobial concentrations tested and resistance breakpoints for *E. coli and Salmonella*.

Antimicrobial	Antimicrobial Concentrations (µg/mL) and Breakpoints										
	0.125	0.25	0.5	1	2	4	8	16	32	64	128
Amoxicillin / Clavulanic acid [a]											
Ampicillin											
Cefazolin											
Cefotaxime											
Cefoxitin											
Ceftiofur											
Ceftriaxone											
Chloramphenicol											
Ciprofloxacin											
Florfenicol											
Gentamicin											
Kanamycin											
Meropenem											
Nalidixic Acid											
Streptomycin											
Tetracycline											
Trimethoprim / Sulfamethoxazole											

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for specific antimicrobials.

Table 2. Range of antimicrobial concentrations tested and resistance breakpoints for *Campylobacter*.

Antimicrobial	Antimicrobial Concentrations (µg/mL) and Breakpoints												
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Ciprofloxacin													
Clindamycin													
Erythromycin													
Florfenicol													
Gentamicin													
Nalidixic Acid													
Telithromycin													
Tetracycline													

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for specific antimicrobials.

Table 3. Range of antimicrobial concentrations tested and resistance breakpoints for *Enterococcus faecalis*.

Antimicrobial	Antimicrobial Concentrations (µg/mL) and Breakpoints																	
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048
Ampicillin																		
Chloramphenicol																		
Daptomycin																		
Erythromycin																		
Flavomycin																		
Gentamicin																		
Kanamycin																		
Linezolid																		
Penicillin																		
Streptomycin																		
Teicoplanin																		
Tetracycline																		
Tigecycline																		
Vancomycin																		

Vertical lines indicate breakpoints for resistance

The white fields denote range of dilutions tested for specific antimicrobials.