Results for today **Ideas for tomorrow**

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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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.

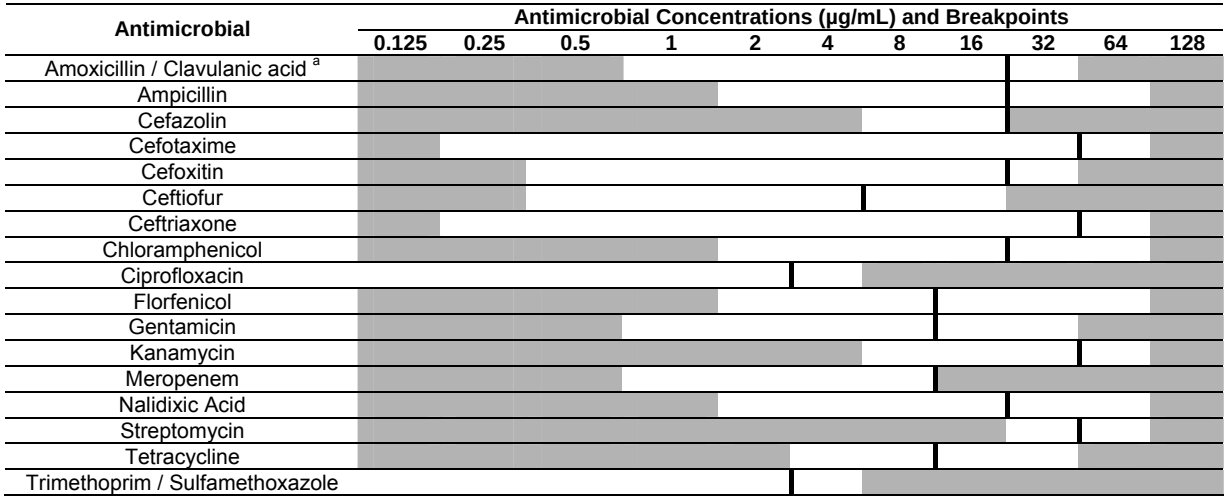
The range of antimicrobial concentrations tested and resistance breakpoints for each antimicrobial/bacterium combination are presented for *E. coli* and *Salmonella* (Table 1), *Campylobacter* (Table 2) and *Enterococcus faecalis* (Table 3). Where available, antimicrobial resistance breakpoint criteria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

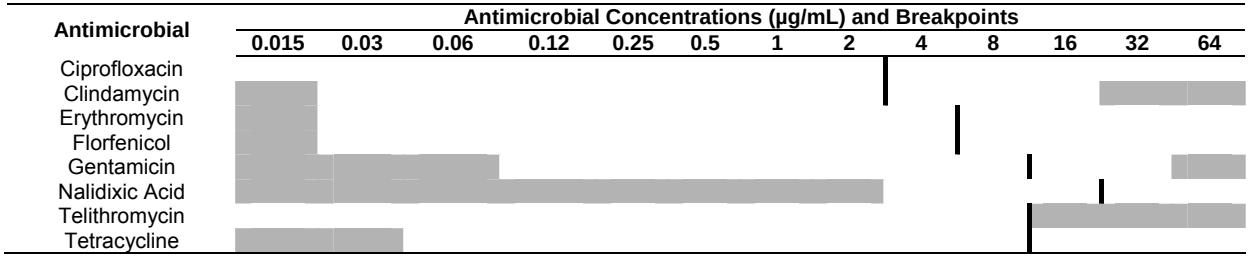
Documentation of the complete survey design and agreed methodology are available in the reports **'Scope and design of a pilot survey for the assessment of antimicrobial resistant (AMR) bacteria in Australian food'** and **'First Deliverable – Pilot survey for antimicrobial resistant (AMR) bacteria in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

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



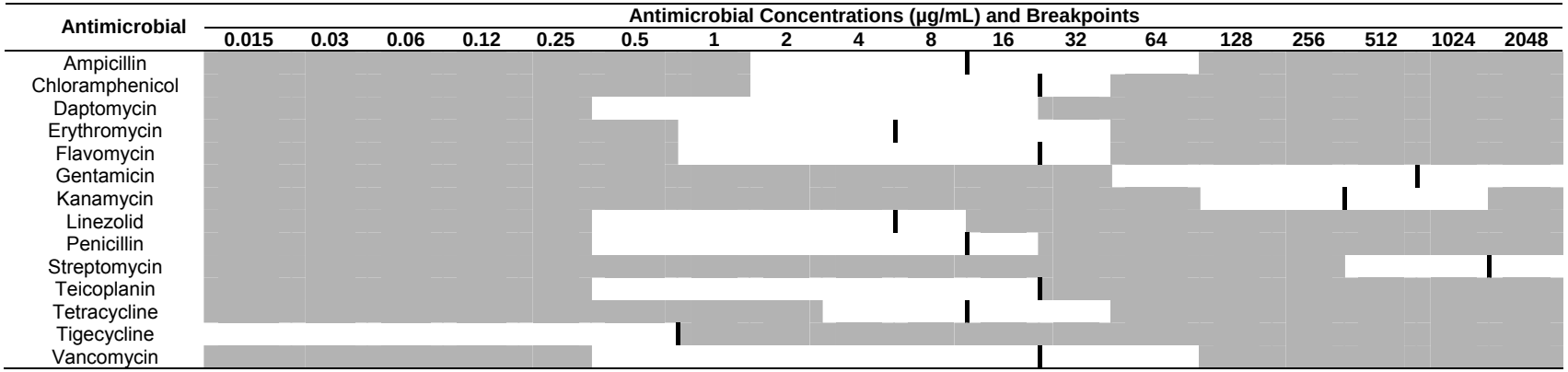
Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for specific antimicrobials.

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



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*.



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

Results

Twelve month prevalence review

Sampling in each of the four capital city areas progressed as planned. Figure 1 shows the actual and anticipated prevalences for each food / bacterium combination at the conclusion of the 12th monthly sampling round. *E. coli* in pork and *E. coli* in lettuce prevalences remained below the anticipated prevalences. Recommended changes to the number of tests conducted for *Campylobacter* in poultry and *E. coli* in pork proposed by FSA as part of the 3 Month Prevalence Report and Monthly Progress Reports were implemented in order to achieve the 100 isolate goal for *Campylobacter* in poultry and *E. coli* in pork. Sampling of lettuce for *E. coli* was reduced as this food / bacterium combination continued to track well below the originally anticipated prevalence of 10% and was not expected to achieve the 100 isolates per food / bacterium combination goal.

At the conclusion of sampling, 7 of the 9 food / bacterium combinations exceeded the 100 isolate goal of the survey using the modified sampling plan. Since the 100 isolate goal was exceeded, the following approaches were used to determine a sub-population on which to conduct AMR testing.

- *Enterococcus* – all *Enterococcus* were tested by PCR to determine if they were *E. faecalis* or *E. faecium*. No *E. faecium* were identified from any food / bacterium combination. Consequently, a subset of 100 *E. faecalis* isolates was randomly selected.
- *Salmonella, E. coli* and *Campylobacter* – randomly selected subsets were designated for all food / bacterium combinations exceeding the 100 isolate goal. All available pork / *E. coli* and lettuce / *E. coli* isolates were tested for AMR.

Bacterial isolates

Details of each bacterial isolate from the survey are provided in the supplementary document **'Supplement 1 – Food AMR Pilot Survey – Bacterial Isolates'**.

Salmonella serotyping

At the time of report preparation, serovar data for 96 of 174 *Salmonella* isolated during the survey have been provided by the project subcontractor. Serovar data is included in the document **'Supplement 1 – Food AMR Pilot Survey – Bacterial Isolates'**. Of those isolates serotyped to date, *S.* Sofia (41%) and *S*. Typhimurium (32%) were the most prevalent serovars. *S.* Montevideo (11%) and *S*. Kiambu (5.2%) were the only other serovars identified at greater than 5% prevalence. Serovars identified with prevalences ≤ 5% include Agona, Infantis, Mbandaka, Muenster, Ohio, Singapore, Tennessee, Sal subsp 1 ser rough:i:1,2 and Sal subsp II ser 4,5,12,27. Among 100 *Salmonella* isolates for which AMR was determined, serovar data is available for 60 isolates. Within this group of 60 isolates, the prevalence of major serovars was *S*. Sofia (38%), *S*. Typhimurium (40%) and *S.* Montevideo (8%).

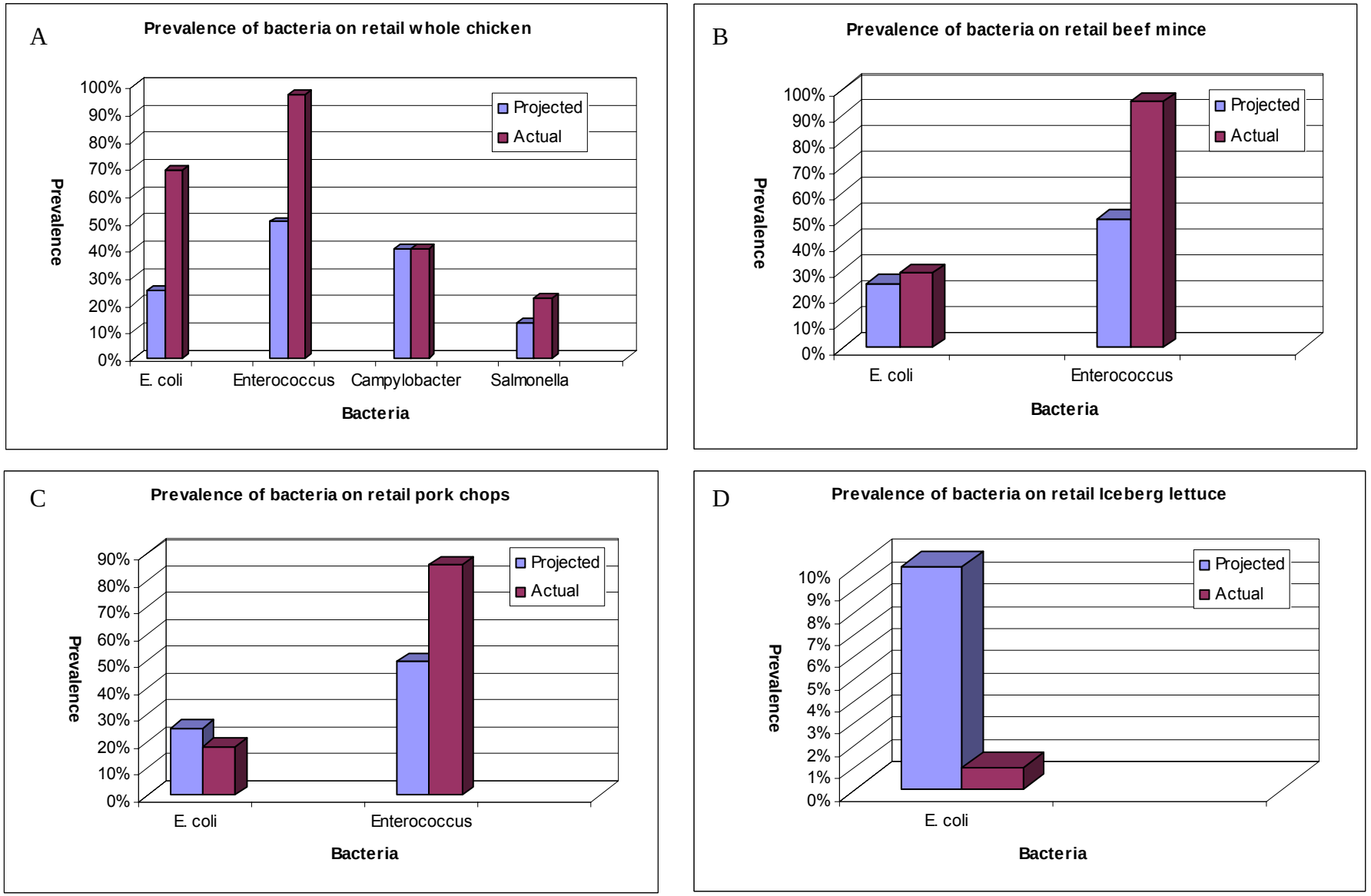


Figure 1. Projected and actual prevalences of bacteria in retail foods (12 month period February 2007 to January 2008 sampling). The initially projected and actual prevalences of bacteria in particular retail foods are shown in for whole chicken (panel A), minced beef (panel B), pork chop (panel C) and Iceberg lettuce (panel D).

Antimicrobial susceptibility testing

Retail poultry – *Salmonella*

A total of 174 *Salmonella* isolates were isolated during the 12 month sampling period. The overall prevalence of *Salmonella* in retail poultry was 21.9% and ranged during monthly sampling from 10.4% to 31.3%. One hundred *Salmonella* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *Salmonella* is presented in Figure 2. The distribution of Minimum Inhibitory Concentrations (MICs) and resistance in *Salmonella* is presented in Table 4. Resistance to one or more antimicrobials was observed in 23% of isolates. Resistance to tetracycline (16%) was most commonly observed. Resistance to amoxicillin / clavulanic acid, ampicillin, cefoxitin, florfenicol, nalidixic acid, streptomycin, and trimethoprim / sulfamethoxazole were observed in no more than five of the 100 isolates tested. Resistance to the remaining antimicrobials tested was not observed.

AMR patterns: A total of 11 AMR patterns were identified amongst the isolates tested (Table 5). The most common patterns observed was resistance to tetracycline alone (12 isolates) and trimethoprim / sulfamethoxazole alone (2 isolates). The remaining 9 patterns were present only in single isolates.

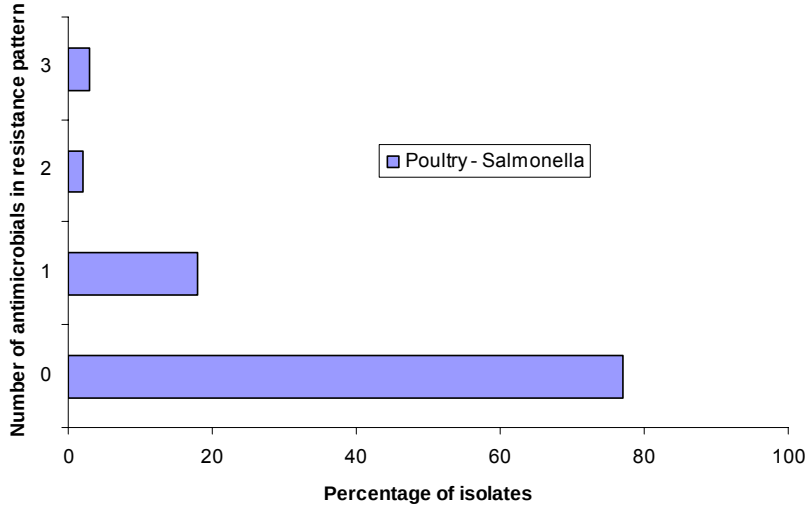


Figure 2. Multiple drug resistance in *Salmonella* from retail poultry samples (n=100)

Table 4. Distribution of MICs and resistance in *Salmonella* from retail poultry.

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs										
					0.125	0.25	0.5	1	2	4	8	16	32	64	128
Amoxicillin / Clavulanic acid [a]	Poultry	100	1.0	[0.03 – 5.45]				47.0	38.0	8.0	6.0		1.0		
Ampicillin	Poultry	100	4.0	[1.10 – 9.93]					86.0	7.0	2.0	1.0	1.0		3.0
Cefazolin	Poultry	100	0.0	[0.00 – 3.62]							96.0	4.0			
Cefotaxime	Poultry	100	0.0	[0.00 – 3.62]		98.0		1.0	1.0						
Cefoxitin	Poultry	100	1.0	[0.03 – 5.45]				1.0	14.0	38.0	42.0	4.0	1.0		
Ceftiofur	Poultry	100	0.0	[0.00 – 3.62]			87.0	13.0							
Ceftriaxone	Poultry	100	0.0	[0.00 – 3.62]		98.0				1.0	1.0				
Chloramphenicol	Poultry	100	0.0	[0.00 – 3.62]					3.0	8.0	89.0				
Ciprofloxacin	Poultry	100	0.0	[0.00 – 3.62]	98.0	1.0		1.0							
Florfenicol	Poultry	100	1.0	[0.03 – 5.45]					1.0	69.0	29.0	1.0			
Gentamicin	Poultry	100	0.0	[0.00 – 3.62]				95.0	4.0		1.0				
Meropenem	Poultry	100	0.0	[0.00 – 3.62]				99.0	1.0						
Nalidixic Acid	Poultry	100	1.0	[0.03 – 5.45]						81.0	18.0			1.0	
Streptomycin	Poultry	100	5.0	[1.64 – 11.28]									95.0	4.0	1.0
Tetracycline	Poultry	100	16.0	[9.43 – 24.68]						84.0		2.0		14.0	
Trimethoprim / Sulfamethoxazole	Poultry	100	3.0	[0.06 – 8.52]	94.0	2.0	1.0			3.0					

Vertical lines indicate breakpoints for resistance.
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.
[a] Concentration of amoxicillin is given, tested with clavulanic acid in concentration 2:1.

Table 5. Multiple antimicrobial resistance phenotypes present in *Salmonella* from retail poultry.

Pattern	Resistance phenotype*	Percentage
0	No pattern	77
1	tet	12
1	sxt	2
1	ffn	1
1	amp	1
1	str	1
1	fox	1
2	aug amp	1
2	str tet	1
3	nal str tet	1
3	str tet sxt	1
3	amp str tet	1
TOTAL		**100**

* Amoxicillin / Clavulanic acid, aug; Ampicillin, amp; Cefazolin, faz; Cefotaxime, fot; Cefoxitin, fox; Ceftiofur, xnl; Ceftriaxone, axo; Chloramphenicol, Ciprofloxacin, cip; Florfenicol, ffn; Gentamicin, gen; Meropenem, mer; Nalidixic Acid, nal; Streptomycin, str; Tetracycline, tet; Trimethoprim / Sulfamethoxazole, sxt.

Retail poultry – *E. coli*

A total of 290 *E. coli* were isolated during the 12 month sampling period. The overall prevalence of *E. coli* in retail poultry was 69.0% and ranged during monthly sampling from 51.4% to 80.0%. One hundred *E. coli* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *E. coli* is presented in Figure 3. The distribution of MICs and resistance in *E. coli* is presented in Table 18. Resistance to one or more antimicrobials was observed in 65% of isolates. Resistance to tetracycline (47%), ampicillin (38%), trimethoprim / sulfamethoxazole (22%) and streptomycin (19%) were most commonly observed. Resistance to kanamycin and gentamicin was observed in 8% and 4% of isolates respectively. Resistance to amoxicillin / clavulanic acid, cefazolin, florfenicol and chloramphenicol was observed in two or less isolates.

AMR patterns: A total of 21 AMR resistance patterns were identified (Table 6). Twenty-two percent of the isolates tested were resistant to three or more antimicrobials and account for 10 of the 21 patterns identified. The most commonly observed patterns were tetracycline alone (14%), ampicillin-tetracycline (11%), ampicillin alone (6%) and ampicillin-tetracycline- trimethoprim / sulfamethoxazole (5%). Eight of the 21 patterns observed were present only in single isolates.

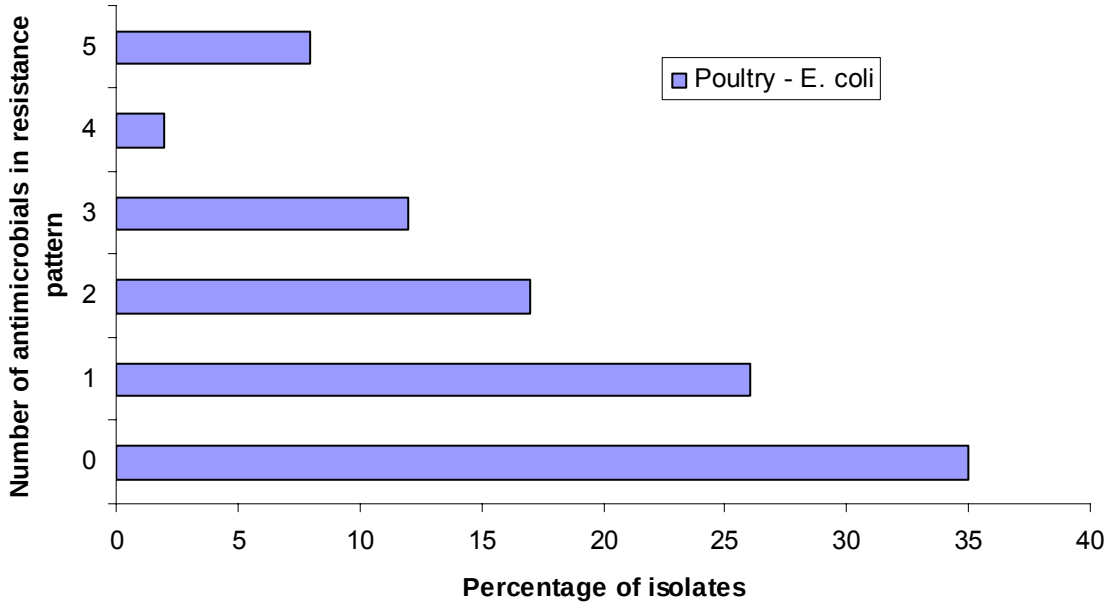


Figure 3. Multiple drug resistance in *E. coli* from retail poultry samples (n=100)

Table 6. Multiple antimicrobial resistance phenotypes present in *E. coli* from retail poultry.

Pattern	Resistance phenotype*	Percentage
0	No pattern	35
1	tet	14
1	amp	6
1	ffn	2
1	sxt	2
1	str	2
2	amp tet	11
2	amp str	2
2	aug faz	1
2	amp sxt	1
2	kan tet	1
2	str tet	1
3	amp tet sxt	5
3	kan tet sxt	2
3	amp str sxt	2
3	kan str tet	1
3	amp str tet	1
3	amp faz tet	1
4	amp str tet sxt	2
5	amp kan str tet sxt	3
5	amp gen str tet sxt	4
6	chl ffn kan str tet sxt	1

* Amoxicillin / Clavulanic acid, aug; Ampicillin, amp; Cefazolin, faz; Cefotaxime, fot; Cefoxitin, fox; Ceftiofur, xnl; Ceftriaxone, axo; Chloramphenicol, Ciprofloxacin, cip; Florfenicol, ffn; Gentamicin, gen; Meropenem, mer; Nalidixic Acid, nal; Streptomycin, str; Tetracycline, tet; Trimethoprim / Sulfamethoxazole, sxt.

Retail poultry – *Enterococcus*

A total of 199 *Enterococcus* were isolated during the 12 month sampling period. The overall prevalence of *Enterococcus* in retail poultry was 96.6% and ranged during monthly sampling from 88.2% to 100.0%. Screening of *Enterococcus* isolates by PCR determined that 92.0% of isolates were *E. faecalis*. *E. faecium* was not identified using PCR. One hundred *E. faecalis* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *Enterococcus* is presented in Figure 4. The distribution of MICs and resistance in *Enterococcus* is presented in Table 15. Resistance to one or more antimicrobials was observed in 81% of isolates. Resistance to tetracycline (76%) and erythromycin (48%) were observed most often. Resistance to clinically significant antimicrobials such as linezolid, gentamicin and vancomycin was not observed.

AMR patterns: A total of 15 AMR patterns were identified (Table 7). Fifty-two percent of the isolates tested were resistant to two or more antimicrobials and account for 11 of the 15 patterns identified. The most commonly observed patterns were tetracycline alone (24%) and erythromycin-tetracycline (36%). Seven of the 15 patterns observed were present only in single isolates.

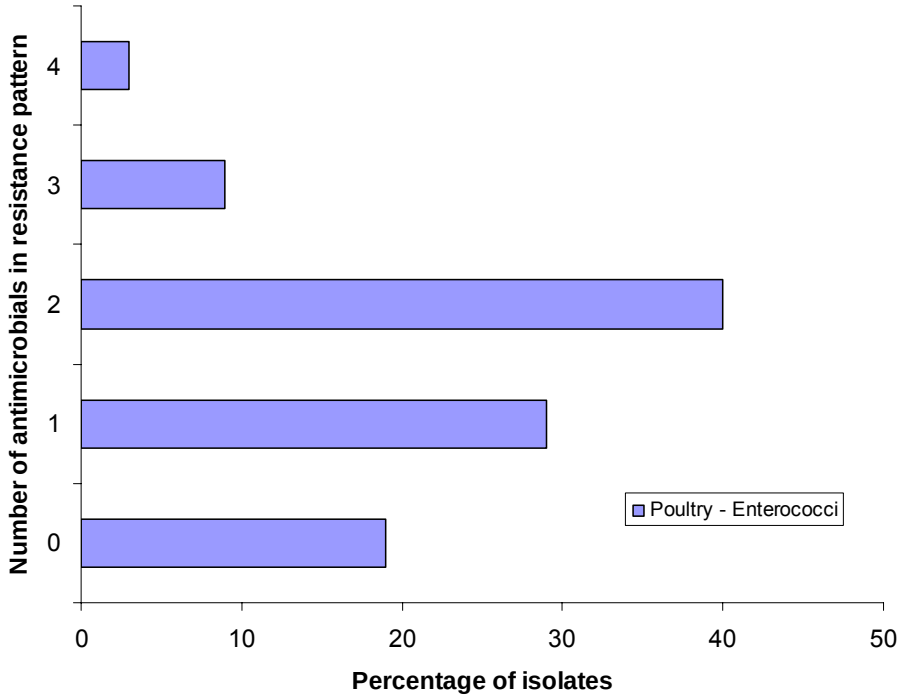


Figure 4. Multiple drug resistance in *Enterococcus faecalis* from retail poultry samples (n=100)

Table 7. Multiple antimicrobial resistance phenotypes present in *Enterococcus faecalis* from retail poultry.

Pattern	Resistance phenotype*	Percentage
0	No pattern	19
1	tgc	2
1	tet	24
1	str	1
1	ery	2
2	tet tgc	2
2	ery tet	36
2	flv tet	2
3	kan str tet	1
3	ery kan tet	4
3	ery tet tgc	2
3	flv kan tet	1
3	ery str tet	1
4	ery kan str tet	2
4	chl ery kan tet	1

* Ampicillin, amp; Chloramphenicol, chl; Daptomycin, dap; Erythromycin, ery; Flavomycin, flv; Gentamicin, gen; Kanamycin, kan; Linezolid, lzd; Penicillin, pen; Streptomycin, str; Teicoplanin, tei; Tetracycline, tet; Tigecycline, tgc; Vancomycin, van.

Retail poultry – *Campylobacter* spp.

A total of 175 *Campylobacter* isolates were isolated during the 12 month sampling period. The overall prevalence of *Campylobacter* in retail poultry was 40.0% and ranged during monthly sampling from 13.6% – 65.2%. One hundred *Campylobacter* isolates were randomly selected for AMR testing and speciation. Screening by PCR of *Campylobacter* isolates selected for AMR testing determined that 60% of isolates were *C. jejuni* with the remaining 40% of isolates identified as *C. coli.*

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *Campylobacter coli and Campylobacter jejuni* is presented in Figure 5 and Figure 6 respectively. The distribution of MICs and resistance in *Campylobacter coli and Campylobacter jejuni* are presented in Table 8.and Table 9 respectively. The overall level of antimicrobial resistance was very low. AMR was observed in two isolates of *Campylobacter coli and* three isolates of *Campylobacter jejuni*. Resistance to clindamycin (*C. coli,* 5%; *C. jejuni,* 1.7%), erythromycin (*C. coli,* 5%; *C. jejuni,* 3.3%), telithromycin (*C. coli,* 2.5%; *C. jejuni,* 3.3%) and tetracycline (*C. jejuni,* 1.7%) were observed. No resistance to ciprofloxacin, florfenicol, gentamicin or nalidixic acid was observed.

AMR patterns: A limited number of AMR patterns were identified (Table 10 and Table 11). The observed patterns were tetracycline alone (*C. jejuni,* 1.7%), erythromycin-telithromycin (*C. jejuni,* 1.7%), clindamycin-erythromycin (*C. coli,* 2.5%) and clindamycin-erythromycin-telithromycin (*C. coli,* 2.5%; *C. jejuni,* 1.7%).

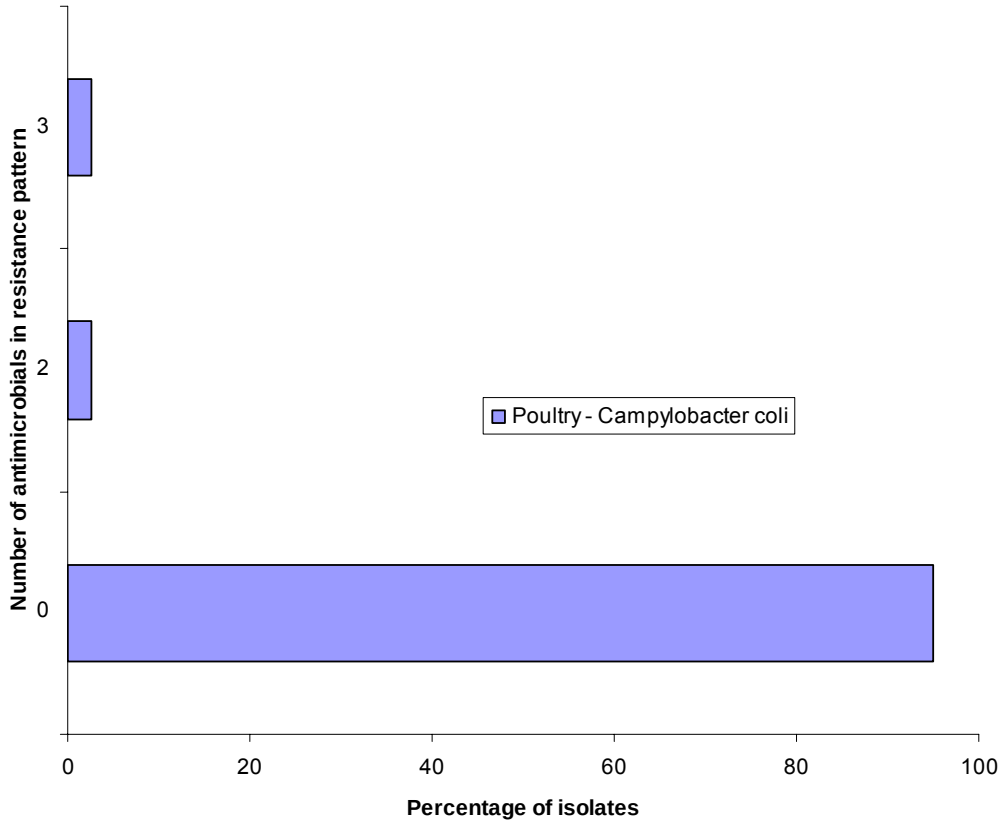


Figure 5. Multiple drug resistance in *Campylobacter coli* from retail poultry samples (n=40).

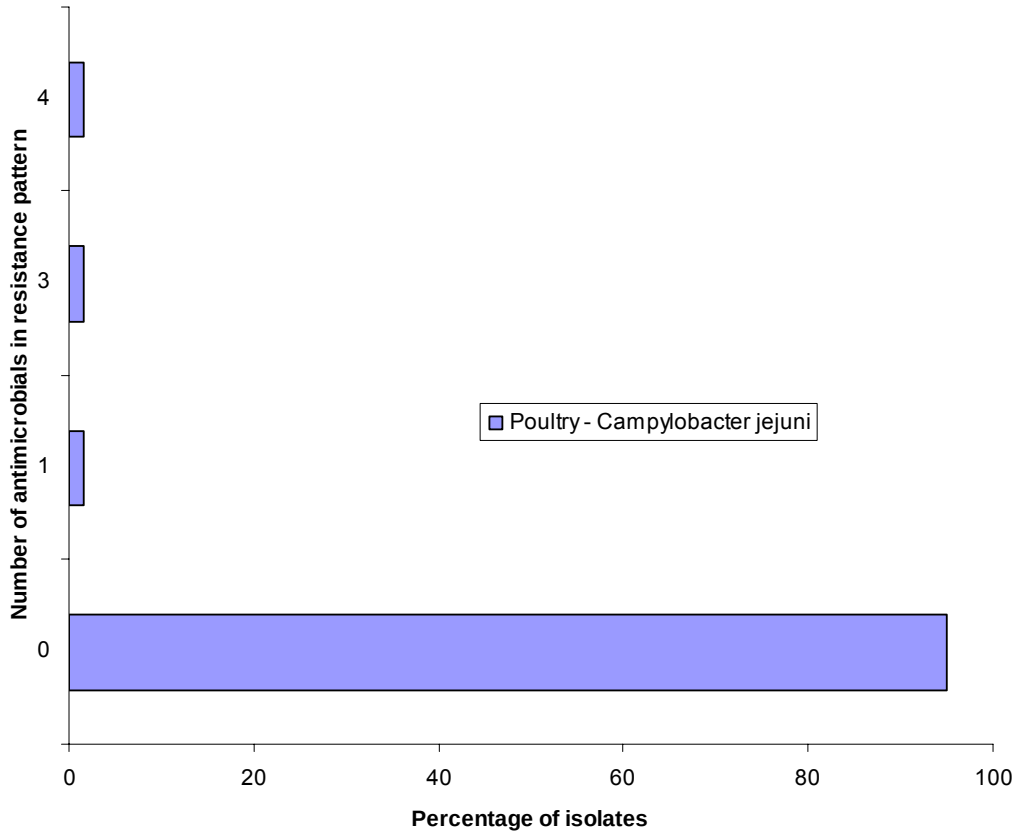


Figure 6. Multiple drug resistance in *Campylobacter jejuni* from retail poultry samples (n=60)

Table 8. Distribution of MICs and resistance in *Campylobacter coli* from retail poultry

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs												
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Ciprofloxacin	Poultry	40	0.0	[0.00 – 8.81]		7.5	37.5	22.5	32.5									
Clindamycin	Poultry	40	5.0	[0.61 – 16.92]		7.5	15.0	12.5	30	25.0	5.0		2.5			2.5		
Erythromycin	Poultry	40	5.0	[0.61 – 16.92]			5.0	17.5	20.0	22.5	15.0	15.0						5.0
Florfenicol	Poultry	40	0.0	[0.00 – 8.81]				2.5	12.5	10.0	45.0	27.5	2.5					
Gentamicin	Poultry	40	0.0	[0.00 – 8.81]				12.5	20.0	55.0	10.0	2.5						
Nalidixic Acid	Poultry	40	0.0	[0.00 – 8.81]									52.5	45.0	2.5			
Telithromycin	Poultry	40	2.5	[0.06 – 13.16]	2.5	2.5	5.0	10.0	25.0	17.5	15.0	10.0	10.0		2.5			
Tetracycline	Poultry	40	0.0	[0.00 – 8.81]			2.5	15.0	40.0	22.5	15.0	5.0						

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

Table 9. Distribution of MICs and resistance in *Campylobacter jejuni* from retail poultry

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs												
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
Ciprofloxacin	Poultry	60	0.0	[0.00 – 5.96]	3.3	18.3	45.0	26.7	6.7									
Clindamycin	Poultry	60	1.7	[0.04 – 8.94]		11.7	21.7	36.7	13.3	10.0	1.7	3.3	1.7					
Erythromycin	Poultry	60	3.3	[0.41 – 11.53]		5.0	13.3	33.3	31.7	8.3	5.0							3.3
Florfenicol	Poultry	60	0.0	[0.00 – 5.96]				5.0	6.7	38.3	40.0	10.0						
Gentamicin	Poultry	60	0.0	[0.00 – 5.96]				50.0	28.3	20.0	1.7							
Nalidixic Acid	Poultry	60	0.0	[0.00 – 5.96]									70.0	30.0				
Telithromycin	Poultry	60	3.3	[0.41 – 11.53]			16.7	11.7	40.0	23.3	3.3	1.7			3.3			
Tetracycline	Poultry	60	1.7	[0.04 – 8.94]			1.7	21.7	36.7	21.7	13.3	1.7		1.7	1.7			

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration

Table 10. Multiple antimicrobial resistance phenotypes present in *Campylobacter coli* from retail poultry.

Pattern	Resistance phenotype*	Percentage
0	No pattern	95.0
2	cli ery	2.5
3	cli ery tel	2.5

* Clindamycin, cli; Erythromycin, ery; Telithromycin, tel.

Table 11. Multiple antimicrobial resistance phenotypes present in *Campylobacter jejuni* from retail poultry.

Pattern	Resistance phenotype*	Percentage
0	No pattern	95.0
1	tet	1.7
2	ery tel	1.7
3	cli ery tel	1.7

* Clindamycin, cli; Erythromycin, ery; Telithromycin, tel; Tetracycline, tet.

Retail beef – *E. coli*

A total of 121 *E. coli* were isolated during the 12 month sampling period. The overall prevalence of *E. coli* in retail beef was 29.7% and ranged during monthly sampling from 13.9% – 36.4%. One hundred *E. coli* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *E. coli* is presented in Figure 7. The distribution of MICs and resistance in *E. coli* is presented in Table 18. Resistance to one or more antimicrobials was observed in 19% of isolates. Resistance to ampicillin (11%), streptomycin (7%) and tetracycline (7%) were most often observed. Resistance to amoxicillin / clavulanic acid (3%), cefazolin (3%), kanamycin (2%), and trimethoprim / sulfamethoxazole (5%) were also observed.

AMR patterns: A total of 13 AMR patterns were identified (Table 12). Resistance to ampicillin alone was the most commonly observed AMR pattern (5%) and only 9% of isolates were resistant to more than one antimicrobial. Resistance to streptomycin alone (2%) and ampicillin--streptomycin-tetracycline-trimethoprim / sulfamethoxazole (2%) were the only other AMR patterns found in multiple isolates. The largest multiple AMR pattern identified was ampicillin-kanamycin-streptomycin-tetracycline-trimethoprim / sulfamethoxazole which was present in a single isolate.

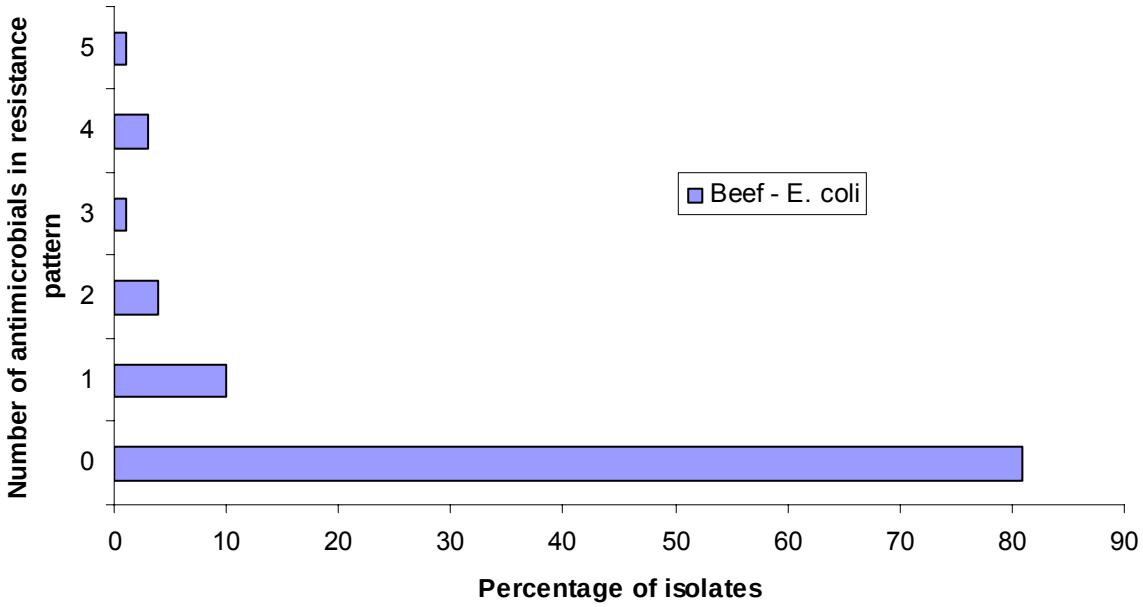


Figure 7. Multiple drug resistance in *E. coli* from retail beef samples (n=100)

Table 12. Multiple antimicrobial resistance phenotypes present in *E. coli* from retail beef.

Pattern	Resistance phenotype*	Percentage
0	No pattern	81
1	amp	5
1	str	2
1	faz	1
1	aug	1
1	tet	1
2	amp tet	1
2	aug faz	1
2	amp sxt	1
2	str tet	1
3	aug amp faz	1
4	kan str tet sxt	1
4	amp str tet sxt	2
5	amp kan str tet sxt	1

* Amoxicillin / Clavulanic acid, aug; Ampicillin, amp; Cefazolin, faz; Cefotaxime, fot; Cefoxitin, fox; Ceftiofur, xnl; Ceftriaxone, axo; Chloramphenicol, Ciprofloxacin, cip; Florfenicol, ffn; Gentamicin, gen; Meropenem, mer; Nalidixic Acid, nal; Streptomycin, str; Tetracycline, tet; Trimethoprim / Sulfamethoxazole, sxt;

Retail beef – *Enterococcus*

A total of 198 *Enterococcus* were isolated during the 12 month sampling period. The overall prevalence of *Enterococcus* in retail beef was 95.7% and ranged during monthly sampling from 85.0% to 100.0%. Screening of *Enterococcus* isolates by PCR determined that 87.9% of isolates were *E. faecalis*. *E. faecium* was not identified using PCR. One hundred *E. faecalis* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *Enterococcus* is presented in Figure 8. The distribution of MICs and resistance in *Enterococcus* is presented in Table 15. Resistance to one or more antimicrobials was observed in 27% of isolates. Resistance to the antimicrobials tetracycline (15%) and tigecycline (10%) was observed. Isolates with resistance to chloramphenicol, erythromycin, flavomycin, kanamycin and streptomycin were observed with a prevalence ≤ 7%. Resistance to the clinically significant antimicrobials linezolid and vancomycin was not observed; however, gentamicin resistance (1%) was observed in a single isolate.

AMR patterns: A total of 10 AMR patterns were identified (Table 13). Resistance to 2 or more antimicrobials was observed in 6% of isolates. The most commonly observed patterns were tetracycline alone (9%) and tigecycline alone (7%).. The largest AMR patterns observed were resistance to chloramphenicol-erythromycin-kanamycin - streptomycin-tetracycline (5 antimicrobials; 1 isolate; 1%) and chloramphenicol-erythromycin-gentamicin-kanamycin-streptomycin-tetracycline-tigecycline (7 antimicrobials; 1 isolate; 1%).

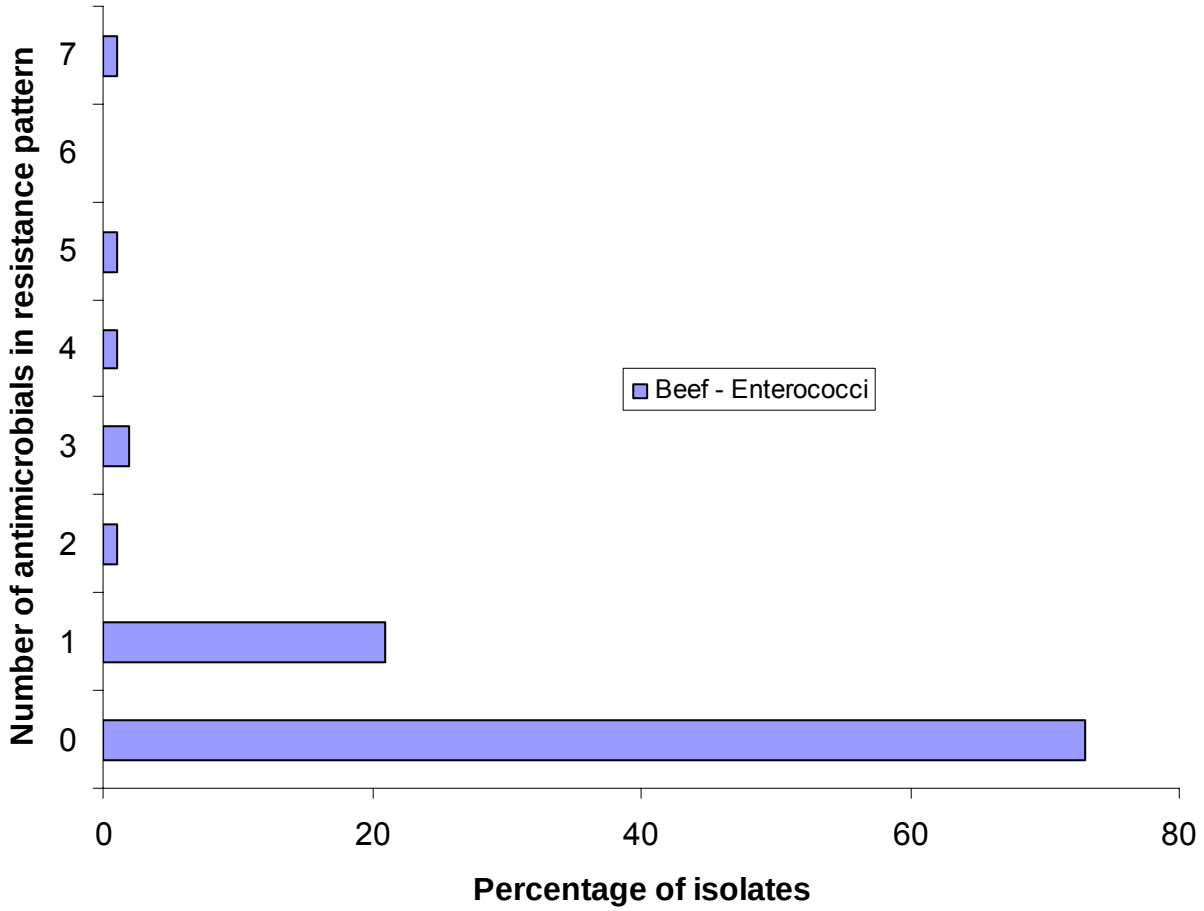


Figure 8. Multiple drug resistance in *Enterococcus faecalis* from retail beef samples (n=100)

Table 13. Multiple antimicrobial resistance phenotypes present in *Enterococcus faecalis* from retail beef.

Pattern	Resistance phenotype*	Percentage
0	No pattern	73
1	ery	2
1	flv	3
1	tet	9
1	tgc	7
2	flv tet	1
3	chl ery tet	1
3	ery tet tgc	1
4	flav kan str tet	1
5	chl ery kan str tet	1
7	chl ery gen kan str tet tgc	1

* Ampicillin, amp; Chloramphenicol, chl; Daptomycin, dap; Erythromycin, ery; Flavomycin, flv; Gentamicin, gen; Kanamycin, kan; Linezolid, lzd; Penicillin, pen; Streptomycin, str; Teicoplanin, tei; Tetracycline, tet; Tigecycline, tgc; Vancomycin, van.

Retail pork – *E. coli*

A total of 92 *E. coli* were isolated during the 12 month sampling period. The overall prevalence of *E. coli* in retail pork was 18.1% and ranged during monthly sampling from 5.9% to 26.5%. The 92 *E. coli* isolates were tested for AMR. The reduction in pork / *E. coli* isolates available for AMR testing correspondingly results in a minor decrease from 95% to approximately 93.5% probability of detecting 1 AMR isolate in 92 if AMR prevalence nominally occurs at 3% prevalence (see FRSC communication note Appendix C).

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *E. coli* is presented in Figure 9. The distribution of MICs and resistance in *E. coli* is presented in Table 18. Resistance to one or more antimicrobials was observed in 80.4% of isolates. Resistance to tetracycline (44.5%), ampicillin (28.2%), streptomycin (17.4%), chloramphenicol (13%) and trimethoprim / sulfamethoxazole (13%) were most often observed. Resistance to florfenicol (8.7%), amoxicillin / clavulanic acid (3.3%), cefazolin (3.3%), kanamycin (3.3%) and gentamicin (1.1%) were also observed.

AMR patterns: A total of 24 AMR patterns were identified (Table 14). Resistance to tetracycline alone was the most commonly observed AMR pattern (13%). Twenty-two percent of isolates were resistant to 3 or more antimicrobials and comprised 14 of the 24 AMR patterns identified. Five of the 14 patterns were found in multiple isolates. The largest AMR patterns identified included resistance to ampicillin-streptomycin-tetracycline-trimethoprim / sulfamethoxazole in conjunction with combinations of chloramphenicol, florfenicol and kanamycin resistance.

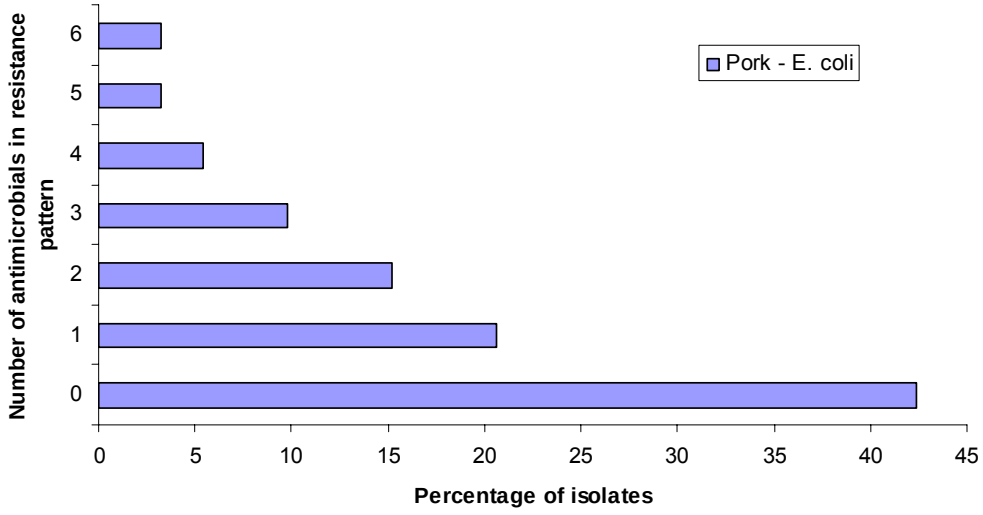


Figure 9. Multiple drug resistance in *E. coli* from retail pork samples (n=92)

Table 14. Multiple antimicrobial resistance phenotypes present in *E. coli* from retail pork.

Pattern	Resistance phenotype*	Percentage
0	No pattern	42
1	tet	13
1	amp	4
1	str	2
1	ffn	1
2	amp tet	8
2	str tet	3
2	gen str	1
2	aug faz	1
2	tet sxt	1
2	chl tet	1
3	amp chl tet	3
3	aug amp faz	2
3	amp str tet	2
3	amp chl sxt	1
3	amp kan tet	1
4	chl str tet sxt	2
4	amp str tet sxt	1
4	amp chl ffn tet	1
4	chl ffn tet sxt	1
5	chl ffn str tet sxt	2
5	amp chl ffn tet sxt	1
6	amp ffn kan str tet sxt	1
6	amp chl ffn str tet sxt	1
6	amp chl kan str tet sxt	1

* Amoxicillin / Clavulanic acid, aug; Ampicillin, amp; Cefazolin, faz; Cefotaxime, fot; Cefoxitin, fox; Ceftiofur, xnl; Ceftriaxone, axo; Chloramphenicol, Ciprofloxacin, cip; Florfenicol, ffn; Gentamicin, gen; Meropenem, mer; Nalidixic Acid, nal; Streptomycin, str; Tetracycline, tet; Trimethoprim / Sulfamethoxazole, sxt.

Retail pork – *Enterococcus*

A total of 178 *Enterococcus* were isolated during the 12 month sampling period. The overall prevalence of *Enterococcus* in retail pork was 86.0% and ranged during monthly sampling from 70.6% to 94.7%. Screening of *Enterococcus* isolates by PCR determined that 83.1% of isolates were *E. faecalis*. *E. faecium* was not identified using PCR. One hundred *E. faecalis* isolates were randomly selected for AMR testing.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *Enterococcus* is presented in Figure 10. The distribution of MICs and resistance in *Enterococcus* is presented in Table 15. Resistance to one or more antimicrobials was observed in 22% of isolates. Resistance to tetracycline (17%) was observed most often. Isolates with resistance to chloramphenicol, erythromycin, flavomycin, kanamycin, streptomycin and tigecycline were observed with a prevalence ≤ 7%. Resistance to the clinically significant antimicrobials gentamicin, linezolid and vancomycin was not observed.

AMR patterns: A total of 11 AMR patterns were identified (Table 16). Resistance to 2 or more antimicrobials was observed in 11% of isolates. The largest AMR patterns observed were resistance to chloramphenicol-erythromycin-kanamycin -streptomycin-tetracycline (5 antimicrobials; 2 isolates; 2%) and erythromycin-flavomycin-kanamycin- streptomycin-tetracycline (5 antimicrobials; 1 isolate; 1%).

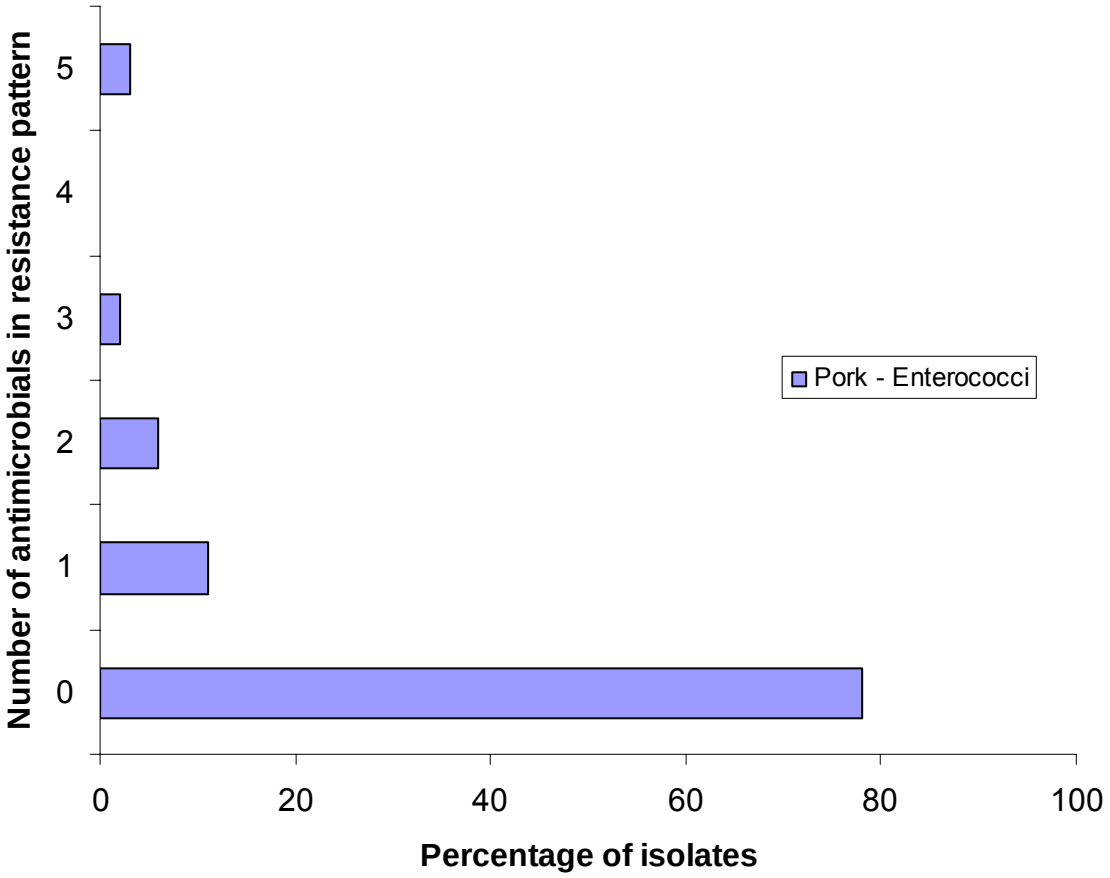


Figure 10. Multiple drug resistance in *Enterococcus faecalis* from retail pork samples (n=100)

Table 15. Distribution of MICs and resistance in *Enterococcus faecalis* from retail poultry, beef, and pork.

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs																
					0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Ampicillin	Poultry	100	0.0	[0.00 – 3.62]								98.0	2.0										
	Beef	100	0.0	[0.00 – 3.62]								96.0	4.0										
	Pork	100	0.0	[0.00 – 3.62]								98.0	2.0										
Chloramphenicol	Poultry	100	1.0	[0.03 – 5.45]								6.0	1.0	74.0	18.0	1.0							
	Beef	100	3.0	[0.62 – 8.52]								9.0	15.0	66.0	7.0		3.0						
	Pork	100	2.0	[0.24 – 7.04]								4.0	4.0	86.0	4.0	1.0	1.0						
Daptomycin	Poultry	100	0.0	[0.00 – 3.62]						16.0	39.0	42.0	3.0										
	Beef	100	0.0	[0.00 – 3.62]						39.0	32.0	22.0	7.0										
	Pork	100	0.0	[0.00 – 3.62]						34.0	26.0	36.0	4.0										
Erythromycin	Poultry	100	48.0	[37.90 – 58.22]							45.0	7.0		2.0	3.0		43.0						
	Beef	100	6.0	[2.23 – 12.60]							85.0	7.0	2.0	1.0			5.0						
	Pork	100	7.0	[2.86 – 13.89]							84.0	8.0	1.0				7.0						
Flavomycin	Poultry	100	3.0	[0.62 – 8.52]							12.0	53.0	29.0	3.0			3.0						
	Beef	100	7.0	[2.86 – 13.89]							12.0	39.0	28.0	12.0	2.0	2.0	5.0						
	Pork	100	7.0	[2.86 – 13.89]							84.0	8.0	1.0				7.0						
Gentamicin	Poultry	100	0.0	[0.00 – 3.62]													100.0						
	Beef	100	1.0	[0.03 – 5.45]													99.0				1.0		
	Pork	100	0.0	[0.00 – 3.62]													99.0		1.0				
Kanamycin	Poultry	100	9.0	[4.20 – 16.40]														88.0	3.0	1.0	1.0	7.0	
	Beef	100	3.0	[0.62 – 8.52]														97.0				3.0	
	Pork	100	4.0	[1.10 – 9.93]														96.0				4.0	
Linezolid	Poultry	100	0.0	[0.00 – 3.62]						4.0	7.0	89.0											
	Beef	100	0.0	[0.00 – 3.62]						10.0	2.0	87.0	1.0										
	Pork	100	0.0	[0.00 – 3.62]						5.0	7.0	87.0	1.0										
Penicillin	Poultry	100	0.0	[0.00 – 3.62]						9.0	20.0	35.0	36.0										
	Beef	100	0.0	[0.00 – 3.62]						30.0	17.0	21.0	32.0										
	Pork	100	0.0	[0.00 – 3.62]						16.0	23.0	22.0	36.0	3.0									
Streptomycin	Poultry	100	5.0	[1.64 – 11.28]																95.0		1.0	4.0
	Beef	100	3.0	[0.62 – 8.52]																97.0		2.0	1.0
	Pork	100	5.0	[1.64 – 11.28]																95.0		2.0	3.0
Teicoplanin	Poultry	100	0.0	[0.00 – 3.62]						99.0	1.0												
	Beef	100	0.0	[0.00 – 3.62]						100.0													
	Pork	100	0.0	[0.00 – 3.62]						99.0	1.0												
Tetracycline	Poultry	100	76.0	[66.43 – 83.98]									23.0	1.0	1.0	3.0	72.0						
	Beef	100	15.0	[8.65 – 23.53]									82.0	3.0	2.0		13.0						
	Pork	100	17.0	[10.23 – 25.82]									81.0	2.0	4.0	2.0	11.0						
Tigecycline	Poultry	100	6.0	[2.23 – 12.60]		3.0	5.0	12.0	34.0	40.0	6.0												
	Beef	100	10.0	[4.90 – 17.62]	2.0		9.0	22.0	40.0	17.0	10.0												
	Pork	100	3.0	[0.62 – 8.52]	2.0	4.0	7.0	22.0	34.0	28.0	3.0												
Vancomycin[1]	Poultry	100	0.0	[0.00 – 3.62]						4.0	10.0	60.0	26.0										
	Beef	100	0.0	[0.00 – 3.62]						3.0	36.0	40.0	21.0										
	Pork	100	0.0	[0.00 – 3.62]						1.0	26.0	45.0	28.0										

Vertical lines indicate breakpoints for resistance.
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration.
[1] Five vancomycin resistant *E. faecalis* isolates from each retail meat source (N=15) were randomly chosen and tested for the presence of *vanA* and *vanB* genes using Polymerase Chain Amplification. All 15 vancomycin resistant *E. faecalis* isolates were negative for both *vanA* and *vanB* (Bradbury and Collignon, pers. comm.).

Table 16. Multiple antimicrobial resistance phenotypes present in *Enterococcus faecalis* from retail pork.

Pattern	Resistance phenotype*	Percentage
0	No pattern	78
1	flv	2
1	tet	6
1	tgc	2
1	flv	1
2	flv tet	1
2	str tet	2
2	ery tet	3
3	flv tet tgc	1
3	ery kan tet	1
5	ery flv kan str tet	1
5	chl ery kan str tet	2

* Ampicillin, amp; Chloramphenicol, chl; Daptomycin, dap; Erythromycin, ery; Flavomycin, flv; Gentamicin, gen; Kanamycin, kan; Linezolid, lzd; Penicillin, pen; Streptomycin, str; Teicoplanin, tei; Tetracycline, tet; Tigecycline, tgc; Vancomycin, van.

Retail lettuce – *E. coli*

A total of seven *E. coli* were isolated during the 12 month sampling period. The overall prevalence of *E. coli* in retail lettuce was 1.0% and ranged from during monthly sampling 0.0% to 2.5%. The seven *E. coli* isolates were tested for AMR.

Antimicrobial drug resistance: The prevalence of multiple drug resistance in *E. coli* is presented in Figure 11. The distribution of MICs and resistance in *E. coli* is presented in Table 18. Resistance to one or more antimicrobials was observed in 5 of 7 isolates (71%). Resistance to ampicillin (57.1%) was observed most often. Resistance to amoxicillin / clavulanic acid (28.6%), cefazolin (28.6%), streptomycin (14.3%), tetracycline (28.6%) and trimethoprim / sulfamethoxazole (14.3%) was also identified.

AMR patterns: A total of 4 AMR patterns were identified in 5 isolates (Table 17). Resistance to ampicillin alone was identified in two isolates. The largest AMR patterns identified were resistance to ampicillin-streptomycin-tetracycline-trimethoprim / sulfamethoxazole (4 antimicrobials; 1 isolate; 14.3%) and amoxicillin / clavulanic acid-ampicillin-cefazolin-tetracycline (4 antimicrobials; 1 isolate; 14.3%).

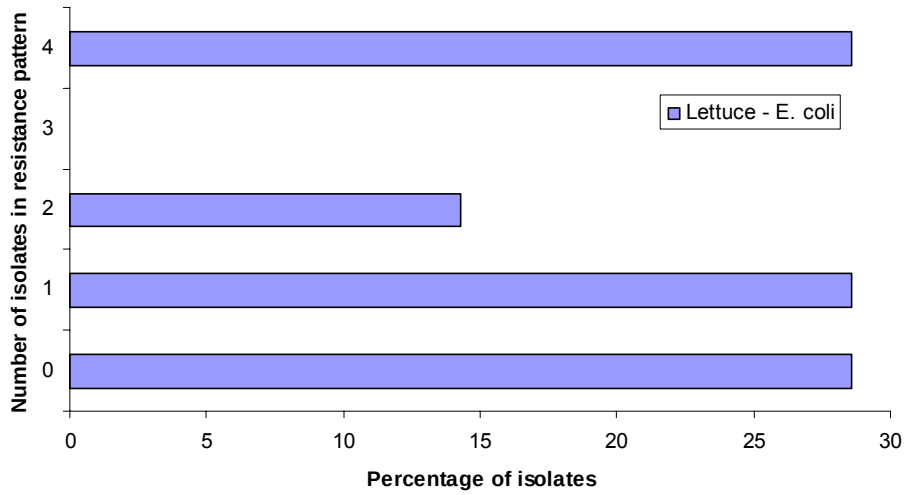


Figure 11. Multiple drug resistance in *E. coli* from retail lettuce samples (n=7)

Table 17. Multiple antimicrobial resistance phenotypes present in *E. coli* from retail lettuce.

Pattern	Resistance phenotype*	Percentage
0	No pattern	29
1	amp	29
2	aug faz	14
4	amp str tet sxt	14
4	aug amp faz tet	14

* Amoxicillin / Clavulanic acid, aug; Ampicillin, amp; Cefazolin, faz; Cefotaxime, fot; Cefoxitin, fox; Ceftiofur, xnl; Ceftriaxone, axo; Chloramphenicol, Ciprofloxacin, cip; Florfenicol, ffn; Gentamicin, gen; Meropenem, mer; Nalidixic Acid, nal; Streptomycin, str; Tetracycline, tet; Trimethoprim / Sulfamethoxazole, sxt.

Table 18. Distribution of MICs and resistance in *E. coli* from retail poultry, beef, pork and lettuce.

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs										
					0.125	0.25	0.5	1	2	4	8	16	32	64	128
Amoxicillin / Clavulanic acid [a]	Poultry	100	1.0	[0.03 – 5.45]				3.0	16.0	57.0	22.0	1.0	1.0		
	Beef	100	3.0	[0.62 – 8.52]				3.0	20.0	63.0	9.0	2.0	2.0	1.0	
	Pork	92	3.3	[0.68 – 9.23]				4.1	7.6	55.4	26.1	6.5	2.2	1.1	
	Lettuce	7	14.3	[0.36 – 57.87]				14.3	42.9		28.6		14.3		
Ampicillin	Poultry	100	38.0	[29.09 – 47.80]					35.0	24.0	1.0	2.0	1.0	3.0	34.0
	Beef	100	11.0	[5.62 – 18.83]					46.0	35.0	3.0	5.0	2.0	2.0	7.0
	Pork	92	28.2	[19.36 – 38.61]					26.1	40.2	3.3	2.2	4.3		23.9
	Lettuce	7	57.2	[18.41 – 90.10]						28.6	14.3			14.3	42.9
Cefazolin	Poultry	100	2.0	[0.24 – 7.04]							96.0	2.0	2.0		
	Beef	100	3.0	[0.62 – 8.52]							90.0	7.0	3.0		
	Pork	92	3.3	[0.68 – 9.23]							90.2	6.5	3.3		
	Lettuce	7	28.6	[3.67 – 70.96]							71.4		28.6		
Cefotaxime	Poultry	100	0.0	[0.00 – 3.62]		100.0									
	Beef	100	0.0	[0.00 – 3.62]		98.0		2.0							
	Pork	92	0.0	[0.00 – 3.93]		100.0									
	Lettuce	7	0.0	[0.00 – 40.96]		71.4	28.6								
Cefoxitin	Poultry	100	0.0	[0.00 – 3.62]					25.0	55.0	17.0	3.0			
	Beef	100	0.0	[0.00 – 3.62]					21.0	55.0	22.0	2.0			
	Pork	92	0.0	[0.00 – 3.93]					8.7	57.6	29.3	4.3			
	Lettuce	7	0.0	[0.00 – 40.96]					42.9	14.3	42.9				
Ceftiofur	Poultry	100	0.0	[0.00 – 3.62]			99.0	1.0							
	Beef	100	0.0	[0.00 – 3.62]			98.0	1.0	1.0						
	Pork	92	0.0	[0.00 – 3.93]			100.0								
	Lettuce	7	0.0	[0.00 – 40.96]			100.0								
Ceftriaxone	Poultry	100	0.0	[0.00 – 3.62]		98.0	2.0								
	Beef	100	0.0	[0.00 – 3.62]		97.0	1.0	2.0							
	Pork	92	0.0	[0.00 – 3.93]		97.8		2.2							
	Lettuce	7	0.0	[0.00 – 40.96]		100.0									
Chloramphenicol	Poultry	100	1.0	[0.03 – 5.45]						37.0	59.0	3.0	1.0		
	Beef	100	0.0	[0.00 – 3.62]					6.0	26.0	67.0	1.0			
	Pork	92	13.0	[6.93 – 21.68]					2.2	18.5	58.7	7.6	8.7	4.3	
	Lettuce	7	0.0	[0.00 – 40.96]					28.6	57.1	14.3				
Ciprofloxacin	Poultry	100	0.0	[0.00 – 3.62]	98.0	2.0									
	Beef	100	0.0	[0.00 – 3.62]	99.0	1.0									
	Pork	92	0.0	[0.00 – 3.93]	97.8	1.1	1.1								
	Lettuce	7	0.0	[0.00 – 40.96]	85.7	14.3									
Florfenicol	Poultry	100	2.0	[0.24 – 7.04]					8.0	62.0	28.0	2.0			
	Beef	100	0.0	[0.00 – 3.62]					7.0	40.0	53.0				
	Pork	92	8.7	[3.83 – 16.42]					3.3	41.3	46.7	8.7			
	Lettuce	7	0.0	[0.00 – 40.96]					42.9	42.9	14.3				
Gentamicin	Poultry	100	4.0	[1.10 – 9.93]				83.0	13.0				4.0		
	Beef	100	0.0	[0.00 – 3.62]				93.0	7.0						
	Pork	92	1.1	[0.03 – 5.91]				87.0	10.9		1.1		1.1		
	Lettuce	7	0.0	[0.00 – 40.96]				100.0							
Kanamycin	Poultry	100	8.0	[3.52 – 15.16]							84.0	8.0			8.0
	Beef	100	2.0	[0.24 – 7.04]							94.0	4.0			2.0
	Pork	92	3.3	[0.68 – 9.23]							83.7	12.0	1.1	1.1	2.2

Antimicrobial	Product	N =	% Resistant	[95% CI]	Distribution (%) of MICs										
					0.125	0.25	0.5	1	2	4	8	16	32	64	128
	Lettuce	7	0.0	[0.00 – 40.96]							85.7	14.3			
Meropenem	Poultry	100	0.0	[0.00 – 3.62]				99.0	1.0						
	Beef	100	0.0	[0.00 – 3.62]				100.0							
	Pork	92	0.0	[0.00 – 3.93]				100.0							
	Lettuce	7	0.0	[0.00 – 40.96]				100.0							
Nalidixic Acid	Poultry	100	0.0	[0.00 – 3.62]					52.0	47.0	1.0				
	Beef	100	0.0	[0.00 – 3.62]					44.0	54.0	2.0				
	Pork	92	0.0	[0.00 – 3.93]					29.3	66.3	4.3				
	Lettuce	7	0.0	[0.00 – 40.96]						85.7	14.3				
Streptomycin	Poultry	100	19.0	[11.84 – 28.07]									81.0	4.0	15.0
	Beef	100	7.0	[2.86 – 13.89]									93.0	4.0	3.0
	Pork	92	17.4	[10.28 – 26.70]									82.6	8.7	8.7
	Lettuce	7	14.3	[0.36 – 57.87]									85.7	14.3	
Tetracycline	Poultry	100	47.0	[36.94 – 57.24]						53.0		5.0	8.0	34.0	
	Beef	100	7.0	[2.86 – 13.89]						91.0	2.0			7.0	
	Pork	92	44.5	[34.19 – 55.30]						54.3	1.1	1.1	4.3	39.1	
	Lettuce	7	28.6	[3.67 – 70.96]						71.4			14.3	14.3	
Trimethoprim / Sulfamethoxazole	Poultry	100	22.0	[14.33 – 31.39]	65.0	9.0	3.0	1.0			22.0				
	Beef	100	5.0	[1.64 – 11.28]	90.0	2.0	2.0		1.0	3.0	2.0				
	Pork	92	13.0	[6.93 – 21.68]	67.4	16.3	3.3			4.3	8.7				
	Lettuce	7	14.3	[0.36 – 57.87]	85.7						14.3				

Vertical lines indicate breakpoints for resistance
The white fields denote range of dilutions tested for each antimicrobial. Values above the range denote MIC values greater than the highest concentration in the range. MICs equal to or lower than the lowest concentration tested are given as the lowest concentration
[a] Concentration of amoxicillin given, tested with clavulanic acid in concentration 2:1

Discussion

The pilot survey for AMR bacteria in Australian food is designed to provide data that can be used to estimate the prevalence of AMR bacteria in food purchased at retail outlets. The survey was limited to those food / bacterium combinations where the expected prevalence of the target organism was projected to be >10%. Four retail foods; poultry, beef, pork and lettuce along with four target organisms; *Campylobacter, Salmonella, E. coli* and *Enterococcus* constitute the nine food / bacterium combinations included in the survey. The initial sampling plan for the survey utilised available Australian and international prevalence data to estimate the number of samples required to generate 100 isolates. Changes to the sampling plan have occurred during the survey in response to the monthly prevalence data progressively generated. Increases to the number of samples being tested for *Campylobacter* in poultry and *E. coli* in pork have been 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*. Both early and subsequent data indicated that the prevalence of *E. coli* on lettuce was likely to be 9-10 fold lower than initially anticipated. Following the sampling modifications indicated, seven food / bacterium combinations met and exceeded projected prevalences and the 100 isolate goal was successfully reached. Due to reduced prevalences, the 100 isolate goal for pork / *E. coli* and lettuce / *E. coli* combinations were not achieved. With respect to pork / *E. coli*, this does not substantially modify the confidence in AMR detection. However, firm conclusions concerning the prevalence of AMR in lettuce / *E. coli* isolates cannot be made with confidence due to the extremely limited isolation of *E. coli* from this food source.

The results of testing isolates from 12 monthly sampling rounds for AMR indicates 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 for ampicillin (38% and 28.2%), streptomycin (19% and 17.4%), tetracycline (47% and 44.5%) and trimethoprim / sulfamethoxazole (22% and 13%) was notably higher than in 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 *E. faecalis* isolates by high prevalences of resistance to erythromycin (48%) and tetracycline (76%). The absence of detection of *Enterococcus faecium* amongst Enterococcus isolates from all retail meat sources was unexpected. A previous study of retail meat (5) found a

predominance of *E. faecalis* on retail meats including chicken, beef and pork, however, in contrast to the present study both *E. faecalis* and *E. faecium* were routinely isolated. It is not readily apparent why no *E. faecium* were isolated in the present study and this observation merits further investigation.

In *Campylobacter* isolates, low resistance to the test antimicrobials was observed. The prevalence of resistance to tetracycline was 1%. High levels of tetracycline resistance have been observed in similar studies throughout the world and the absence of resistance in Australian *Campylobacter* from poultry is notable (see below).

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) (4), Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (2) and the United States of America National Antimicrobial Resistance Monitoring System (NARMS) (3). While each national AMR monitoring program collects and presents data in specific formats, within these limitations the broad comparisons presented below have been possible. The following comparisons are considered by retail food type reported for year 2005 in each of the abovementioned programs. For the purpose of this discussion variations in AMR prevalence which are ≥ or ≤ 10% are designated as 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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Appendices

Appendix A. Protocols for the preparation of retail product samples and isolation of bacteria of concern for the AMR in retail foods pilot surveillance program.

Sample preparation

Poultry (rinse fluid)

- Place whole bird into a sterile plastic bag of suitable size
- Add 500 ml of buffered peptone water (BPW) into the plastic bag
- Shake and massage sample vigorously for 2 min
- Release the rinse fluid into a sterile sample container by cutting off the corner of the bag and allowing the fluid to drain into a container

Beef (initial suspension)

- Place 25g of minced beef into a sterile stomacher bag
- Add 225 ml of BPW
- Stomach for 1 min

Pork (initial suspension)

- Aseptically remove 25g of pork adipose tissue and place in a sterile stomacher bag
- Add 225 ml of BPW
- Stomach for 1 min

Lettuce (initial suspension)

- Aseptically cut a cross-section through the entire lettuce at approximately 5cm to 7cm from the stem end.
- Prepare this stem end portion by cutting and mixing and then remove 25g as the test sample portion and place into a sterile stomacher bag
- Add 225 mL BPW
- Stomach for 1 min

Bacterial isolation

Escherichia coli

- inoculate 50 mL of rinse fluid or initial suspension in 50mL of double strength EC broth;
- incubate aerobically at 37°C for 18-24 hours;
- streak one loopful of incubated EC broth-rinse fluid mix onto eosin methylene blue (EMB) agar and incubate at 37°C for 18-24 hours;
  select a typical *E. coli* colony (dark green metallic sheen by reflected light and dark purple centres by transmitted light) and streak for isolation on tryptic soy agar containing 5% sheep blood (TSA-B), incubate as above;
- examine the TSA-B plate for purity. If it is not pure repeat the previous step;
- perform rapid biochemical identification of isolate using spot indole test in conjunction with Simmons citrate tube test or use an appropriate commercially available biochemical identification kit (eg Microbact 12E);
- store confirmed isolates in duplicate at -70°C.

Enterococcus spp.

- inoculate 50 mL of rinse fluid or initial suspension into 50 mL of double strength Enterococcosel broth;
- incubate aerobically at 37°C for 18-24 hours;
- If no growth or blackening of the Enterococcosel broth-rinse fluid mix can be observed, sample is negative and can be discarded;
- Streak one loopful of broths exhibiting growth or blackening onto Enterococcosel agar plates and incubate aerobically at 37°C for 24-48 hours;
- examine Enterococcosel agar plates for typical Enterococci colonies (aesculin hydrolysis) and plate onto Columbia agar containing 5% sheep blood (CBA). Incubate aerobically at 37°C for 24 – 48 hours;
- examine CBA plate for purity. If it is not pure repeat the previous step;
  confirm isolates as *Enterococcus* spp;
- identify *Enterococci* spp. biochemically or by PCR;
- store confirmed isolates in duplicate at -70°C.

Campylobacter spp.

- inoculate 50 mL of rinse fluid into 50 mL of double strength Preston broth without antibiotic supplement and incubate at 37°C for 2 hours;

- after 2 hours incubation add 0.4 mL of antibiotic supplement (B2.4 AS5013.6) to 100 mL of broth culture. Broths are then incubated under microaerophilic conditions at 42°C for 46 hours;
- plate a loopful of the broth culture onto modified CCDA agar plates (with antibiotic supplement) and incubate at 42°C for 48hrs under microaerophilic conditions;
- examine m-CCDA plates for smooth, flat translucent, colourless to grey-brown colonies with an irregular edge and plate onto blood agar;
- confirm identity using gram stain, motility, oxidase and catalase and identify species of *Campylobacter* using commercial identification kit;
- store confirmed isolates in duplicate at -70°C.

Salmonella spp.

- incubate 100 mL of rinse fluid aerobically at 37°C for 18-24 hours;
- transfer 0.1 mL of the enrichment to 10 mL of Rappaport-Vassiliadis medium with soya (RVS) and incubate aerobically at 41.5°C for 24 hours (do not exceed 42.5°C);
- transfer 1 mL of the enrichment to 10 mL of Muller-Kaufmann tetrathionate-novobiocin broth (MKTTn) and incubate aerobically at 37°C for 24 hours;
- plate a loopful of RVS and MKTTn enrichment onto xylose lysine deoxycholate agar (XLD) and brilliant green agar (BGA) and incubate aerobically at 37°C for 24 hours; examine XLD and BGA plates for typical *Salmonella* colonies; colonies will have a black centre surrounded by a lightly transparent zone of red on XLD and will be red colonies surrounded by bright red medium on BGA. Plate typical *Salmonella* colonies onto nutrient agar and incubate at 37°C for 24 hours;
- confirm isolates as *Salmonella* spp. biochemically and serologically;
- store confirmed isolates in duplicate at -70°C

NB: all strains considered to be *Salmonella* must be sent to the approved *Salmonella* serotyping laboratory at MDU, Melbourne University for definitive typing.

Storage of isolates

Scrape the surface growth from a pure culture into a commercial cryostorage system such as MicroBank or Protect™. Snap freeze and store in duplicate at – 70°C.

Appendix B. Sensititre custom and standard Campylobacter plate formats for antimicrobial susceptibility testing

AUSVN – Gram negative bacteria

	1	2	3	4	5	6	7	8	9	10	11	12
A	**CIP**	CIP	CIP	CIP	CIP	CIP	**AMP**	AMP	AMP	AMP	AMP	AMP
	0.125	0.25	0.5	1	2	4	2	4	8	16	32	64
B	**NAL**	NAL	NAL	NAL	NAL	NAL	**SXT**	SXT	SXT	SXT	SXT	SXT
	2	4	8	16	32	64	0.12/2.38	0.25/4.75	0.5/9.5	1/19	2/38	4/76
C	**FFN**	FFN	FFN	FFN	FFN	FFN	**AUG2**	AUG2	AUG2	AUG2	AUG2	AUG2
	2	4	8	16	32	64	1/0.5	2/1	4/2	8/4	16/8	32/16
D	**XNL**	XNL	XNL	XNL	XNL	XNL	**CHL**	CHL	CHL	CHL	CHL	CHL
	0.5	1	2	4	8	16	2	4	8	16	32	64
E	**GEN**	GEN	GEN	GEN	GEN	GEN	**FAZ**	FAZ	**FOX**	FOX	FOX	FOX
	1	2	4	8	16	32	8	16	0.5	1	2	4
F	**AXO**	AXO	AXO	AXO	AXO	AXO	AXO	AXO	AXO	FOX	FOX	FOX
	0.25	0.5	1	2	4	8	16	32	64	8	16	32
G	**TET**	TET	TET	TET	**KAN**	KAN	KAN	KAN	**MERO**	MERO	MERO	MERO
	4	8	16	32	8	16	32	64	1	2	4	8
H	**FOT**	FOT	FOT	FOT	FOT	FOT	FOT	FOT	FOT	**STR**	STR	**POS**
	0.25	0.5	1	2	4	8	16	32	64	32	64	**CON**

ANTIMICROBIALS

AUG2	Amoxicillin / clavulanic acid 2:1 ratio
AMP	Ampicillin
FAZ	Cefazolin
FOT	Cefotaxime
FOX	Cefoxitin
XNL	Ceftiofur
AXO	Ceftriaxone
CHL	Chloramphenicol
CIP	Ciprofloxacin
FFN	Florfenicol
GEN	Gentamicin
KAN	Kanamycin
MERO	Meropenem
NAL	Nalidixic Acid
POS	Positive Control
STR	Streptomycin
TET	Tetracycline
SXT	Trimethoprim / sulfamethoxazole

AUSVP – Gram positive bacteria

	1	2	3	4	5	6	7	8	9	10	11	12
A	**TGC**	TGC	TGC	TGC	TGC	TGC	**AMP**	AMP	AMP	AMP	AMP	AMP
	0.015	0.03	0.06	0.12	0.25	0.5	2	4	8	16	32	64
B	**PEN**	PEN	PEN	PEN	PEN	PEN	**DAP**	DAP	DAP	DAP	DAP	DAP
	0.5	1	2	4	8	16	0.5	1	2	4	8	16
C	**SYN**	SYN	SYN	SYN	SYN	SYN	**VIR**	VIR	VIR	VIR	VIR	VIR
	1	2	4	8	16	32	1	2	4	8	16	32
D	**FLV**	FLV	FLV	FLV	FLV	FLV	**TEI**	TEI	TEI	TEI	TEI	TEI
	1	2	4	8	16	32	0.5	1	2	4	8	16
E	**GEN**	GEN	GEN	GEN	GEN	GEN	**LIN**	LIN	LIN	LIN	LIN	LIN
	64	128	256	512	1024	2048	1	2	4	8	16	32
F	**ERY**	ERY	ERY	ERY	ERY	ERY	**TET**	TET	TET	TET	**STR**	STR
	1	2	4	8	16	32	4	8	16	32	512	1024
G	**KAN**	KAN	KAN	KAN	VAN	VAN	VAN	VAN	VAN	VAN	VAN	STR
	128	256	512	1024	0.5	1	2	4	8	16	32	2048
H	**CHL**	CHL	CHL	CHL	CHL	**LZD**	LZD	LZD	LZD	LZD	VAN	**POS**
	2	4	8	16	32	0.5	1	2	4	8	64	**CON**

ANTIMICROBIALS

AMP	Ampicillin
CHL	Chloramphenicol
DAP	Daptomycin
ERY	Erythromycin
FLV	Flavomycin
GEN	Gentamicin
KAN	Kanamycin
LIN	Lincomycin
LZD	Linezolid
PEN	Penicillin
POS	Positive Control
SYN	Quinupristin / dalfopristin
STR	Streptomycin
TEI	Teicoplanin
TET	Tetracycline
TGC	Tigecycline
VAN	Vancomycin
VIR	Virginiamycin

CAMPY – *Campylobacter*

	1	2	3	4	5	6	7	8	9	10	11	12
A	**AZI**	AZI	AZI	AZI	AZI	AZI	AZI	AZI	AZI	AZI	AZI	AZI
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
B	AZI	**CIP**	CIP	CIP	CIP	CIP	CIP	CIP	CIP	CIP	CIP	CIP
	64	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
C	CIP	CIP	**ERY**	ERY	ERY	ERY	ERY	ERY	ERY	ERY	ERY	ERY
	32	64	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16
D	ERY	ERY	**GEN**	GEN	GEN	GEN	GEN	GEN	GEN	GEN	GEN	**TET**
	32	64	0.12	0.25	0.5	1	2	4	8	16	32	0.06
E	TET	TET	TET	TET	TET	TET	TET	TET	TET	TET	**FFN**	FFN
	0.12	0.25	0.5	1	2	4	8	16	32	64	0.03	0.06
F	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	FFN	**NAL**	NAL
	0.12	0.25	0.5	1	2	4	8	16	32	64	4	8
G	NAL	NAL	NAL	**TEL**	TEL	TEL	TEL	TEL	TEL	TEL	TEL	TEL
	16	32	64	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
H	TEL	**CLI**	CLI	CLI	CLI	CLI	CLI	CLI	CLI	CLI	CLI	**POS**
	8	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	**CON**

ANTIMICROBIALS

AZI	Azithromycin
CIP	Ciprofloxacin
ERY	Erythromycin
GEN	Gentamicin
TET	Tetracycline
FFN	Florfenicol
NAL	Nalidixic Acid
TEL	Telithromycin
CLI	Clindamycin
POS	Positive Control

Appendix C. FRSC AMR working group queries and response

Dear FRSC AMR Working Group

After reading the 12 monthly report from Food Science Australia (FSA), distributed by email, a couple of members had a few queries. Robert Barlow from FSA has kindly provided the following responses for the information of members:

1. Pat Blackall wrote "I note that the report predicts a shortfall of 4-6 isolates in the pork *E. coli* isolates. As there is no comment about the need for any altered sampling, I assume that the research group believes that this shortfall will not be of any significance?"

FSA has responded:
"It is unfortunate that achieving the 100 isolate goal for *E. coli* in pork appears unlikely despite increasing the number of tests to be conducted during the latter part of the survey. Based on current projections, a shortfall of 4-6 isolates is expected and consequently the impact on the final results has been questioned. The selection of 100 isolates as the target for each food / bacterium combination is based on having a 95% probability of detecting 1 AMR isolate in 100 at 3% prevalence. The equation used to generate this statement can be used to understand the significance of any shortfalls. If 90 isolates is used as the worse case scenario for *E. coli* in pork then the probability of detecting 1 AMR isolate in 90 at 3% prevalence is reduced to 93.5%. To put this in the context of the original proposition, the '93.5% probability of detecting 1 AMR isolate in 90 at 3% prevalence', is equivalent to saying that 'there is a 95% probability of detecting 1 AMR isolate in 100 at ~3.3% prevalence'.
We believe the reduction in confidence of detecting AMR is not sufficient enough to warrant the collection of further isolates and therefore additional sampling should not be considered at this point.

Appendix D. Identification of survey strengths, limitations and lessons learned

Identification of any specific strengths and limitations of the survey

The pilot survey for AMR bacteria in food has been conducted as a response to the recommendations outlined in the JETACAR report. It forms part of a three-pronged approach into investigating the prevalence of AMR bacteria in food production animals, retail foods, and clinical settings. The completion of the survey for AMR bacteria in food provides a snapshot view of the prevalence of AMR in nine food / bacterium combinations. The survey has representatively sampled the retail supply chain at the point of sale servicing approximately two-thirds of Australia's population and although not specifically designed to address seasonality, the completion of the survey over a 12 month period may provide seasonal and annual data on AMR and bacterial prevalence. However, it must be noted that the survey was designed to determine the AMR prevalence in 100 isolates per food / bacterium combination and not to determine seasonal or annual prevalences around AMR or bacterial contamination of retail foods. A survey designed to determine data in addition to total AMR prevalence in 100 isolates of each food / bacterium combination would require a different sampling regimen. In particular, the number of samples collected and the areas of collection would require substantial increase. Despite the peripheral limitations, the survey has, as designed, determined the level of AMR prevalence in bacteria from nine food / bacterium combinations. Additionally, the use of internationally recognised methods for the detection, isolation and AMR characterisation of isolation permits direct comparison with similar studies conducted overseas. Such comparisons will provide insight into the significance of AMR bacteria in Australian retail foods and will be used in the future to determine AMR trends over time and hence assist in evaluating the efficiency of interventions or changes in food chain antimicrobial use in Australian food producing systems.

The system of monthly progress reporting to DOHA/FRSC employed in the current survey has been beneficial for overall project success. The positive factors associated with monthly reporting have included:

- Early recognition and addressing of operational challenges
- Opportunity for provision of early expert opinion and advice
- Routine reporting to prompt timely management and reporting of any emerging issues.

A brief discussion of any lessons learned in relation to the methodology used to undertake the services

As previously mentioned, the methodologies employed to complete the survey are internationally recognised and therefore permit direct comparison with similar overseas studies. The use of standard methods for the isolation of bacteria from food and the use of standardised AMR testing equipment and procedures must therefore be an integral part of any future survey of this kind. That aside, there are some lessons that have been learnt whilst undertaking the services. These lessons deal specifically with the subcontractor-contractor interaction. The approach taken in this survey required sampling and testing for bacteria of concern to occur in each of four capital cities. Upon collection of six month's isolates, the subcontractor was responsible for delivery of the isolates to Food Science Australia for subsequent AMR testing of up to 100 isolates per food / bacterium combination. This approach differs slightly from that used in overseas studies where all samples collected are sent to regional testing laboratories where testing for bacteria of concern and AMR occurs at once. Whilst the lack of AMR testing infrastructure meant that the overseas approach was not possible it is easy to see in hindsight that substantial inefficiencies occur when the AMR testing is not completed at the time of bacterial isolation. Furthermore, the inability to recover some isolates (*Campylobacter* in particular) meant that the original sampling plan which was based on anticipated prevalence is somewhat compromised. Indeed the inability to recover *Campylobacter* from Protect™ beads in combination with a lower than expected prevalence required a significant increase in the number of samples tested for *Campylobacter* in the second half of the survey. Future surveys should establish an approach or infrastructure support such that bacterial isolation and AMR testing can occur at the same time and in the same laboratory. This would reduce the inefficiencies observed in the current survey and would ensure that 100% of isolates selected for AMR analysis were available for testing.

It is also recommended that future AMR surveillance be conducted by a single integrated project team with a high level of awareness of purpose of sample collection, standardised practices and overall project goals. The operation of an integrated project team will promote simplified lines of communication, resource allocation and responsibility for timely delivery. In summary, while it is recognised that factors and costs for optimal survey design, management and scientific integrity will often be constrained by limited resources (primarily financial), the following recommendations are strongly made for any future AMR surveillance programs:

- Overall project quality be enhanced through the operation of a single, integrated project team
- The number of persons in key project management/communication positions should be minimised in order to promote clear communication, accountability and project delivery.

Supplementary file note

Supplement 1 – Food AMR Pilot Survey – Bacterial Isolates

Details of each bacterial isolate from the survey are provided in the supplementary document **'Supplement 1 – Food AMR Pilot Survey – Bacterial Isolates'**.