

## 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 12<sup>th</sup> 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  $\leq 5\%$  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%).

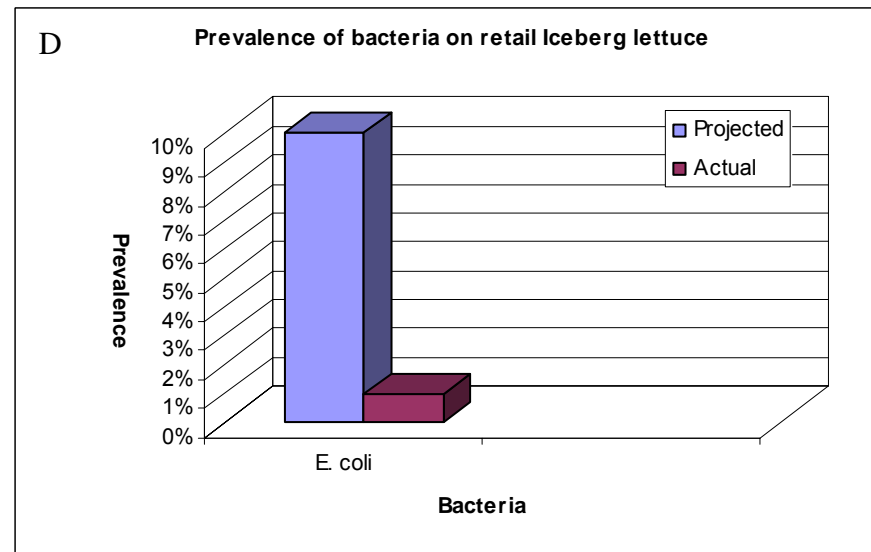
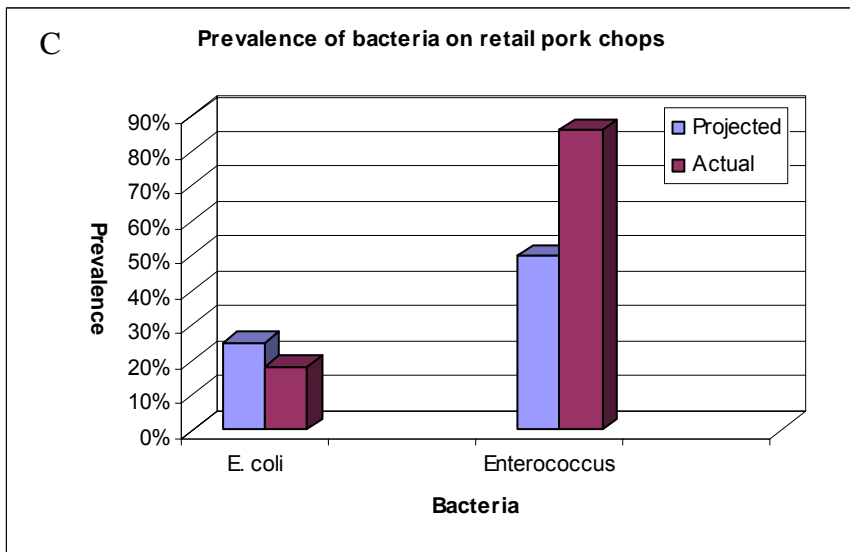
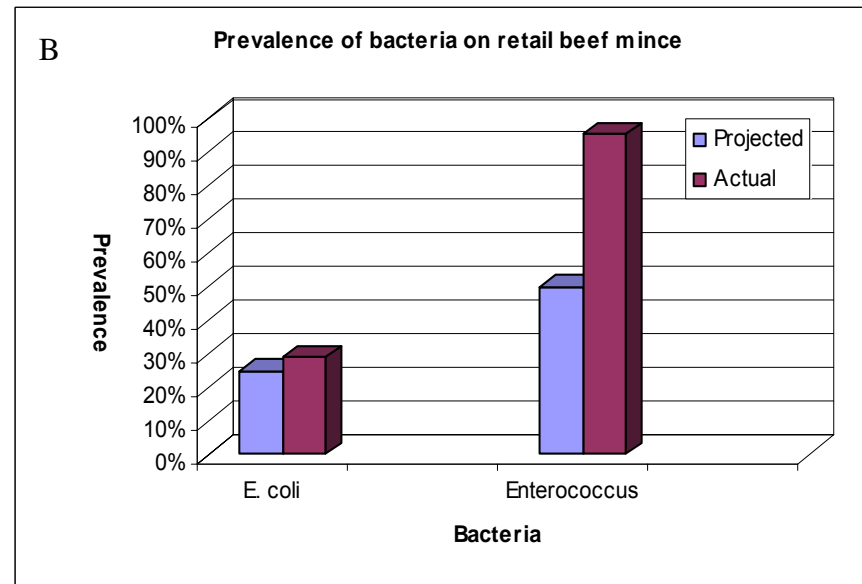
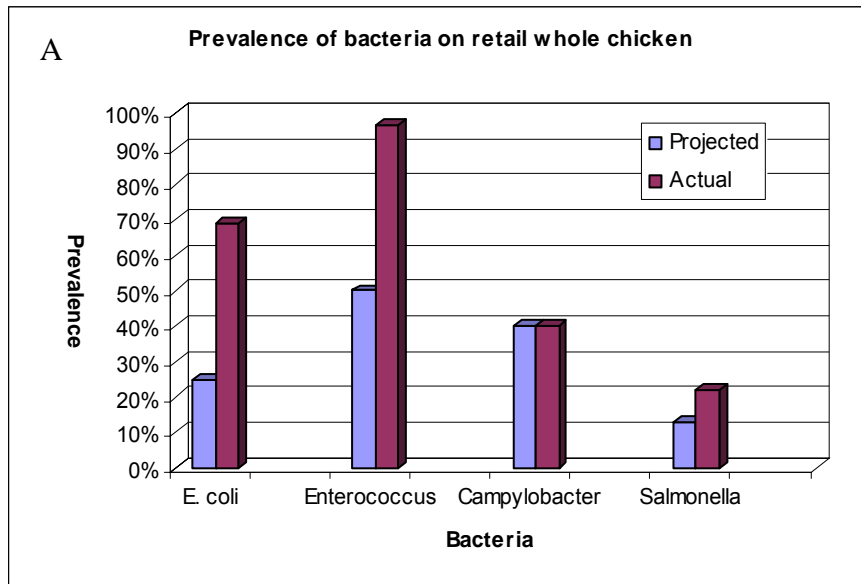


Figure 1. Projected and actual prevalences of bacteria in retail foods (12 month period February 2007 to January 2008 sampling). The initially projected and actual prevalences of bacteria in particular retail foods are shown in for whole chicken (panel A), minced beef (panel B), pork chop (panel C) and 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 2). 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.

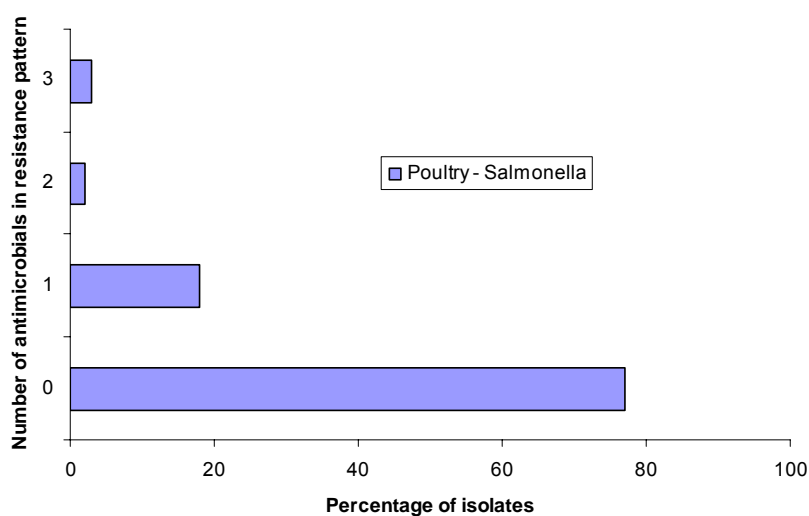


Figure 2. Multiple drug resistance in *Salmonella* from retail poultry samples (n=100)

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 <sup>a</sup>	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.

<sup>a</sup> 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
<b>TOTAL</b>		<b>100</b>

\* 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 6Table). 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.

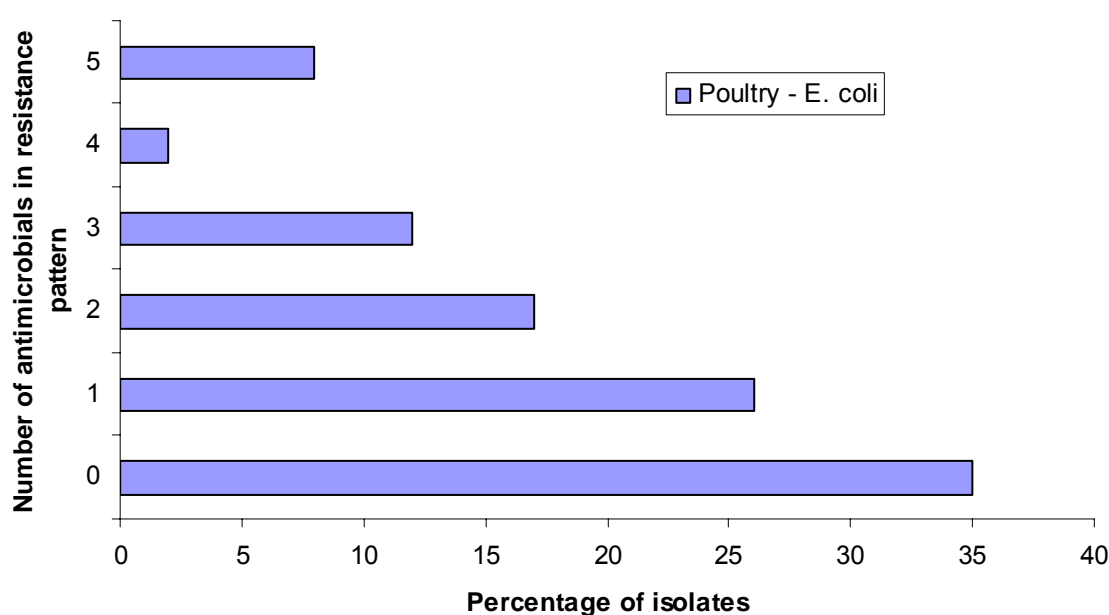


Figure 3. Multiple drug resistance in *E. coli* from retail poultry samples (n=100)

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.

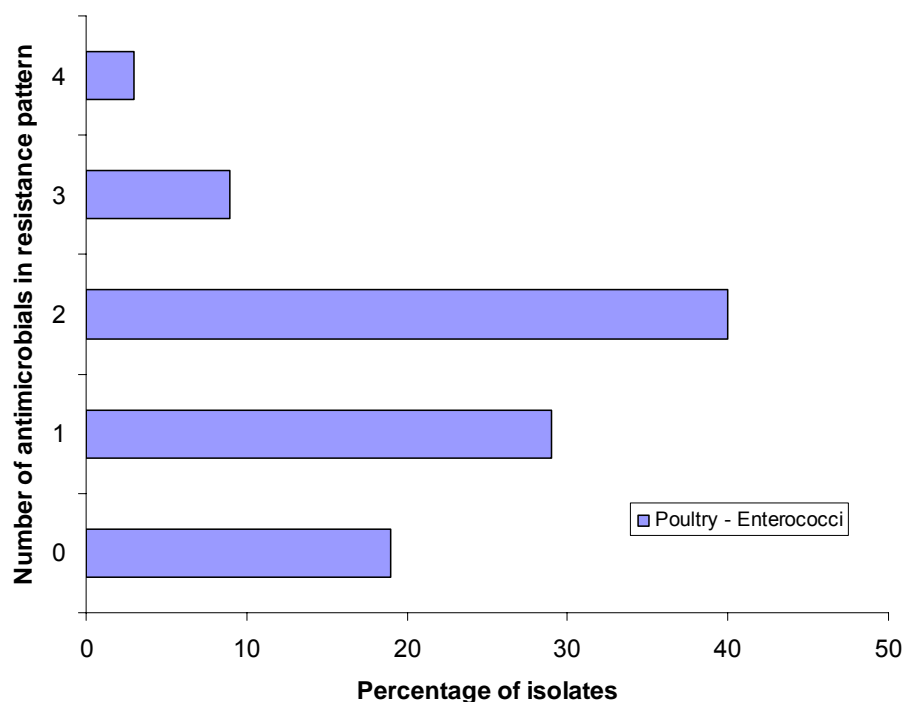


Figure 4. Multiple drug resistance in *Enterococcus faecalis* from retail poultry samples (n=100)

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 and Table 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%).

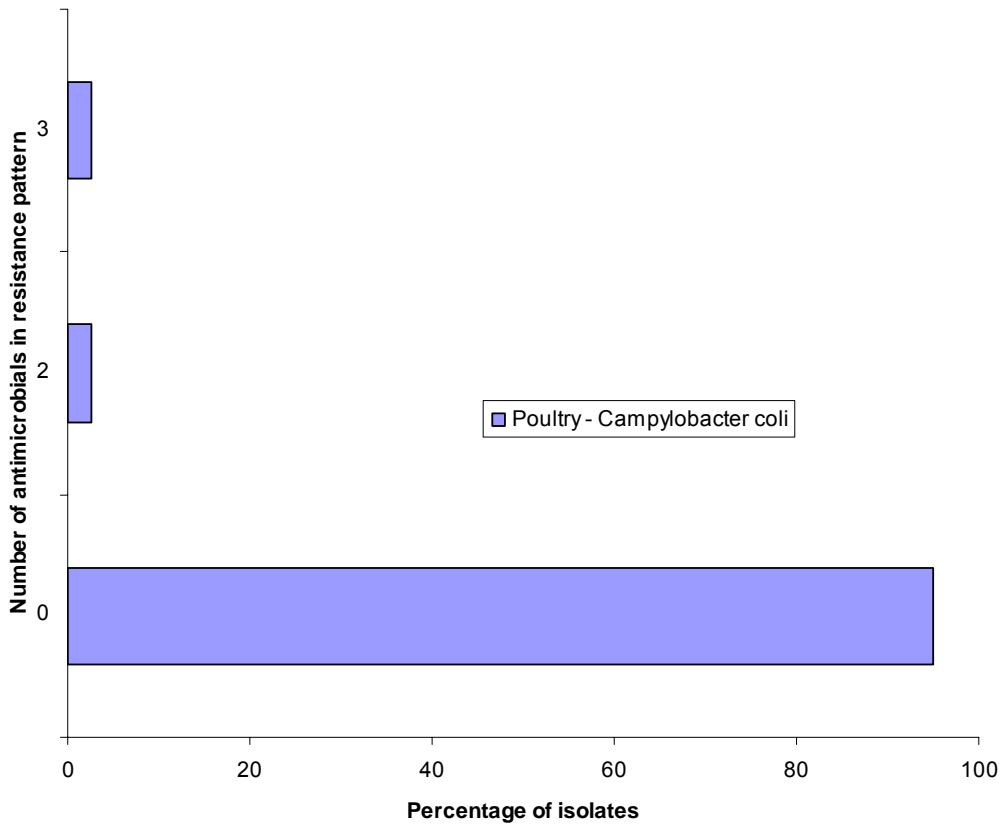


Figure 5. Multiple drug resistance in *Campylobacter coli* from retail poultry samples (n=40).

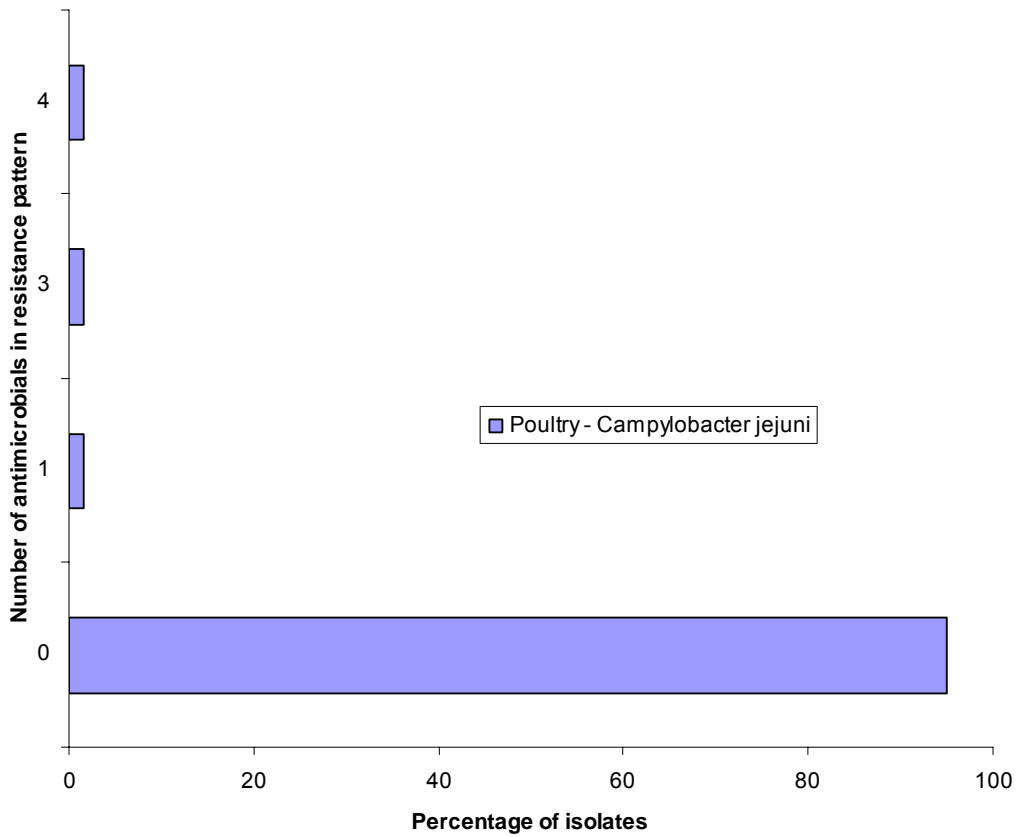


Figure 6. Multiple drug resistance in *Campylobacter jejuni* from retail poultry samples (n=60)

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.

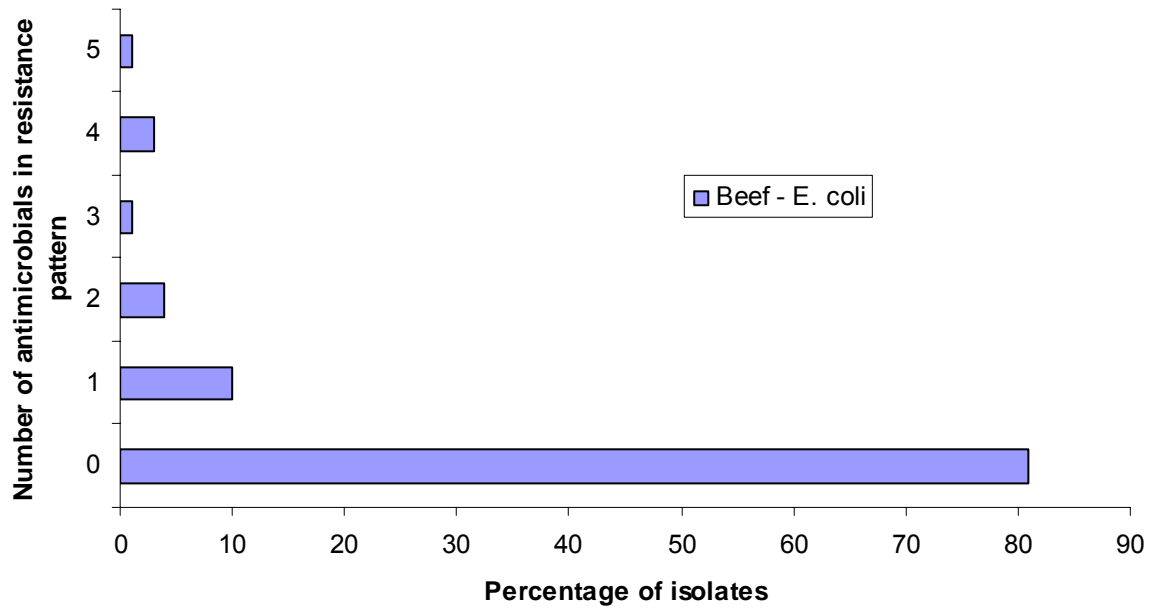


Figure 7. Multiple drug resistance in *E. coli* from retail beef samples (n=100)

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  $\leq 7\%$ . 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%).

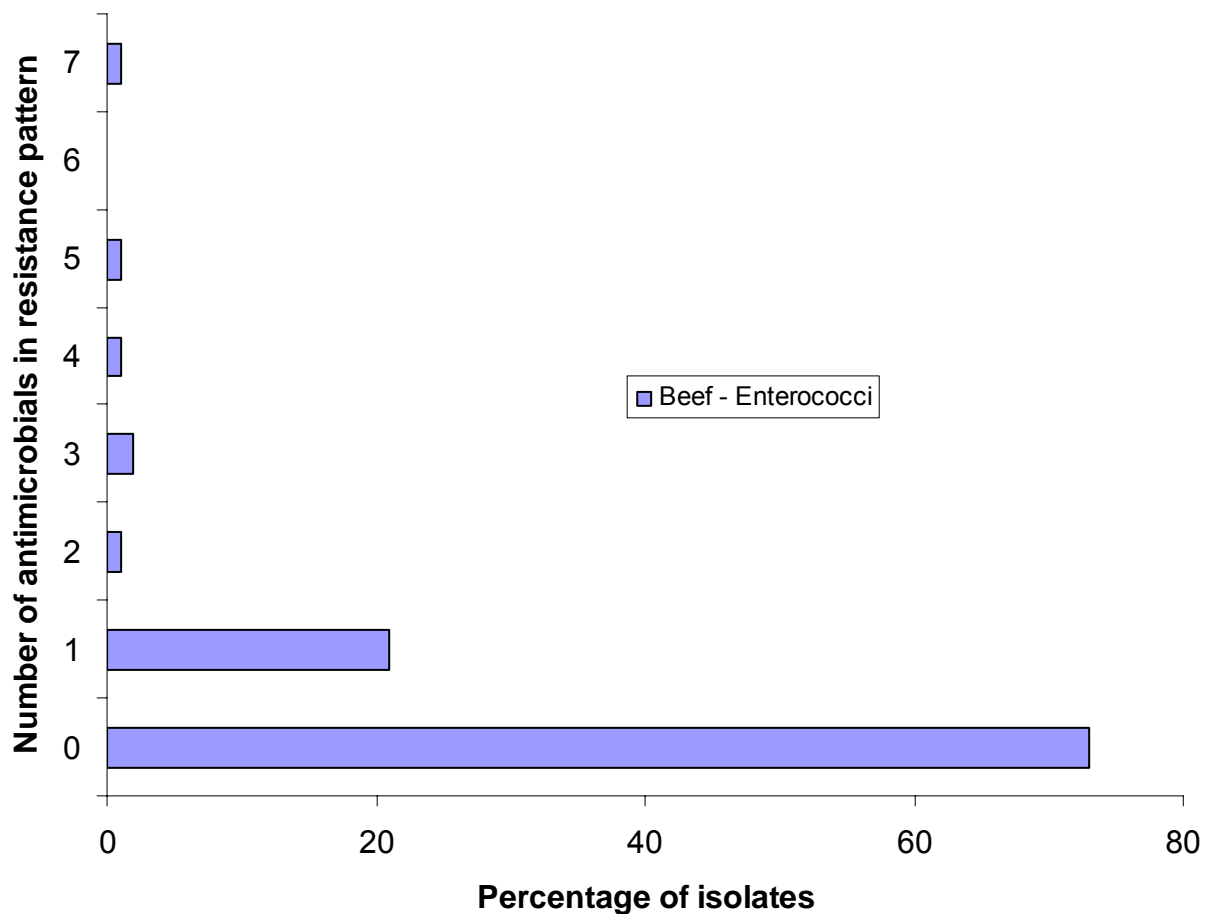


Figure 8. Multiple drug resistance in *Enterococcus faecalis* from retail beef samples (n=100)

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.