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Title: First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals

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Highlights

- First detection of extended-spectrum cephalosporin and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals.
- These isolates resistant to critically important antimicrobials (CIAs) belong to internationally disseminated, multidrug-resistant zooanthroponotic clonal lineages.
- Frequency of resistance to CIAs among *E. coli* isolates causing clinical disease in Australian food-producing animals is defined.

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First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals

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ABSTRACT

This study aimed to define the frequency of resistance to critically important antimicrobials (CIAs) [i.e. extended-spectrum cephalosporins (ESCs), fluoroquinolones (FQs) and carbapenems] among *Escherichia coli* isolates causing clinical disease in Australian food-producing animals. Clinical *E. coli* isolates (*n* = 324) from Australian food-producing animals [cattle (*n* = 169), porcine (*n* = 114), poultry (*n* = 32) and sheep (*n* = 9)] were compiled from all veterinary diagnostic laboratories across Australia over a 1-year period. Isolates underwent antimicrobial susceptibility testing to 18 antimicrobials using the Clinical and Laboratory Standards Institute disk diffusion method. Isolates resistant to CIAs underwent minimum inhibitory concentration determination, multilocus sequence typing (MLST), phylogenetic analysis, plasmid replicon typing, plasmid identification, and virulence and antimicrobial resistance gene typing. The 324 *E. coli* isolates from different sources exhibited a variable frequency of resistance to tetracycline (29.0–88.6%), ampicillin (9.4–71.1%), trimethoprim/sulfamethoxazole (11.1–67.5%) and streptomycin (21.9–69.3%), whereas none were resistant to imipenem or amikacin. Resistance was detected, albeit at low frequency, to ESCs (bovine isolates, 1%; porcine isolates, 3%) and FQs (porcine isolates, 1%). Most ESC- and FQ-resistant isolates represented globally disseminated *E. coli* lineages (ST117, ST744, ST10 and ST1). Only a single porcine *E. coli* isolate (ST100) was identified as a classic porcine enterotoxigenic *E. coli* strain (non-zoonotic animal pathogen) that exhibited ESC resistance via acquisition of bla_{CMY-2}. This study uniquely establishes the presence of resistance to CIAs among clinical *E. coli* isolates from Australian food-producing animals, largely attributed to globally disseminated FQ- and ESC-resistant *E. coli* lineages.

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1. Introduction

The World Health Organization (WHO) has recently highlighted the major public health risks posed by resistance to critically important antimicrobials (CIAs) such as extended-spectrum cephalosporins (ESCs), fluoroquinolones (FQs) and carbapenems among Enterobacteriaceae [1]. Concerns are heightened when such resistance occurs in food-producing animals because of the potential risk of transmission to humans through the food chain and/or the environment [2,3]. Plasmid-mediated ESC resistance (mediated by *bla*$_{\text{CMY-2}}$) was first detected in *Escherichia coli* from US livestock in 1996 and in *Salmonella* Newport shortly thereafter in Canada [4,5]. Similarly, in Asia and Europe, ESC resistance in *E. coli* isolated from livestock has been attributed to the emergence and spread of plasmid-mediated *bla*$_{\text{CTX-M}}$ genes and *bla*$_{\text{CMY-2}}$ [6–8]. In several countries in these regions, extensive use of FQs in some food-animal species has been linked to the emergence of FQ-resistant *E. coli* and *Salmonella* [9,10]. More recently, carbapenemases (NDM-1, VIM, OXA-23) have been detected in Enterobacteriaceae isolated from livestock systems both in Asia and Europe [3].

Recent studies have suggested that the ecology of antimicrobial resistance among Enterobacteriaceae isolated from food-producing animals in Australia is different to that in other parts of the world [11,12]. Resistance to ESCs, FQs and carbapenems has yet to be reported among Enterobacteriaceae from Australian livestock [11,12]. This has been attributed to Australia's geographic isolation, restrictions placed on the importation of live animals and some foods, and strong regulation governing the use of CIAs [13,14]. The latter includes bans on the use of FQs and carbapenems in any

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food-producing animal and of ceftiofur (an ESC) for mass medication [13]. In this study, we sought to define the frequency of resistance to these three critically important classes of antimicrobial among *E. coli* isolates causing clinical disease in Australian food-producing animals.

2. Materials and methods

2.1. Bacterial strains

A collection of 324 clinical *E. coli* isolates from Australian food-producing animals was compiled within the first national Australian veterinary antimicrobial resistance survey, which took place over 12 months (January 2013 to January 2014) with the co-operation of all veterinary diagnostic laboratories (*n* = 22) in all Australian states and territories. The study isolates were from bovine (*n* = 169), porcine (*n* = 114), poultry (*n* = 32) and ovine (*n* = 9) and were considered by the diagnostic microbiologist to be involved in the aetiology of the presenting disease.

2.2. Phenotypic detection of antimicrobial resistance

All isolates underwent disk diffusion susceptibility testing as per Clinical and Laboratory Standards Institute (CLSI) guidelines to 18 antimicrobials of veterinary and human health importance, including amoxicillin/clavulanic acid, amikacin, ampicillin, apramycin, cefoxitin, ceftazidime, ceftiofur, cefalotin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, imipenem, neomycin, spectinomycin, streptomycin, trimethoprim/sulfamethoxazole (SXT) and tetracycline. The breakpoints used were those recommended in CLSI document VET01-S2 [15] and M100-S24 [16]. For antimicrobials that lacked published CLSI breakpoints,

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Australian veterinary laboratory breakpoints were used [apramycin and neomycin, resistant (R), ≤12 mm; and florfenicol, R, ≤14 mm]. Isolates that demonstrated resistance to ciprofloxacin, ceftiofur or ceftazidime underwent minimum inhibitory concentration (MIC) testing by microbroth dilution to ciprofloxacin, enrofloxacin, pradofloxacin, ceftriaxone, ceftiofur, cefovecin, ceftazidime and moxifloxacin as per CLSI guidelines [15]. In addition, all ESC- and FQ-resistant isolates underwent ciprofloxacin and enrofloxacin MIC testing in the presence of the efflux pump inhibitor Phe-Arg-β-naphthylamide (PAβN) at 64 mg/L [17]. Isolates resistant to at least three antimicrobial classes were classed as multidrug-resistant (MDR).

2.3. Molecular characterisation of Escherichia coli resistant to critically important antimicrobials

All ESC- and FQ-resistant isolates underwent PCR-based phylotyping [18], identification of $bla_{CTX\text{-}M}$ and $bla_{CMY\text{-}2}$ genes by PCR and amplicon sequencing [12], plasmid replicon typing [19,20], screening for virulence genes typical of bovine and porcine enterotoxigenic *E. coli* (ETEC) (*f4*, *f5*, *f6*, *f18*, *lt1*, *sta*, *stb* and *stx2e*) [21] and multilocus sequence typing (MLST) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) as previously described. PCR and amplicon-sequencing of the quinolone resistance-determining region (QRDR) for the *gyrA*, *gyrB*, *parC* and *parE* genes and plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB*, *qnrS*, *qepA*, *aac(6')-Ib* and *aac(6')-Ib*-cr were performed as previously described [17,22].

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2.4. Plasmid characterisation

Plasmid characterisation was performed by *S*1 pulsed-field gel electrophoresis (PFGE) and in-gel hybridisation. Genomic DNA in agarose blocks was digested with the restriction enzyme *S*1 (Invitrogen, Abingdon, UK). DNA fragments were separated by PFGE. In-gel hybridisation was done with a bla_{CMY} and $bla_{CTX\text{-}M}$ probe labelled with ^{32}P by random priming using 9-mer oligomers and a commercial kit (Stratagene, Amsterdam, The Netherlands).

2.5. Statistical analysis

The prevalence of resistance amongst the animal species groups was evaluated using Fisher's exact test.

3. Results

3.1. Phenotypic characterisation of antimicrobial resistance

The 324 *E. coli* isolates from food animal sources exhibited a high prevalence of resistance to tetracycline, ampicillin, SXT and streptomycin, whereas none were resistant to imipenem or amikacin (Table 1). Resistance to ESCs (ceftiofur and/or ceftazidime) was detected in five isolates (1.5%) [two bovine (1.2%) and three porcine (2.6%)] (Tables 1 and 2). These ESC-resistant phenotypes were confirmed by MIC testing to ESCs (ceftiofur, ceftriaxone, cefovecin and/or ceftazidime; Table 2). One of the porcine isolates (#03/13/4/59) was also resistant to ciprofloxacin. The FQ-resistant phenotype was confirmed by ciprofloxacin, enrofloxacin, moxifloxacin and pradofloxacin MIC testing (Table 2).

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Overall, in comparison with bovine, ovine and poultry isolates, porcine isolates demonstrated a higher resistance prevalence for most antimicrobial agents (Table 1). These differences between species were statistically significant ($P < 0.05$) for 13 of the 18 antimicrobials evaluated in this study. Porcine isolates demonstrated a remarkably high frequency of resistance to tetracycline, ampicillin, SXT, streptomycin, chloramphenicol, apramycin, florfenicol and gentamicin (Table 1). Among the isolates characterised in this study, 79% of porcine, 33% of poultry, 26% of bovine and 22% of ovine isolates qualified as MDR.

3.2. Molecular characterisation of Escherichia coli resistant to critically important antimicrobials

Molecular characterisation of the ESC-resistant isolates revealed that bla_{CMY-2}, $bla_{CTX-M-14}$ and $bla_{CTX-M-9}$ were the genes that encoded ESC resistance among the *E. coli* isolates in this study. Molecular characterisation also showed that only one isolate (ST100) was a classic porcine ETEC isolate based on the presence of ETEC virulence genes *f4*, *f6*, *lt1* and *stb* (Table 2). The other four isolates each belonged to a distinct clonal lineage such as ST1-D, ST10-A, ST117-F and ST744-A. The ST744-A isolate was also resistant to FQs. This isolate demonstrated known mutations in the QRDRs, including a double mutation in *gyrA* (83Ser→Leu; 87Asp→Asn) and a single mutation in *parC* (80Ser→Ile). In addition, its ciprofloxacin and enrofloxacin MICs showed a two- to four-fold decrease in the presence of the efflux inhibitor PAβN. No PMQR genes were identified.

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3.3. Characterisation of plasmids from Escherichia coli resistant to critically important antimicrobials

Plasmid characterisation by *S*1 PFGE revealed a number of different plasmids among the ESC-resistant *E. coli* isolates. Each ESC-resistant *E. coli* strain contained one to five plasmids (Table 3). Probe hybridisation revealed that *bla*$_{CMY-2}$ and *bla*$_{CTX-M-14}$ were carried on plasmids, whilst *bla*$_{CTX-M-9}$ was present on the chromosomal DNA. One of the strains (#03/13/4/91) also carried *bla*$_{CMY-2}$ on the chromosomal DNA (Table 3). The ESC-resistant plasmids were of different sizes, ranging from 75 kb to 250 kb (Table 3). One of the isolates (#02/13/1/13) appeared to have lost a number of plasmids, including the *bla*$_{CMY-2}$-bearing plasmid, following storage at –80 °C (Table 3). However, this isolate still maintained one of the ESC resistance plasmids containing *bla*$_{CTX-M-14}$.

4. Discussion

Here we report the first detection of ESC- and FQ-resistant *E. coli* from Australian food-producing animals. The presence of *bla*$_{CTXM-14}$, *bla*$_{CTX-M-9}$ and *bla*$_{CMY-2}$ in *E. coli* from food-producing animals is of potential public health significance because of the risk of direct transfer of such strains to humans via the food chain or environment and/or through the mobilisation of plasmids that can potentially transfer the ESC resistance genes to other Gram-negative bacteria, including *Salmonella* spp.

Four of the five ESC-resistant (± FQ-resistant) *E. coli* isolates represented clonal lineages previously isolated both from animals and humans. ST117 strains are important pathogens identified predominantly as avian pathogenic *E. coli* in the USA

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and Europe, but also as a sporadic cause of human extraintestinal infections in Canada, Chile, France, Spain and Brazil [23,24] (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). ST10 is an extremely diverse and broad-host-range lineage causing extraintestinal infections in hospitalised and community-dwelling humans in The Netherlands and Canada [24–26], and is also detected in poultry, wild birds and pigs as well as retail chicken and pork meat [24–27]. ST1 has also been reported to be both a pig and a human pathogen in Germany (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Finally, the ESC- and FQ-resistant strain (ST744) has been identified previously as an extended-spectrum β-lactamase (ESBL)-producing lineage associated with wild birds in Bangladesh and with human extraintestinal infection in Laos [28]. This demonstrates that these ESC-resistant *E. coli* strains are potentially strains that may move bidirectionally between humans and animals.

To our knowledge, these ESC-resistant *E. coli* strains belonging to the sequence types identified in this study have not been reported previously in Australia either from food-producing animals or as a cause of human infection. Their low frequency among clinical isolates from Australian animals suggests that they have potentially been introduced. This plausibly could occur via either human carriers or migratory wild birds due to Australian quarantine restrictions on the importation of livestock and regulation on the use of CIAs in livestock [13,14,25,27,28]. Australia has banned the importation of livestock since the 1970s [14], and FQs cannot legally be administered to food-producing animals in this country [13]. Furthermore, ESC-resistant clones identified in this study have also been detected among humans and birds in other continents as previously described. Therefore, it is unlikely that strains such as

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ST744-A, which is resistant both to ESCs and FQs, evolved from an animal-associated susceptible progenitor strain under local FQ selection pressure.

The detection of a single ESC-resistant porcine ETEC (ST100-A) strain carrying *bla*$_{CMY-2}$ is potentially significant for animal health. Our previous study on porcine ETEC identified ST100 as a prominent clonal lineage among Australian porcine ETEC isolates of serogroup O149, and the majority of these clones are resistant to a wider range of antimicrobials that are regularly used to treat post-weaning diarrhoea [12]. However, none of the O149/ST100 ETEC isolates in that study were ESC-resistant [12]. This is the first time ESC resistance has been reported in Australian porcine ETEC. In Australia, ceftiofur (an ESC) is used as a last-line off-label antimicrobial for parenteral treatment of seriously ill pigs with MDR ETEC infection. Therefore, detection of ESC-resistant porcine O149/ST100 ETEC has potential animal health implications in Australia owing to the limited therapeutic options. Detection of ESC resistance in a single Australian porcine ETEC isolate indicates a need for both ongoing surveillance at the national level and closer scrutiny of the off-label use of ESCs to avoid the spread of ESC-resistant ETEC in Australian pig herds.

Despite the detection of ESC-resistant *E. coli* clones from food-producing animals in Australia, it is difficult to evaluate the public health impact of the detected ESC-resistant *E. coli* clones. This is because there are limited data regarding the CTX-M and CMY types detected among human clinical *E. coli* isolates in Australia. The Australian human Gram-negative surveillance reports the ESC-resistant isolates as CTX-M or CMY without identifying the specific types [29]. Therefore, it is difficult to

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track the movement of ESC-resistant clones and ESC resistance-encoding plasmids from animals to humans. Further work is therefore required to evaluate the public health impact of the ESC-resistant *E. coli* clones identified in this study.

In summary, this study establishes the presence of resistance to CIAs among clinical *E. coli* isolates from Australian food-producing animals, largely attributed to globally disseminated FQ- and ESC-resistant *E. coli* lineages.

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Competing interests: SA and DJT have received research grants and contracts from Zoetis and Novartis; DJ has received funds from Meat and Livestock Australia for research advising on food safety issues in red meat production; JRJ has received

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research grants, contracts or honoraria from Crucell, ICET, Merck, Syntiron and Tetraphase, and has patent applications related to detection of certain *E. coli* strains. All other authors declare no competing interests.

Ethical approval: Not required.

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ACCEPTED MANUSCRIPT

References

[1] World Health Organization. *Antimicrobial resistance: global report on surveillance 2014*. Geneva, Switzerland: WHO; 2014.

[2] Laxminarayan R, Duse A, Wattal C, Zaidi AKM, Wertheim HFL, Sumpradit N, et al. Antibiotic resistance? The need for global solutions. Lancet Infect Dis 2013;13:1057–98.

[3] Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? J Antimicrob Chemother 2014;69:287–91.

[4] Bradford PA, Petersen PJ, Fingerman IM, White DG. Characterization of expanded-spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. J Antimicrob Chemother 1999;44:607–10.

[5] Allen KJ, Poppe C. Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by β-lactamase CMY-2 in *Salmonella* isolated from food-producing animals in Canada. Can J Vet Res 2002;66:137–44.

[6] Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 2012;18:646–55.

[7] Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. Prevalence of extended-spectrum cephalosporinase (ESC)-producing

Accepted Manuscript

ACCEPTED MANUSCRIPT

Escherichia coli in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. J Antimicrob Chemother 2012;67:582–8.

[8] Zheng H, Zeng Z, Chen S, Liu Y, Yao Q, Deng Y, et al. Prevalence and characterisation of CTX-M β-lactamases amongst *Escherichia coli* isolates from healthy food animals in China. Int J Antimicrob Agents 2012;39:305–10.

[9] Wang X-M, Jiang H-X, Liao X-P, Liu J-H, Zhang W-J, Zhang H, et al. Antimicrobial resistance, virulence genes, and phylogenetic background in *Escherichia coli* isolates from diseased pigs. FEMS Microbiol Lett 2010;306:15–21.

[10] Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 2011;24:718–33.

[11] Abraham S, Groves MD, Trott DJ, Chapman TA, Turner B, Hornitzky M, et al. *Salmonella enterica* isolated from infections in Australian livestock remain susceptible to critical antimicrobials. Int J Antimicrob Agents 2014;43:126–30.

[12] Abraham S, Trott DJ, Jordan D, Gordon DM, Groves MD, Fairbrother JM, et al. Phylogenetic and molecular insights into the evolution of multidrug-resistant porcine enterotoxigenic *Escherichia coli* in Australia. Int J Antimicrob Agents 2014;44:105–11.

[13] Cheng AC, Turnidge J, Collignon P, Looke D, Barton M, Gottlieb T. Control of fluoroquinolone resistance through successful regulation, Australia. Emerg Infect Dis 2012;18:1453–60.

[14] Turner AJ. Quarantine, exports and animal disease in Australia 1901–2010. Aust Vet J 2011;89:366–71.

ACCEPTED MANUSCRIPT

[15] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; second informational supplement*. Document VET01-S2. Wayne, PA: CLSI; 2013.

[16] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement*. Document M100-S24. Wayne, PA: CLSI; 2014.

[17] Platell JL, Cobbold RN, Johnson JR, Heisig A, Heisig P, Clabots C, et al. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. Antimicrob Agents Chemother 2011;55:3782–7.

[18] Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 2013;5:58–65.

[19] Abraham S, Chapman TA, Zhang R, Chin J, Mabbett AE, Totsika M, et al. Molecular characterization of *Escherichia coli* that cause symptomatic and asymptomatic urinary tract Infections. J Clin Microbiol 2012;50:1027–30.

[20] Carattoli A, Bertini A, Villa L, Falbo V, Hopkins K, Threlfall E. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005;63:219–28.

[21] Casey TA, Bosworth BT. Design and evaluation of a multiplex polymerase chain reaction assay for the simultaneous identification of genes for nine different virulence factors associated with *Escherichia coli* that cause diarrhea and edema disease in swine. J Vet Diagn Invest 2009;21:25–30.

ACCEPTED MANUSCRIPT

[22] Gibson JS, Cobbold RN, Kyaw-Tanner MT, Heisig P, Trott DJ. Fluoroquinolone resistance mechanisms in multidrug-resistant *Escherichia coli* isolated from extraintestinal infections in dogs. Vet Microbiol 2010;146:161–6.

[23] Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis 2010;16:88–95.

[24] Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, et al. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg Infect Dis 2012;18:415–21.

[25] Manges AR, Johnson JR. Food-borne origins of *Escherichia coli* causing extraintestinal infections. Clin Infect Dis 2012;55:712–9.

[26] Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 2011;17:873–80.

[27] Poirel L, Potron A, De La Cuesta C, Cleary T, Nordmann P, Munoz-Price LS. Wild coastline birds as reservoirs of broad-spectrum-β-lactamase-producing Enterobacteriaceae in Miami Beach, Florida. Antimicrob Agents Chemother 2012;56:2756–8.

[28] Hasan B, Sandegren L, Melhus A, Drobni M, Hernandez J, Waldenstrom J, et al. Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry, Bangladesh. Emerg Infect Dis 2012;18:2055–8.

[29] Turnidge JD, Gottlieb T, Mitchell DH, Coombs GW, Daley DA, Bell JM; Australian Group on Antimicrobial Resistance. Community-onset Gram-

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negative surveillance program annual report, 2012. Commun Dis Intell Q Rep 2014;38:E54–8.

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Table 1

Percentage of clinical *Escherichia coli* isolates from different food animal species expressing phenotypic resistance to each of 18 antimicrobials

Antimicrobial	Frequency of resistance (%)				*P*-value [a]
	Bovine (*n* = 169)	Ovine (*n* = 9)	Porcine (*n* = 114)	Poultry (*n* = 32)	
AMC	4.14	0.00	14.91	0.00	0.008
AMK	0.00	0.00	0.00	0.00	N/A
AMP	39.05	22.22	71.05	9.38	<0.001
APM	0.59	0.00	34.21	3.13	<0.001
CAZ	0.59	0.00	0.88	0.00	1.000
CEF	8.28	0.00	24.56	6.25	0.005
CFT	1.18	0.00	2.63	0.00	0.693
CHL	1.18	11.11	44.74	0.00	<0.001
CIP	0.00	0.00	0.88	0.00	0.480
FFC	0.59	0.00	26.32	3.13	<0.001
FOX	2.37	0.00	11.40	0.00	0.012
GEN	1.18	0.00	28.95	3.13	<0.001
IPM	0.00	0.00	0.00	0.00	N/A
NEO	17.16	11.11	35.96	3.13	0.002
SPT	0.59	0.00	21.93	0.00	<0.001
STR	26.04	33.33	69.30	21.88	<0.001
SXT	23.08	11.11	67.54	37.50	<0.001
TET	28.99	33.33	88.60	75.00	<0.001

AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; APM, apramycin; CAZ, ceftazidime; CEF, cefalotin; CFT, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; FFC, florfenicol; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; NEO, neomycin; SPT, spectinomycin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; N/A, not applicable.

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[a] *P*-value tests the equality of prevalence of resistance for each drug across all animal species.

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Table 2

Minimum inhibitory concentrations (MICs) and molecular characteristics of extended-spectrum cephalosporin (ESC)-resistant *Escherichia coli* from Australian food-producing animals

Isolate ID	Source		MIC (mg/L)										ESC resistance gene	ETEC VGs	Plasmid replicon (Inc)	Phylotype	ST
	Host	Site/specimen	CFT	CVN	CAZ	CTX	MOX	PRD	CIP	CIP + EPI	ENR	ENR + EPI					
#02/13/1/13	Calf	Liver	>64	>64	>64	>64	0.06	0.008	0.008	<0.004	0.03	<0.004	*bla*$_{CTX-M-14}$, *bla*$_{CMY-2}$	None found	FIB, I1	F	ST117
#01/13/2/25	Calf	Faeces	64	16	0.25	32	0.06	0.015	0.008	0.004–0.008	0.03	<0.004	*bla*$_{CTX-M-9}$	None found	None found	A	ST10
#03/13/4/59	Pig	Small intestine	>64	>64	4	>64	16	4	16	4	32	2	*bla*$_{CTX-M-14}$	None found	FIB, A/C, Y, I1	A	ST744

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#03/13/4/91	Pig	Colon	32	>64	64	32	0.06	0.015	0.015	N/G	0.06	N/G	*bla*$_{CMY-2}$	*f4*, *f6*, *lt1*, *stb*	FIC, FIB	A	ST100
#03/13/4/134	Pig	Small intestine	16	>64	64	32	0.015–0.03	0.004	0.008	<0.004	0.015	<0.004	*bla*$_{CMY-2}$	None found	B/O, I1	D	ST1

CFT, ceftiofur; CVN, cefovecin; CAZ, ceftazidime; CTX, ceftriaxone; MOX, moxifloxacin; PRD, pradofloxacin; CIP, ciprofloxacin; EPI, efflux pump inhibitor (Phe-Arg-β-naphthylamide at 64 mg/L); ENR, enrofloxacin; ETEC VGs, enterotoxigenic *E. coli* virulence genes; ST, sequence type; N/G, no growth.

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Table 3

Plasmid characteristics of extended-spectrum cephalosporin-resistant *Escherichia coli* from Australian food-producing animals

Isolate ID	*bla*$_{CTX-M}$	*bla*$_{CMY-2}$	Chromosomal	*bla*$_{CMY-2}$ plasmid size	*bla*$_{CTX-M}$ plasmid size	No. of plasmids
#01/13/2/25	+		*bla*$_{CTX-M-9}$			2
#03/13/4/134		+		75 kb		4
#03/13/4/59	+				140 kb (*bla*$_{CTX-M-14)}$	4
#03/13/4/91		+	*bla*$_{CMY-2}$	200 kb		5
#02/13/1/13	+	+		100 kb	250 kb (*bla*$_{CTXM-14)}$	5
#02/13/1/13 [a]	+				250 kb (*bla*$_{CTXM-14)}$	1

[a] This clone demonstrated different colony morphology after storage.

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