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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 disc 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.

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_{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*

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isolated from livestock has been attributed to the emergence and spread of plasmid-mediated bla_{CTX-M} genes and bla_{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 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 disc 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, 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-M} and bla_{CMY-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].

2.4. Plasmid characterisation

Plasmid characterisation was performed by *S1* pulsed-field gel electrophoresis (PFGE) and in-gel hybridisation. Genomic DNA in agarose blocks was digested with the restriction enzyme *S1* (Invitrogen, Abingdon, UK). DNA fragments were separated by PFGE. In-gel hybridisation was done with a bla_{CMY} and bla_{CTX-M} probe labelled with 32P 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,

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.

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