Antibiotic resistance among verocytotoxigenic *Escherichia coli* (VTEC) and non-VTEC isolated from domestic animals and humans

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Two hundred verocytotoxigenic and 216 non-verocytotoxigenic *Escherichia coli* (VTEC and non-VTEC), isolated from a variety of sources were tested for their resistances to 11 antimicrobial agents. The strains included isolates from domestic food animals and both symptomatic and asymptomatic infections in man. A much higher level of resistance was found among the non-VTEC than among the VTEC, regardless of source. The resistant VTEC isolated from animals were predominantly from specimens associated with sick animals. Antibiotic resistance was detected in only four of the 59 (6.8 %) VTEC of human origin, whereas more of the human non-VTEC possessed antibiotic resistance determinants. It was particularly noteworthy that 24/87 (28 %) strains isolated from healthy babies, who had neither contact with antibiotics nor had gastrointestinal symptoms for at least 2 weeks prior to the specimen being taken, were resistant to one or more of the antibiotics tested.

Received 15 February 2002

Accepted 7 October 2002

INTRODUCTION

Extensive studies over the years, both by ourselves and others around the world, have shown that verocytotoxigenic *Escherichia coli* (VTEC) are commonly isolated from the faeces of ruminants such as cattle and sheep (Beutin *et al.*, 1993; Djordjevic *et al.*, 2001; Dorn *et al.*, 1989; Fagan *et al.*, 1999; Hornitzky *et al.*, 2001; Kudva *et al.*, 1997; Richter *et al.*, 1997; Wells *et al.*, 1991). In addition, there has been increasing concern of the possible development of resistance to antimicrobial agents in the *Enterobacteriaceae*, especially *E. coli*, as a result of the use of such agents in animal feed (Willis, 2000). With the recent emergence of strains of *Salmonella* Typhimurium DT104, resistant to four or more antimicrobial agents (Threlfall *et al.*, 1997), the problem of such resistant organisms evolving and being transmitted through the food chain from cattle and sheep to man is becoming increasingly important throughout the world (Glynn *et al.*, 1998; Levy & Cruz, 1999). In his review, Willis (2000) concludes that 'there is now good evidence that the use of antibiotics in agriculture is contributing to the problem of antibiotic resistance amongst pathogenic bacteria.'

In her book, Garrett (1995) discusses the problems associated with the acquisition of antibiotic resistance factors by the intestinal organisms of domestic animals, as a result of the use of antibiotics in animal husbandry. She then discusses the emergence of VTEC, especially *E. coli* O157 : H7, and suggests that these may well also have emerged due to such antibiotic use. It seemed that if this hypothesis were true then VTEC isolated from cattle and sheep would be resistant to antibiotics. It was specifically to address this question that this study was initiated, especially as there are few studies in which the antibiotic sensitivities of VTEC have been determined. Most of these deal with human VTEC. Zhao *et al.* (2001) demonstrated that 39/50 (78 %) VTEC, mainly O157 : H7, but also including a range of serotypes, exhibited resistance to two or more antimicrobial classes. Most of these were isolated from cattle or ground beef. Only eight of the strains were isolated from man. Multiple resistances to streptomycin, sulfamethoxazole and tetracycline were most often observed. Stephan & Kuhn (1999) identified VTEC in 4/145 (2.8 %) *E. coli* isolates from cows with mastitis. One isolate showed multiple antibiotic resistances to ampicillin, neomycin, tetracycline and sulphonamide. Earlier studies in Spain (Gonzales & Blanco, 1989) of VTEC and non-VTEC isolated from calves with diarrhoea showed that the majority (81 %) of VTEC were resistant to at least one of six antimicrobial agents tested with 41 % being resistant to five

Abbreviations: HC, haemorrhagic colitis; HUS, haemolytic uraemic syndrome; UTI, urinary tract infection; VT, verocytotoxigenic; VTEC, verocytotoxigenic *Escherichia coli*.

antimicrobial agents. There appear to have been no studies reported on antimicrobial sensitivities of ovine VTEC.

The paucity of data on the resistance of VTEC to antibiotics prompted us to determine the extent to which VTEC isolated from domestic animals, especially cattle and sheep, and man were resistant to a range of antibiotics. These resistance patterns were then compared to those produced by non-VTEC isolated from the same species.

METHODS

Strains of *E. coli*. The 416 *E. coli* strains used in this study were all from the culture collection of the National *Escherichia coli* Reference Laboratory, Microbiological Diagnostic Unit, Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne, Australia. The selection was largely based on availability, and in attempting to obtain a reasonable basis for the comparison between VTEC and non-VTEC, to also test as great a variety of sources and serotypes as possible.

Most of the 109 bovine and 53 ovine strains were from earlier studies (Fagan *et al.*, 1999; Djordjevic *et al.*, 2001; Hornitzky *et al.*, 2001). They also included seven bovine strains from Hong Kong (Leung *et al.*, 2001). The bovine strains included 16 strains isolated from the faeces of healthy pre-slaughter pasture beef cattle, 38 from healthy pre-slaughter feedlot cattle and 23 from faecal samples submitted from cattle for diagnostic purposes. Also included were 20 isolates from the rumens of healthy cattle and five beef isolates. Of the 53 ovine strains, 13 were from pre-slaughter adult sheep, 37 from pre-slaughter lambs and three from ovine diagnostic samples. Of the 47 porcine strains, 27 were from samples submitted from pigs for diagnostic purposes and 20 were from healthy pigs. All these animal strains had been isolated in the years 1996–2001 except the diagnostic porcine samples, some of which had been isolated in the 1970s and the remainder in the 1990s.

The 207 human strains were all collected as part of the work of the laboratory (Bettelheim, 2001). The 87 strains from healthy babies had been collected between 1989 and 1992 from healthy infants under 1 year of age who had not had gastrointestinal symptoms or been treated with antibiotics for any reason for at least 2 weeks prior to obtaining the specimen. Apart from age and gender, these were the only data we were ethically permitted to take. Of the remaining 120 strains, which had all been isolated during the last 6 years, 12 were from healthy adults, 29 were isolated from cases of diarrhoea, 13 were from cases of haemorrhagic colitis (HC), 33 were from cases of haemolytic uraemic syndrome (HUS), one from a case of human septicæmia and 10 from urinary tract infections (UTIs). For 22 human isolates, the symptoms were not adequately given.

The bovine non-VTEC were obtained from Dr C. S. McSweeney, CSIRO, Longpocket, Brisbane, Australia. Apart from the seven bovine strains from Hong Kong, all other strains were isolated in Australia.

Characterization of *E. coli*. The strains were characterized as *E. coli* on the basis of their reactions in triple-sugar-iron (TSI) agar (Oxoid), motility-indole-ornithine (MIO) medium (Oxoid), ONPG (Oxoid) and urease broth (Oxoid). Strains were considered to be *E. coli* if the results obtained with these tests conformed to the description of the species. Those producing aberrant results were subjected to additional tests. The *E. coli* were then O and H serotyped (Bettelheim & Thompson, 1987; Chandler & Bettelheim, 1974). Supernate preparations were tested on Vero cells (Konowalchuk *et al.*, 1977) and also tested by ELISA using mAbs 13C4 and 11E10 directed against VT1 and VT2, respectively (Acheson *et al.*, 1990).

Antimicrobial testing. The *E. coli* isolates were tested for their sensitivity to a range of antimicrobials by the plate/replicator method. The antimicrobials used were ampicillin (32 mg ml⁻¹), streptomycin (25 mg l⁻¹), tetracycline (20 mg l⁻¹), chloramphenicol (10 mg l⁻¹), sulphathiazole (550 mg l⁻¹), trimethoprim (50 mg l⁻¹), kanamycin (10 mg l⁻¹), nalidixic acid (50 mg l⁻¹), spectinomycin (50 mg l⁻¹), gentamicin (2.5 mg l⁻¹) and ciprofloxacin (2 mg l⁻¹). The results obtained were treated as epidemiological markers and the *E. coli* were recorded as resistant to an antimicrobial rather than sensitive to the antimicrobial. The antimicrobials were incorporated in lysed blood isosensitest agar (Oxoid). Plates and cultures were tested by a method similar to that used for MIC using a Clements antibiotic sensitivity replicator with 32 prongs.

To perform the test, the *E. coli* and controls were grown up in double strength nutrient broth (Oxoid) in an orbital shaker at 100 r.p.m. for 1.5 h, at 37 °C. The organisms were then diluted 1:10 (v/v) in tryptone water (Oxoid) and added to the wells of the antibiotic sensitivity replicator, which were filled with 0.5 ml nutrient broth containing 0.05 % agar. The *E. coli* were inoculated, by means of the replicator, onto each of the antimicrobial agar plates and then incubated at 37 °C overnight. The plates were examined the following day and no (or very little) growth of the *E. coli* on the plate was recorded as demonstrating sensitivity to that specific antimicrobial. This method had originally been developed to determine the resistance patterns of *Salmonella* isolates, therefore, two standard strains, *Salmonella* Heidelberg and *Salmonella* Hadar with known resistances were used as positive controls and a fully sensitive strain of *Salmonella* Typhimurium was used as negative control. All strains were tested against all the antimicrobials listed above; however, only the strains for which antibiotic resistances were observed are listed in Tables 2 and 3.

RESULTS

Relationship of antibiotic resistance to verotoxigenicity of *E. coli*

Of the 416 *E. coli* used in this study, 200 (48.1 %) were VTEC and 216 (51.9 %) were non-VTEC. Thirty-one of 200 (15.5 %) of the VTEC and 70/216 (32.9 %) of the non-VTEC were resistant to at least one antibiotic (Table 1). Of the VTEC isolates, which showed antibiotic resistance, 5/31 (16.1 %) were of bovine origin and these were all isolated from diagnostic samples. None was identified in sheep. Seventy-one percent (22/31) of porcine origin were resistant and consisted of 7/11 (63.6 %) from healthy pigs and 15/20 (75.0 %) from porcine diagnostic samples. Four of 59 (6.8 %) resistant VTEC were of human origin. Similar ranges of resistance patterns were observed with the porcine isolates from the 1970s and 1990s.

Of the non-VTEC, 71/216 (32.9 %) showed antibiotic resistance. Two of 26 (7.7 %) were of bovine origin, 1/26 (3.8 %) was of ovine origin and 14/16 (87.5 %) were from pigs (eight healthy and six porcine diagnostic samples). Of the human isolates 54/148 (36.5 %) showed resistance (Table 1).

Resistance patterns of VTEC

Tetracycline (either alone or combined with other antibiotic resistances) was the most common antibiotic against which VTEC showed resistance (17/31; 54.8 %) and was found in isolates from human infection (2), sick pigs (8), healthy pigs

Table 1. Relationship of antibiotic resistances to verocytotoxinogenicity of *E. coli* isolates

Source of strains		No. of strains VT-negative and fully sensitive	No. of strains VT-positive and fully sensitive	No. of strains VT-negative with at least one resistance	No. of strains VT- positive with at least one resistance	Total no. of strains tested
Bovine	Bovine diagnostic samples	1	16	1	5	23
	Healthy cattle	23	62	1	0	86
Ovine	Ovine diagnostic samples	0	3	0	0	3
	Healthy sheep	25	24	1	0	50
Porcine	Porcine diagnostic samples	1	5	6	15	27
	Healthy pigs	1	4	8	7	20
Human	Healthy human	5	2	5	0	12
	Symptomatic human	26	53	25	4	108
	Healthy babies	63	0	24	0	87
Total		145	169	71	31	416

(6) and a bovine diagnostic isolate. Resistance to both streptomycin and sulphathiazole was noted in 15/31 (48.4%) and was the second most frequently found resistance pattern, often in combination with other resistances. This resistance was found in VTEC from bovine diagnostic samples (4), human infections (3) and sick pigs (8). Chloramphenicol resistance was only detected in VTEC from porcine diagnostic samples (Table 2).

Resistance patterns of non-VTEC

Antibiotic resistance was more commonly found in non-VTEC isolates from sick animals and man. Non-VTEC were most commonly resistant to sulphathiazole (44/216; 20.4%), ampicillin (40/216; 18.5%) and tetracycline (34/216; 15.7%). Resistances to nalidixic acid and ciprofloxacin were the least prevalent, being found in one strain from a human UTI case, which also had six other resistances.

DISCUSSION

The main aim of this study was to ascertain to what extent VTEC isolated from cattle and sheep, which may enter the food chain, are resistant to antibiotics. While it is currently not considered appropriate to treat VTEC infections, such as HUS with antibiotics (Wong *et al.*, 2000; Zimmerhackl, 2000), there are significant disadvantages for VTEC infections to be caused by multiple resistant strains. In the presence of antibiotics such strains may have a selective advantage and therefore be more likely to cause an outbreak.

Of the 109 bovine *E. coli* isolates studied, of which 84 were VTEC, antibiotic resistance was detected in only five of these strains (Table 1). These five strains were all isolated from sick animals with gastrointestinal conditions. It is further noteworthy, that three of these strains belonged to serotypes O5:H- and O111:H8 (Table 2), which are rarely encountered among healthy cattle in Australia (M. A. Hornitzky and others, unpublished). However, VTEC with serotype O5:H- have been more frequently recovered from faeces

from cattle with diarrhoea in Germany (Wieler *et al.*, 1996). VTEC of serotype O5:H- have been commonly isolated from healthy sheep (Djordjevic *et al.*, 2001; Ramachandran *et al.*, 2001). Strains belonging to the third VTEC serotype, O26:H11, have been commonly isolated from cattle (Hornitzky *et al.*, 2001) and rarely from healthy sheep (Djordjevic *et al.*, 2001; Ramachandran *et al.*, 2001). These three VTEC serotypes have been associated with human disease around the world. Interestingly, none of the 38 *E. coli* isolates, of which 36 were VTEC, recovered from feedlot cattle displayed antibiotic resistance. Of the two bovine non-VTEC resistant to antibiotics, one was also isolated from a bovine diagnostic sample and the second was isolated from a beef specimen (Table 3). The former belonged to a serotype not reported in the literature as associated with human disease and the latter to an O-untypable group; therefore, no further conclusions can be drawn.

These results suggest that bovine *E. coli* from healthy Australian cattle do not present a high risk of spreading antibiotic-resistant *E. coli* to man. Our data are in contrast to high levels (78 and 49%) of antibiotic-resistant VTEC recently reported by Zhao *et al.* (2001) and Khan *et al.* (2002). However, given the high rates of antibiotic-resistant VTEC from diarrhoeagenic calves reported in Spanish studies (Gonzales & Blanco, 1989), further studies of *E. coli* isolates from cattle with gastrointestinal diseases are needed to determine if such isolates pose the greatest risk of developing multiple antibiotic resistances. These conclusions are even more appropriate when examining the antibiotic sensitivity of 53 ovine isolates in this study (Table 1). All but one strain, a non-VTEC, were fully sensitive to all antibiotics used. This strain from a healthy adult sheep (Table 3) belonged to serotype O117:H21, which has not been associated with human disease.

Compared with cattle and sheep, there were a greater number of antibiotic-resistant VTEC as well as non-VTEC isolated from pigs (Tables 2 and 3). However, the majority (21/27; 77.8%) were isolated from sick animals. Fourteen of 27

Table 2. Antibiotic resistance patterns of VTEC isolates

Abbreviations: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; Su, sulphathiazole; Tm, trimethoprim; K, kanamycin; Sp, spectinomycin. Symbols: +, resistant; –, sensitive.

Source of strains		Serotype	No. of isolates	Resistance to:								No. of resistances
				A	S	T	C	Su	Tm	K	Sp	
Bovine	Bovine diagnostic samples	O104:H11	1	+	+	+	–	+	–	+	–	5
	Bovine diagnostic samples	O5:H-	1	+	+	–	–	+	+	–	–	4
	Bovine diagnostic samples	O26:H11	1	+	+	–	–	+	–	+	–	4
	Bovine diagnostic samples	O5:H-	1	+	+	–	–	+	–	–	–	3
	Bovine diagnostic samples	O111:H8	1	–	–	–	–	–	+	–	–	1
Human	Human diarrhoea	O111:H-	1	–	–	+	–	–	–	–	–	1
	Human HC	O26:H-	1	+	+	+	–	+	+	+	–	6
	Human HUS	O82:H8	1	+	+	–	–	+	–	–	+	4
	Human HUS	O18ac:H7	1	+	+	–	–	+	–	–	–	3
Porcine	Healthy pig	O157:H-	5	–	–	+	–	–	–	–	–	1
	Healthy pig	O130:H11	1	–	–	–	–	–	–	+	–	1
	Healthy pig	O75:H-	1	–	–	+	+	–	–	–	–	2
	Porcine diagnostic samples	Ont:H18	1	+	+	+	–	+	+	+	–	6
	Porcine diagnostic samples	OR:H4	1	–	+	+	–	+	–	+	+	5
	Porcine diagnostic samples	O141:H-	1	–	+	+	–	+	–	–	+	4
	Porcine diagnostic samples	Ont:H4	1	+	+	+	–	–	–	+	–	4
	Porcine diagnostic samples	OR:H4	1	–	+	–	+	+	–	–	+	4
	Porcine diagnostic samples	O141:H4	1	–	+	–	–	+	–	–	+	3
	Porcine diagnostic samples	Ont:H9	1	–	+	–	–	+	–	–	+	3
	Porcine diagnostic samples	OR:H4	1	–	–	+	–	+	–	–	–	2
	Porcine diagnostic samples	Ont:H-	3	–	–	+	+	–	–	–	–	2
	Porcine diagnostic samples	OR:H4	2	–	+	–	–	+	–	–	–	2
	Porcine diagnostic samples	OR:H4	2	–	+	–	–	–	–	–	–	1

(51.9 %) of these were resistant to tetracycline, an antibiotic commonly used in the Australian pig industry either as a growth promotant or for the control of enteric and respiratory diseases (Johnston, 1987). Thirteen of these 27 (48.1 %) porcine *E. coli* isolates were resistant to kanamycin. Neomycin, a structurally closely related molecule, has been routinely used as a broad-spectrum antimicrobial for the treatment of enteritis in the Australian pig industry (Johnston, 1987). Certain VTEC are accepted porcine pathogens and it is therefore not surprising to find resistant strains presumably as a result of veterinary therapy. Only seven VTEC isolates, three of which were resistant to one or more antibiotics, were recovered from healthy swine in this study. Further studies are required to establish the level of antibiotic resistance among *E. coli* derived from healthy swine. A recent study (Schroeder *et al.*, 2002) on *E. coli* O157 isolates from humans, cattle, swine and food showed a similar tendency with the highest number of resistant strains being of porcine origin, with those of human and bovine origin having a low number of resistance determinates. This contrasts with results reported among VTEC recovered from human infections in England and Wales between 1995 and 1998 where approximately 20 % of these strains were resistant to at least one antimicrobial agent (Willshaw *et al.*, 2001). It is likely that the health status of meat-producing animals prior to slaughter,

animal husbandry and herd management practices are likely to impinge significantly on the ability of antibiotic-resistant bacteria to make their way into the human food chain.

The adult human isolates which were studied in parallel included strains isolated from cases of UTI, where antibiotic treatment is the rule (Sotto *et al.*, 2001) as well as strains from cases of HUS, where it is not. Only four VTEC out of the 59 human VTEC isolated were found with antibiotic resistance (Table 1). These included two serotypes, O26:H- and O111:H-, which are commonly isolated from human VTEC infections around the world and interestingly were also shown to be resistant when isolated from cattle in this study. The other two antibiotic-resistant VTEC were found to belong to rarer serotypes O82:H8 and O18ac:H7.

A much higher percentage of resistant human *E. coli* (36.5 %; 54/148) isolates compared to animal isolates (25 %; 17/68) was noted among the non-VTEC (Table 3). Many of these antibiotic-resistant human isolates belonged to serotypes commonly associated with human infections such as UTI (Bettelheim, 1997) and enteropathogenic *E. coli*. Nonetheless, many of these isolates demonstrated multiple resistances to antibiotics. Of interest was the observation that 37/54 (66 %) resistant isolates were resistant to ampicillin, an antibiotic where resistance was poorly recorded among

Table 3. Antibiotic resistance patterns of non-VTEC isolates

Abbreviations: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; Su, sulphathiazole; Tm, trimethoprim; K, kanamycin; Na, nalidixic acid; G, gentamicin; Cp, ciprofloxacin; Sp, spectinomycin. Symbols: +, resistant; –, sensitive.

Source of strains		Serotype	No. of isolates	Resistance to:											No. of resistances	
				A	S	T	C	Su	Tm	K	Na	G	Cp	Sp		
Bovine	Bovine diagnostic sample	O21 : H21	1	—	—	+	—	—	—	—	—	—	—	—	1	
	Bovine meat	Ont : H21	1	—	+	+	+	+	—	—	—	—	—	+	5	
Ovine	Healthy sheep	O117 : H21	1	—	+	+	—	+	—	—	—	—	—	—	3	
Porcine	Healthy pig	Ont : H11	4	—	+	+	—	+	—	+	—	—	—	+	5	
	Healthy pig	Ont : H11	1	—	—	+	—	+	—	+	—	—	—	+	4	
	Healthy pig	O157 : H32	1	+	—	+	+	—	—	—	—	—	—	—	3	
	Healthy pig	O126 : H11	1	—	+	+	—	+	—	—	—	—	—	—	3	
	Healthy pig	O75 : H-	1	—	—	+	+	—	—	—	—	—	—	—	2	
	Porcine diagnostic samples	O157 : H-	1	—	+	+	—	+	+	+	—	+	—	—	7	
	Porcine diagnostic samples	O149 : H-	1	—	—	+	+	+	+	+	—	—	—	+	6	
	Porcine diagnostic samples	O21 : H-	1	+	+	+	—	—	—	+	—	—	—	+	5	
	Porcine diagnostic samples	O157 : H19	1	—	+	+	—	+	—	—	—	—	—	+	4	
	Porcine diagnostic samples	O157 : H32	1	+	—	+	+	—	—	—	—	—	—	—	3	
	Porcine diagnostic samples	O20 : H-	1	—	—	+	—	—	—	+	—	—	—	—	2	
	Human	Human symptoms unknown	O111 : H2	1	+	+	—	—	+	+	+	—	+	—	—	6
		Human symptoms unknown	O6 : Hnt	1	+	+	+	—	+	+	—	—	—	—	—	5
		Human symptoms unknown	O111 : H4	1	+	+	+	—	+	+	—	—	—	—	—	5
		Human symptoms unknown	O5 : H4	1	—	—	+	—	+	—	—	—	—	—	—	2
		Human symptoms unknown	O55 : H-	1	—	+	—	—	+	—	—	—	—	—	—	2
		Human symptoms unknown	O4 : H-	1	+	—	—	—	—	—	—	—	—	—	—	1
		Human symptoms unknown	O157 : H-	1	+	—	—	—	—	—	—	—	—	—	—	1
		Human diarrhoea	O111 : H2	1	+	+	—	—	—	+	—	—	+	—	—	4
Human diarrhoea		O111 : H4	1	—	+	+	—	—	—	—	—	—	—	—	2	
Human diarrhoea		O123 : H-	1	—	—	+	—	+	—	—	—	—	—	—	2	
Human diarrhoea		O111 : H8	1	—	—	+	—	—	—	—	—	—	—	—	1	
Human diarrhoea		O111 : H-	1	—	—	+	—	—	—	—	—	—	—	—	1	
Human HC		O55 : H5	1	+	+	+	+	+	+	—	—	—	—	—	6	
Human HUS		O6 : H-	1	+	+	—	—	+	—	—	—	—	—	—	3	
Human HUS		O6 : H1	1	+	—	—	—	+	—	—	—	—	—	+	3	
Human HUS		O11 : H-	1	—	—	—	—	—	+	—	—	—	—	—	1	
Human HUS		O86 : H18	1	+	—	—	—	+	—	—	—	—	—	—	2	
Human UTI		O2 : H-	1	—	—	—	—	—	+	—	—	—	—	—	1	
Human UTI		O6 : H1	1	+	+	—	—	+	—	—	—	—	—	+	4	
Human UTI		O6 : H1	1	+	—	+	—	—	+	—	—	—	—	—	3	

Continued on following page

Table 3. cont.

Source of strains	Serotype	No. of isolates	Resistance to:											No. of resistances
			A	S	T	C	Su	Tm	K	Na	G	Cp	Sp	
Human UTI	O6:H1	1	—	+	—	—	+	+	—	—	—	—	—	3
Human UTI	O6:H1	1	+	—	—	—	+	—	—	—	—	—	+	3
Human UTI	O20:H-	1	—	—	+	—	+	+	—	—	—	—	—	3
Human UTI	O25:H-	1	+	—	+	—	+	+	—	—	—	—	—	4
Human UTI	O172:H23	1	+	—	+	+	—	+	+	+	+	+	—	8
Human healthy baby	O4:H1	1	+	—	+	—	+	+	—	—	—	—	—	4
Human healthy baby	OR:H2	1	+	+	—	—	+	—	—	—	—	—	—	3
Human healthy baby	Ont:H-	1	+	+	—	—	+	—	—	—	—	—	—	3
Human healthy baby	O6:H31	1	+	—	+	—	+	—	—	—	—	—	—	3
Human healthy baby	O25:H4	1	+	—	+	—	+	—	—	—	—	—	—	3
Human healthy baby	O6:H1	1	—	+	—	—	+	+	—	—	—	—	—	3
Human healthy baby	OR:H4	1	—	+	—	—	+	—	—	—	—	—	+	3
Human healthy baby	O1:H7	1	+	—	+	—	—	—	—	—	—	—	—	2
Human healthy baby	O2:H1	1	+	—	—	—	+	—	—	—	—	—	—	2
Human healthy baby	O2:H4	1	+	—	+	—	—	—	—	—	—	—	—	2
Human healthy baby	O4:H5	1	+	—	—	—	+	—	—	—	—	—	—	2
Human healthy baby	O6:H-	2	+	—	—	—	+	—	—	—	—	—	—	2
Human healthy baby	O21:H-	1	+	—	—	—	—	+	—	—	—	—	—	2
Human healthy baby	O25:H1	2	+	—	—	—	+	—	—	—	—	—	—	2
Human healthy baby	Ont:H1	1	+	—	—	—	+	—	—	—	—	—	—	2
Human healthy baby	Ont:H1	1	—	+	—	—	+	—	—	—	—	—	—	2
Human healthy baby	OR:H34	1	+	—	+	—	—	—	—	—	—	—	—	2
Human healthy baby	O7:H15	1	+	—	—	—	—	—	—	—	—	—	—	1
Human healthy baby	O11:H-	1	+	—	—	—	—	—	—	—	—	—	—	1
Human healthy baby	O62:H48	1	+	—	—	—	—	—	—	—	—	—	—	1
Human healthy baby	O81:H-	1	—	—	+	—	—	—	—	—	—	—	—	1
Human healthy baby	O154:H-	1	+	—	—	—	—	—	—	—	—	—	—	1
Human healthy adult	Ont:H18	1	+	+	—	+	+	+	—	—	—	—	—	5
Human healthy adult	O75:H7	1	+	—	—	—	+	—	—	—	—	—	+	3
Human healthy adult	O6:H1	1	—	—	—	—	+	—	—	—	—	—	+	2
Human healthy adult	O16:H6	1	—	—	—	—	+	—	—	—	—	—	—	1
Human healthy adult	O120:H4	1	—	—	—	—	—	+	—	—	—	—	—	1

animal isolates. This trend was even more striking among the infant isolates, where 22/29 (75.9 %) were resistant to ampicillin.

The high prevalence of antibiotic resistance among the strains isolated from healthy babies under 1 year of age, 24/87 (28 %), who had not had contact with antibiotics for at least 2 weeks prior to the specimen being taken, may be considered surprising (Bettelheim *et al.*, 1974a). It is suggested that such resistant *E. coli* originated from the mother's faecal flora or from the hospital environment and nursing staff (Bettelheim *et al.*, 1974b) at the time the baby was born, although it is possible that resistant strains persisted longer than 2 weeks in babies that may have been treated with antibiotics. Alternatively, these babies may have become colonized from food containing antibiotic-resistant organisms or from the environment. Studies on the acquisition of *E. coli* by neonates carried out in the United Kingdom (Bettelheim *et al.*, 1974a, b, c), where antibiotic resistance was used as an additional phylogenetic marker, demonstrated that 33/102 (32.4 %) isolates were resistant to at least one of the antibiotics tested. This pattern of antibiotic resistance observed in the neonates was similar to the pattern of resistance in isolates recovered from the mothers. This suggests that infants may acquire antibiotic-resistant *E. coli* from their mothers, which then remain a part of their microbial flora for considerable periods after birth. The percentage (32.4 %) of resistance found then is within the same order of magnitude as that found in the current study (28 %) and suggests that a quarter to a third of *E. coli* resident in the human intestinal tract at any one time may carry antibiotic resistance characteristics. Consistent with these observations, a recent study of the presence of integron-associated resistance in *Enterobacteriaceae* recovered from patients recently admitted to hospital showed that 19 % carried integrons (Leverstein-van Hall *et al.*, 2002). These authors concluded that integrons, most probably acquired from bacteria linked to the food chain, are widespread in the community. A similar Australian study reported that 59/120 human urinary isolates belonging to the *Enterobacteriaceae*, of which 90 were *E. coli*, possessed integrons containing cassettes that confer resistance to trimethoprim and aminoglycosides (White *et al.*, 2001). Studies by Lidin-Janson *et al.* (1977) reported that 102/709 (14.4 %) *E. coli* recovered from 771 rectal swabs from healthy school children were resistant to antibiotics and these authors concluded that the resistant strains were likely to be animal-derived strains. These studies highlight the need to determine baseline prevalence rates of antibiotic-resistant bacteria and the genes responsible for conferring resistance among healthy adult humans.

These studies strongly suggest that the development of antibiotic-resistant *E. coli*, either VTEC or non-VTEC, should not be a major concern under the current Australian animal husbandry regime for cattle and sheep. However, more extensive studies are required to establish the levels of antibiotic resistance among populations of bacteria from meat-producing animals across a range of production systems. The identification of resistant VTEC belonging to

serotypes (O5:H- and O111:H8) which are uncommonly found in healthy Australian cattle, and which are accepted human pathogens (Starr *et al.*, 1998; Paton *et al.*, 1996) and may also be animal pathogens (Hornitzky *et al.*, 2000) in Australia, suggests that the use of antibiotics to treat sick animals may play a role in the emergence of antibiotic-resistant VTEC. Generally, most of the resistant serotypes isolated from the healthy babies belonged to types commonly isolated from healthy humans, such as those belonging to O-groups O1, O2, O4, O6, O7, O25 and O75 (Bettelheim, 1997) and not to typical bovine or ovine serotypes.

ACKNOWLEDGEMENTS

We wish to acknowledge with thanks the excellent technical assistance of Ms Irene Petalotis.

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