Table 3. MIC of STEC strains isolated from food-producing animals in Japan

Anti-microbial agent	Breakpoint ^a (mg l ⁻¹)	Bovine isolates $(n = 65)$			Swine isolates $(n = 25)$		
		MIC ₅₀ (mg l ⁻¹)	MIC ₉₀ (mg l ⁻¹)	No. of resistant isolates (%)	MIC ₅₀ (mg l ⁻¹)	MIC ₉₀ (mg l ⁻¹)	No. of resistant isolates (%)
Ampicillin	12.5	3.13	25	7 (10.8)	3.13	> 50	4 (16.0)
Cefazolin	25	1.56	1.56	0 (0.0)	1.56	1.56	0 (0.0)
Cefuroxime	50	3.13	3.13	0 (0.0)	3.13	6.25	0 (0.0)
Ceftiofur	3.13	0.39	0.39	0 (0.0)	0.39	0.78	0 (0.0)
Dihydrostreptomycin	50	3.13	> 100	20 (30.8)	25	> 100	11 (44.0)
Kanamycin	12.5	1.56	> 100	8 (12.3)	1.56	> 100	4 (16.0)
Gentamicin	3.13	0.39	0.78	0 (0.0)	0.39	0.78	1 (4.0)
Apramycin	12.5	1.56	3.13	0 (0.0)	3.13	3.13	1 (4.0)
Colistin	1.56	0.39	0.78	2 (3.1)	0.39	0.78	0 (0.0)
Chloramphenicol	50	6.25	6.25	2 (3.1)	25	> 100	12 (48.0)
Oxytetracycline	12.5	3.13	> 100	21 (32.3)	100	> 100	16 (64.0)
Bicozamycin	100	25	25	0 (0.0)	25	25	0 (0.0)
Nalidixic acid	50	3.13	3.13	0 (0.0)	3.13	12.5	0 (0.0)
Oxolinic acid	12.5	0.2	0.39	0 (0.0)	0.39	3.13	0 (0.0)
Enrofloxacin	3.13	≤ 0.05	≤ 0.05	0 (0.0)	≤ 0.05	0.2	0 (0.0)
Ofloxacin	3.13	0.1	0.1	0 (0.0)	0.1	0.2	0 (0.0)
Trimethoprim	12.5	0.2	0.39	2 (3.1)	0.39	> 50	3 (12.0)
Sulphadimethoxine	400	400	> 400	36 (55.4)	> 400	> 400	17 (68.0)

^aBreakpoints are those described by Kijima-Tanaka et al. (2003).

rabbit origin (Oswald et al., 2000), and did not react with eae- ε primers of Blanco et al. (2004) (data not shown).

In contrast with the bovine isolates, serotypes frequently implicated in human disease or eae-positive isolates were not observed in swine isolates. Many of swine isolates possessed the stx2e subtype. Stx2e has been referred to as 'pig oedema disease toxin', and has been demonstrated not share to the same glycolipid-binding specificity with Stx2 (DeGrandis et al., 1989). Stx2 subtype of six O100:NM isolates was not stx2e, but none of these isolates had eae genes. These findings suggested that our swine isolates were genetically not closely associated with human isolates. Similarly, DesRosiers et al. (2001) suggested that swine and human isolates in Canada are not genetically related. However, Nakazawa et al. (1999) reported the first isolation of STEC O157:H7 from pigs in Japan, and continuous monitoring of swine STEC might be necessary.

STEC were isolated from 23% of bovine faecal samples and 14% of swine faecal samples over a wide area of Japan. As for the STEC detection methods other than serogroup of O157, effective methods have not been widely reported; and the use of different detection methods and different sampling design make it difficult to compare STEC prevalence rates reported for various geographic areas. In previous Japanese reports, however, Miyao et al. (1998) had a 24% STEC isolation rate from cattle at a slaughterhouse of central prefecture, Tokyo, Fukushima and Seki (2004) had a 15.9% STEC isolation rate from cattle at a slaughterhouse of western prefecture, Shimane, and Kobayashi et al. (2001) had STEC isolation rates of 19-31% from healthy cattle in central Japan, and our bovine data appear comparable with these. Considering that the real 'STEC prevalence rate' would be higher than the 'STEC detection rate', our data indicate that STEC are spread generally throughout Japan. STEC isolation reports on healthy swine are limited, but our level of 14% is higher than those in Germany (7.5%; Beutin et al., 1993) and Hong Kong (2.1%; Leung et al., 2001) and shows that pigs are also

potential reservoirs of STEC in Japan. STEC were not isolated from the 158 broiler samples. Some reports indicated that colonization and faecal shedding of STEC were observed in chicks after oral challenge (Stavric et al., 1993; Schoeni and Doyle, 1994; La Ragione et al., 2005), and STEC were detected from 'poultry meat of grocery stores' (Doyle and Schoeni, 1987; Samadpour et al., 1994). STEC was not, however, detected from chicken faecal samples at farms or slaughterhouse (Beutin et al., 1993; Chapman et al., 1997; Kobayashi et al., 2002; Wani et al., 2004) or was detected at a low level (1%; Cobeljic et al., 2005). Considering these, chickens might not be potential reservoirs of STEC.

Anti-microbial resistance was higher in the swine isolates than in the bovine ones. Under JVARM, we also monitored the anti-microbial resistance of E. coli as indicator bacteria in a total of 1018 isolates (Kijima-Tanaka et al., 2003), revealing again that resistance was more frequent among swine isolates than bovine isolates. Our STEC results might reflect the resistance situation of E. coli in general. We observed high rates of resistance to sulphadimethoxine, oxytetracycline and dihydrostreptomycin, followed by ampicillin and kanamycin. All of these anti-microbials are commonly used and classically approved in Japan. Chloramphenicol resistance was high in swine isolates but low in bovine isolates, possibly because chloramphenicol has been used in pigs but not in cattle in Japan. The use of chloramphenicol was banned in all animals except pets in Japan in 1998. However, persistently high rates of chloramphenicol-resistance have been reported years after withdrawal in Europe (Aalbaek et al., 1991) and the United States (Bischoff et al., 2002). Bischoff et al. (2002) suggest that this might have happened by co-selection of chloramphenicol resistance with either common virulence genes or other antimicrobial resistance phenotypes. This indicates that continuous monitoring is clearly also necessary for chloramphenicol resistance. Fluoroquinolones and the third generation cephalosporins are currently the most valuable anti-microbials in human medicine in Japan, and their veterinary use

^bMIC₅₀ is MIC values that inhibit 50% of the isolates tested, and MIC₉₀ is MIC values that inhibit 90% of the isolates tested.

Table 4. Anti-microbial resistance patterns of STEC

Source	No. of anti-microbials resisted	Resistance pattern ^a	No. of isolates	Serotype of STEC ^b
Cattle	1	SDM	14	O157:H7 (2), O157:H NT, O26:H21, O26:NM, O26:H NT, O145:NM, O1:H45, O2:NM (2), 84:NM (2), O98:NM, O103:H11
		OTC	1	O84:NM
	2	SDM, OTC	1	O174:H21
	_	SDM, DSM	1	O8:NM
		SDM, ABPC	1	O174:H21
		SDM, CL	1	O26:NM
	3	SDM, OTC, DSM	6	0145:NM (stx2), O2:NM (4°), O174:NM
	4	SDM, OTC, DSM, KAN	5	0174:NM, 0174:H21(4 ^d)
	•	SDM, OTC, DSM, AMP	1	O84:NM
		SDM, OTC, DSM, CHL	1	O113:H19
		OTC, DSM, AMP, CL	1	O145:NM (stx1)
	5	SDM, OTC, DSM, KAN, AMP	3	$\overline{\text{O26:H11}}$, $\overline{\text{O145:NM}}$ (stx2, eae- βI), O116:NM
	ŭ	SDM, OTC, DSM, CHL, TMP	1	0145:NM (stx2)
		SDM, OTC, DSM, AMP, TMP	1	Rough:NM
Pigs	1	SDM	3	O100:NM (2), O123:H NT
		OTC	2	O2:H NT, O121:H10
		DSM	2	O91:H NT, O100:H NT
	2	SDM, OTC	1	O121:NM
		SDM, DSM	1	NT:H28
	3	SDM, OTC, CHL	3	O2:NM, O121:NM, O123:H NT
		OTC, DSM, CHL	1	O100:NM
	4	SDM, OTC, CHL, TMP	1	O104:NM
		SDM, OTC, DSM, AMP	1	O100:NM
		SDM, OTC, DSM, CHL	1	O2:NM
	5	SDM, OTC, DSM, CHL, AMP	1	O100:NM
		SDM, OTC, DSM, CHL, KAN	1	O100:NM
		SDM, OTC, DSM, CHL, TMP	1	O121:H10
	6	SDM, OTC, CHL, KAN, AMP, TMP	1	O123:H NT
		SDM, OTC, DSM, CHL, KAN, AMP	1	O8:H19
	7	SDM, OTC, DSM, CHL, KAN, GEN, APM	1	NT:NM

^aSDM, sulphadimethoxine; OTC, oxytetracycline; DSM, dihydrostreptomycin; CHL, chloramphenicol; KAN, kanamycin; AMP, ampicillin; TMP, trimethoprim; CL, Colistin; GEN, gentamicin; APM, apramycin.

has been restricted in Japan since their introduction to the veterinary market. It is therefore encouraging that we did not observe resistance to the quinolones, cephalosporins and bicozamycin.

Anti-microbial resistance patterns differed between bovine and swine isolates. Multi-resistant STEC were relatively few among the bovine isolates (23 isolates; 35%), but six of them belonged to the serotypes frequently implicated in human disease (O26:H11, O26:NM and four O145:NM isolates). Hiruta et al. (2000) reported an outbreak of diarrhoea due to multiple-resistant O26:H11; that resistance was encoded by a transmissible plasmid, and that resistance pattern was same as our O26:H11 isolate. Yamamoto and Wakisaka (1998) reported that resistance was more frequently observed in O26 than in O157 among human STEC. We had the same finding in our study of bovine STEC, and careful monitoring of anti-microbial resistance in O26 and O145:NM is therefore needed.

Among the bovine isolates, the prevalent resistance pattern was that of sulphadimethoxine—oxytetracycline—dihydrostreptomycin. This resistance pattern has been reported in the United States as the most frequent pattern of resistance in

O157:H7 of human and bovine origin (Kim et al., 1994; Meng et al., 1998). Yamamoto and Wakisaka (1998) also reported that this resistance pattern was prevalent within STEC of human and bovine origin in Japan. Our present data support these reports, and may demonstrate the similarity between human and bovine isolates. We observed additional patterns of resistance to kanamycin and/or ampicillin in our bovine isolates, including serotypes O26:H11 and O145:NM; such patterns are also frequently observed in human STEC. Furthermore, we observed multiple resistance in O2:NM and O174:NM/H21 isolates. Data on the resistance patterns of STEC other than O157 or O26 are limited, and it is noteworthy that four of our seven bovine O2:NM isolates from distant prefectures showed identical resistance patterns. Among the swine isolates, the resistance patterns varied. Resistance patterns frequently observed in human isolates, such as sulphadimethoxine-oxytetracycline-dihydrostreptomycin, were not observed in swine isolates. None of the swine isolates had serotypes of frequently implicated in human disease, and although resistance was observed more frequently in swine isolates, resistance to the quinolones and cephalosporins was not observed. Therefore, there may be only a low

Numbers in parentheses are numbers of isolates. Stx and eae type were varied in O145:NM isolates, and were stated in parentheses for multi-resistant isolates of this serotype. Bold and underlied serotypes are serotypes frequently implicated in human disease. NM, non-motile isolates. NT, not typeable isolates with O1 to O175 anti-sera, or with commercially available 22 H anti-sera.

^cFour O2: NM isolates were derived from far-spaced prefectures.

^dThree O174:H21isolates were derived from different farms in the same prefecture, and the fourth was from a distant prefecture.

possibility that these swine STEC will become hazardous to humans.

This study is the first national survey of STEC in the veterinary field across Japan. Anti-microbial use in animals, as in humans, inevitably selects for resistant bacteria, which could be transmitted to humans via food, and this is a matter of public health concern. To minimize the negative public health impact of the use of anti-microbials in food-producing animals, appropriate and prudent use of anti-microbials and continuous monitoring should be considered in the future.

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