Jpn. J. Vet. Res. **41**(1): 1–10 (1993)

ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* AND *SALMONELLA* FROM APPARENTLY HEALTHY SLAUGHTERED CATTLE AND PIGS, AND DISEASED ANIMALS IN ZAMBIA

Michael NGOMA[1,2], Atsuko SUZUKI[1], Ikuo TAKASHIMA[2] and Gihei SATO[1]

(Accepted for publication: Jan. 7, 1993)

ABSTRACT

Escherichia (E.) coli and *Salmonella (S.) choleraesuis* (subsp. *choleraesuis* and subsp. *salamae*) from apparently healthy slaughtered cattle and pigs in 1989 in Zambia, were examined for antibiotic resistance and the presence of conjugative R plasmid. *Salmonella* strains from diseased animals (cattle, chickens, leopards, lions and warthogs) were similarly tested. The majority of the cattle had been nomadically kept in so-called "traditional farms" while all the pigs were from commercial farms. More pigs (39 %; 41/105) harboured drug-resistant *E. coli* than cattle (6.7 %; 7/105). Moreover, the number of drug-resistant *E. coli* was higher among strains from pigs (31.2 %; 49/157) than cattle (4.2 %; 7/167). For both cattle and pigs, drug resistance was more frequently observed against tetracycline, streptomycin, sulfadimethoxine and ampicillin than other antibiotics and the single resistance pattern occurred most frequency, especially among pig *E. coli* strains. Drug-resistant *Salmonella* was recorded in 3.6 % (1/28) of strains from slaughtered cattle and 31.3 % (10/32) of those from diseased animals. Drug-resistant *E. coli* from pigs and cattle carried R plasmid at high frequency.

Key words: *Escherichia coli, Salmonella,* R plasmids, antibiotics, animals in Zambia

INTRODUCTION

Contamination of edible meat by drug-resistant *E. coli* and *Salmonella* is a problem of public health importance[9]. Generally speaking, in many countries the use

[1] Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia.

[2] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.

of antibiotics to promote growth in domestic animals, especially in fattening calves, pigs and chickens, has greatly contributed to the appearance of drug-resistant bacteria[1,3,6,7,12,17]. In most developing countries, the bacteria drug resistance status is not clearly defined. Such is true in Zambia concerning the *E. coli* and *Salmonella* drug resistance situation in slaughtered animals. In Zambia, about 80 % of the cattle and the majority of the pigs are traditionally maintained depending on natural pasture with little or no supplementation[13].

In this paper, the drug resistance of *E. coli* and *Salmonella* isolates from abattoir-slaughtered cattle and pigs and *Salmonella* from sick animals in Zambia, was surveyed. The presence of transferrable R plasmid in drug-resistant strains was also examined.

MATERIALS AND METHODS

Tested strains and sampled animals: Examination of caecal contents and mesenteric lymph nodes from 105 cattle and 105 pigs, slaughtered at abattoirs in Lusaka, Zambia, in 1989, yielded isolation of different serovars of *S. choleraesuis* (subsp. *choleraesuis* and subsp. *salamae*)[8] (Table 1) and *E. coli* strains. Twenty-eight *Salmonella* strains from cattle and 39 from pigs were tested in this investigation.

Table 1. *Salmonella* serovars[a] of tested strains

Strains from slaughtered cattle		Strains from slaughtered pigs		Strains from diseased animals		
Serovar	No. of strains	Serovar	No. of strains	Serovar	Animal	No. of strains
Typhimurium	14	Bredeney	22	Dublin	Cattle	11
Heidelberg	2	Braenderup	5	Livingstone	Cattle	5
Othmarschen	2	Infantis	2	Agona	Cattle	3
Bonn	8	Muenchen	4	Agona	Chiken	2
Weltevreden	2	Newport	3	Agona	Lion	2
		Bovismorbificans	1	Agona	Warthog	1
		Elisabethville	2	Newington	Cattle	2
				Anatum	Cattle	1
				Schwarzengrund	Chicken	2
				Bovismorbificans	Cattle	1
				Makoma	Cattle	1
				Virchow	Leopard	1
Total	28		39			32

a) Except for *S. choleraesuis* subsp. *salamae* serovar Makoma (*S.* Makoma), the rest are various serovars under *S. choleraesuis* subsp. *choleraesuis*.

Thirty-two *Salmonella* strains from sick animals (cattle, chickens, leopards, lions and warthogs)[4] were also similarly tested. Tested *E. coli* strains were isolated from 105 cattle (167 strains) and 105 pigs (157 strains). Cattle were from 4 provinces and the majority had been traditionally reared while all the pigs had been commercially kept in 7 piggeries in Lusaka (Table 2). All the tested strains had been stored on Dorset egg slope media at 4 °C in our laboratory. *E. coli* strain K12 ML1410 (nalidixic acid-resistant and methionine-requiring F^- derivative of K-12), used as a recipient in detection of transferrable R plasmid, was kindly supplied by Dr. N. Ishiguro (Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan).

Drugs and drug concentrations for drug sensitivity test: Ten antibiotics were used at the following concentrations: 25 μg/ml for ampicillin (Ap), chloramphenicol (Cp), gentamicin (Gm), kanamycin (Km), nalidixic acid (NA) and tetracycline (Tc); 12.5 μg/ml for streptomycin (Sm); 6.3 μg/ml for colistin (Cl) and furazolidon (Fz); and 800 μg/ml for sulfadimethoxine (Su). The drug concentrations were according to a previous report[16] with some modifications. The agar dilution method was used for detection of drug resistance and heart infusion agar (Eiken Chemical Co., Ltd, Tokyo, Japan) was used except in the case of Su, where Mueller Hinton agar (Eiken Chemical Co., Ltd, Tokyo, Japan) was used. A strain was recorded as resistant when its growth was not inhibited by the drug concentration mentioned above.

Table 2. Origin of cattle and pigs, and number harbouring drug-resistant *E. coli* strains

Animal	Source province/piggery[a]	Farming system	Positive animal/total no. of animals (%)[b]
Cattle	Southern	Traditional	1/59 (1.8)
	Western	Traditional	5/25 (20)
	Central	Traditional	0/4 (0)
	Lusaka	Commercial	1/17 (5.9)
Total			7/105 (6.7)
Pigs	A		21/40 (52.5)
	B		3/10 (30)
	C	All	1/10 (10)
	D	commercial	4/10 (40)
	E		6/10 (60)
	F		4/10 (40)
	G		2/15 (13.3)
Total			41/105 (39)

a) A to G = Piggeries, all in Lusaka Province.
b) Positive animals yielding drug-resistant *E. coli* strains.

Detection of conjugative R plasmid: A test for detection of the R plasmid was conducted on all *E. coli* and *Salmonella* strains (donor) showing drug resistance. The *E. coli* strain K12 ML1410 was used as a recipient strain. The test was done according to the method described by Sato *et al.*[16]. Each of the donor isolates was cultivated in brain heart infusion broth (Eiken Chemical Co., Ltd, Tokyo, Japan) at 37 °C for 18 hours. Broth (2 ml) in a test tube was inoculated with 0.2 ml of each donor broth culture and an equal amount of the recipient culture similarly cultivated. The mixure was incubated at 37 °C for 18 hours. A loopful of the mixed culture was streaked on a selective agar plate containing NA (50 μg/ml) and appropriate concentrations of drugs to which the donor was resistant. Desoxycholate hydrogen sulfide lactose (DHL) agar (Nissui, Tokyo, Japan) was used as a basal medium for Cp, Sm, Km and Ap; heart infusion agar for Tc; and Mueller Hinton agar (Eiken Chemical Co., Ltd, Tokyo, Japan) for Su. To the latter 2 media had been added 1.5 g of lactose and 4 ml of 0.2 % bromothymol blue per 100 ml. The drug concentrations used for selective media were the same as for the drug sensitivity test. The selective media were incubated at 37 °C for 24 hours. To determine transconjugant recipients and their resistance patterns, colonies of transconjugants on each selective medium were purified by successive single-colony isolations on the same selective medium and were examined for drug resistance pattern. When a transconjugant recipient was not found on the selective medium, the mixed culture of donor and recipient, which had been left overnight at 25 °C was reinoculated on the selective medium to detect temperature-sensitive R plasmid.

RESULTS

Drug resistance of tested strains: The sources of the slaughtered cattle and pigs are shown in Table 2. The percentage of cattle heads from which drug resistant *E.coli* was isolated was 6.7 % (7/105) while that of pigs was 39 % (41/105) and there was a statistically significant difference in isolation rates between cattle and pigs ($P < 0.01$). Heads of cattle, from each of the 4 provinces, harbouring drug-resistant *E. coli* ranged from 0 % (Central) to 20 % (Western). The percentage of pigs carrying drug-resistant *E. coli* was from 10 % (1/10) to 52.5 % (21/40) (Table 2).

The resistance of *E. coli* and *Salmonella* to each of the tested antibiotics is shown in Table 3. Only 4.2 % of the 167 cattle *E. coli* as compared to 31.2 % of the 157 from pigs were resistant to at least one of the following antibiotics; Tc, Sm, Su, Ap, Km and Cl. The rate of resistance of cattle *E. coli* strains was greatest to Tc (3.0 %) and Su (3.0 %) followed by Ap (2.4 %) and Sm (1.8 %), and lastly Km (1.2 %) while pig strains were resistant to Tc (18.5 %), Sm (11.5 %), Ap (10.8 %), Su (9.6 %) and finaily Km and Cl (0.6 %). A higher percentage of drug-resistant *E. coli* was detected in pigs, especially against Tc, Sm, Su and Ap.

Of the 28 cattle *Salmonella* strains, only 1 (3.6 %) serovar was resistant to Ap.

None of the 39 pig *Salmonella* strains were resistant to any of the drugs used. A total of 31.3 % of the 32 *Salmonella* strains from diseased animals showed resistance, mainly against Sm. Seven strains of *S. choleraesuis* subsp. *choleraesuis* serovar Dublin (S. Dublin) and 1 of *S.* Anatum from cattle and 1, *S.* Schwarzengrund, from the chicken were resistant to Sm. One *S.* Agona strain from cattle was resistant to 3 drugs, Tc, Sm and Su (Table 3). *Salmonella* strains from slaughtered cattle and pigs appeared to be sensitive even to commonly used drugs. All the *E. coli* and *Salmonella* strains tested were sensitive to Gm, Cp, Fz and NA (data not shown).

In total, 5 drug resistance patterns, in terms of single or multiple resistance, were observed for *E. coli* and 4 patterns for both cattle and pig *E. coli* (Table 4). In cattle *E. coli* strains, single, double and quintuple patterns occured at the same frequency (29 %) followed by a triple pattern (14 %). In pig *E. coli* strains, the single pattern was more frequent (59 %) than the double (20 %), triple (16 %) and quadruple (4 %) ones. The single pattern was the only pattern observed in *Salmonella* from apparently healthy cattle, and in *Salmonella* from diseased animals it was the most frequent (90 %). At present, the single resistance pattern appears to be more common than multiple drug resistance patterns, especially in pig *E. coli* strains.

Conjugative R plasmid: Of 49 resistant *E. coli* strains from pigs, 20.4 % (10/49) carried R plasmid, of which 60 % (6/10) showed the TcSmSu pattern while in cattle *E. coli* R plasmid was detected in 28.6 % (2/7) of the resistant strains tested. Pig *E. coli* with a triple resistance pattern and that of cattle with a quintuple pattern carried conjugative R plasmid at high frequency. Cattle *E. coli* strains with the quintuple resistance pattern also partially transferred triple and double drug resistance. No R plasmids were detected in the *Salmonella* strains from apparently healthy cattle but triple resistance was transferred from the strain of a sick calf (Table 5). Temperature-sensitive R plasmid was not detected under the present experimental conditions (data not shown). The frequency of transferrable R plasmid appears tn be more common in pig *E. coli* strains carrying the TcSmSu drug resistance pattern.

Table 3. Antibiotic resistance of *E. coli* and *Salmonella* from different sources in Zambia

Bacteria	Source	No. of resistant strains/ total no. of strains isolated (%)	No. (%) of strains resistant to:					
			Tc	Sm	Su	Ap	Km	Cl
E. coli	Cattle	7/167 (4.2)	5 (3.0%)	3 (1.8%)	5 (3.0%)	4 (2.4%)	2 (1.2%)	0
	Pigs	49/157 (31.2)	29 (18.5%)	18 (11.5%)	15 (9.6%)	17 (10.8%)	1 (0.6%)	1 (0.6%)
Salmonella	Cattle	1/28 (3.6)	0	0	0	1 (3.6%)	0	0
	Pigs	0/39 (0)	0	0	0	0	0	0
	Diseased animals[a)]	10/32 (31.3)	1 (3.1%)	10 (31.3%)	1 (3.1%)	0	0	0

a) These include 9/24 of cattle strains, 1/2 of 2 chicken strains and none of 1 leopard, 2 lion and 1 warthog strains.

Table 4. Antibiotic resistance patterns of tested E. coli and *Salmonella* strains

Resistance pattern	Drug	No. of cattle *E. coli* strains	No. of pig *E. coli* strains	*Salmonella* from healthy cattle	*Salmonella* from diseased animals
Single	Ap	2	11	1	—
	Tc	—	13	—	—
	Sm	—	3	—	9
	Su	—	1	—	—
	Cl	—	1	—	—
Subtotal (%)		2(29)	29(59)	1(100)	9(90)
Double	TcSu	2	2	—	—
	TcSm	—	3	—	—
	SmSu	—	2	—	—
	ApSu	—	1	—	—
	TcAp	—	1	—	—
	SmAp	—	1	—	—
Subtotal (%)		2(29)	10(20)	—	—
Triple	TcSmSu	1	6	—	1
	TcSmAp	—	1	—	—
	TcSuAp	—	1	—	—
Subtotal (%)		1(14)	8(16)	—	1(10)
Quadruple	TcSmSuAp	—	1	—	—
	TcSmSuKm	—	1	—	—
Subtotal (%)		—	2(4)	—	—
Quintuple	TcSmSuApKm	2	—	—	—
Subtotal (%)		2(29)	—	—	—
Total		7	49	1	10

Table 5. Transferred antibiotic resistance patterns from *E. coli* and *Salmonella* strains

Bacteria	Source	Donor strains:		Transconjugant:		%(No. of strains with R plasmid/total no. of resistant strains tested)
		resistance pattern	no. of strains	resistance pattern	no. of strains[a]	
E. coli	Pig	Sm	1	Sm	1	
	Pig	Su	1	Su	1	
	Pig	ApSu	1	Su	1	
	Pig	TcSmSu	6	TcSmSu	6[b]	
	Pig	TcSuAp	1	TcSu	1	
	Subtotal		10		10	20.4(10/49)
	Cattle	TcSmSuApKm	2	TcSmSuApKm TcSmSu, SmSu	2	28.6(2/7)
Salmonella (*S. Agona*)	Sick calf	TcSmSu	1	TcSmSu	1	10.0(1/10)

a) No. of strains carrying conjugative R plasmids.
b) This pattern constituted 60% (6/10) of pig *E. coli* carrying R plasmid.

DISCUSSION

Although drug resistance in *Salmonella* from sick animals has been reported in Zambia[4], this is the first report on the drug resistance and conjugative R plasmid of *E. coli* and *Salmonella* from abattoir-slaughtered cattle and pigs.

The investigation revealed more pigs harbouring drug resistant *E. coli* than cattle. This was confirmed by different isolation rates, both of animals examined and of strains isolated between pigs and cattle. In Zambia, most of the cattle are nomadically and "traditionally" reared with limited administration of drugs, while in commercially reared pigs drugs are administered more frequently. This difference in rearing pattern between cattle and pigs is likely to be an important factor. Similar findings were made in Indonesia where animal rearing conditions are almost comparable to those in Zambia[10].

For pig *E. coli*, resistance was higher against Tc, Su, Ap and Sm, and not detected against Gm, Cp, Fz and NA. High incidence of *E. coli* resistance against Tc, Sm and Su is a common finding world-wide where these antibiotics are included in animal feeds[1,3,6,15]. The presence of antibiotic-resistant bacteria in domestic animals, especially those under intensive management, due to consumption of feeds containing antibiotics has been pointed out by many authors[1,6,7,9,1,17]. In Zambia, the use of

antibiotics is regulated but some farmers use the drugs indiscriminately. Drugs such as Tc, Sm and Su are more commonly used, especially on commercial farms, for various purposes.

Salmonella strains from both healthy cattle and pigs appear to be sensitive even to the commonly used drugs but the strains from diseased animals were more resistant. In healthy cattle, the limited administration of drugs could account for this. In Indonesia, Nakamura *et al.*[11] could not detect resistance in *Salmonella* against any of the antibiotics used. However, continuous usage of antibiotics may easily results in drug-resistant *Salmonella* as is likely to have occurred in the strains from the diseased animals. The situation in pigs in Zambia needs to be carefully evaluated although *Salmonella* strains from pigs are not resistant at present. Because of the high number of drug resistant *E. coli* and the presence of R plasmids in pigs, transfer of resistance from *E. coli* to *Salmonella* could possibly occur[14].

In total, 5 drug resistance patterns in terms of single or multiple resistance, were observed, four for both cattle and pig *E. coli*, 1 for *Salmonella* from apparently healthy cattle and 2 for *Salmonella* from diseased animals. The single resistance pattern was significantly more often detected among pig *E. coli* than others. However, despite being under intensive management, administration of drugs in pigs is likely to be infrequent in Zambia. Results obtained in Nigeria in domestic animals being fed antibiotic-containing feeds and regularly given antibiotics as prophylactic or chemotherapeutic agents, showed higher degrees of drug resistance[1].

The majority of the detected conjugative R plasmids were in pig *E. coli*. This might be attributed to the differences in managemental care of pigs and cattle in Zambia already mentioned. Intensively reared pigs (also calves and chickens) have been observed to be carriers of large numbers of bacteria with R plasmids[1,5,15]. In this study, similar results were obtained. The transfer of R plasmids carrying multiple resistance to *S.* Typhimurium and the problems associated with it are well known in Great Britain[2]. The high frequency of transferrable resistance observed in pig *E. coli* with TcSmSu resistance and those of cattle with TcSmSuApKm resistance suggests the necessity of further work to monitor possible transfer from *E. coli* to *Salmonella* in both commercially and traditionally kept domestic animals in Zambia, since 1 of the 2 *E. coli* strains carrying R plasmid was from tradionally reared cattle. Although temperature-sensitive R plasmid was not detected, more work needs to be done to substantiate this finding. Temperature-sensitive R plasmid has been widely reported and is known to transfer Tc resistance[5].

In general, the frequency of resistant bacteria is low in both cattle and pigs in Zambia. That is, at present contamination of meats by antibiotic-resistant *E. coli* and *Salmonella* is likely not to be a big problem. Especially for *Salmonella*, at present the commonly used drugs seem to be useful for the treatment of salmonellosis.

REFERENCES

1) ADETOSOYE, I. A. : Infective drug resistance among *Escherichia coli* isolated from clinically healthy domestic livestock. *Vet. Microbiol.* **5** : 333–342, 1980.
2) ANDERSON, S. E. : Drug resistance in *Salmonella typhimurium* and its implications. *Brit. Med. J.* **3** : 333–339, 1968.
3) DUPONT, L. H. & STEELE, H. J. : The human health implication of the use of antimicrobial agents in animal feeds. *Vet. Quart.* **9** : 309–320, 1987.
4) FALADE, S., SATO, G., ULAYA, W. & MWANZA, L. : Serovars and antibiotic sensitivity patterns of *Salmonella* strains isolated from domestic animals in Zambia. *Zimbabwe Vet. J.* **20** : 19–22, 1989.
5) ISHIGURO, N., GOTO, J. & SATO, G. : Genetical relationship between R plasmids derived from *Salmonella* and *Escherichia coli* obtained from a pig farm, and its epidemiological significance. *J. Hyg. Camb.* **84** : 365–379, 1980.
6) KARIUKI, P. D. : Incidence of drug resistance in *Escherichia coli* isolated from scouring calves. *Bull. Epiz. Dis. Afr.* **22** : 115–117, 1974.
7) KELLY, R. W., MOREHOUSE, C. E. & COLLINS, D. J. : The incidence and importance of antibiotic-resistant bacteria in livestock in the Republic of Ireland. *Irish Vet. J.* **26** : 49–57, 1972.
8) LEMINOR, L., VERON, M. & POPOFF, M. : Proposition pour une nomenclature des *Salmonella*. *Ann. Microbiol. (Inst. Pasteur)* **133B** : 245–254, 1982.
9) LINTON, H. A. : Antibiotic resistance : The present situation reviewed. *Vet. Rec.* **100** : 354–360, 1977.
10) NAKAMURA, M. : A report on the animal hygiene situation in Indonesia. *J. Miyagi Pref. Vet. Med. Assoc.* **41** : 121–132, 1988 (In Japanese).
11) NAKAMURA, M., ISTIYANINGSIH, NAKASHIMA, N., KAJI, T. & SATO, S. : Isolation of *Salmonellae* from clinically normal cattle and pigs in Indonesia in 1986 and detection of plasmids in the isolates. *Jpn. J. Vet. Sci.* **51** : 1059–1061, 1989.
12) OJENIYI, A. A. : Drug enrichment of commercial poultry feeds and human health in the tropical developing countries. *Acta Vet. Scand.* **30** : 133–139, 1989.
13) PERRY, D. B., MWANAUMO, B., SCHELS, F. H., EICHER, E. & ZAMAN, R. M. : A study of health and productivity of traditionally managed cattle in Zambia. *Prev. Vet. Med.* **2** : 633–653, 1984.
14) POHL, P. & THOMAS, J. : R plasmid reservoirs and circulation. *Ann. Med. Vet.* **123** : 385–396, 1979.
15) SAIDA, K., IKE, Y. & MITSUHASHI, S. : Drug resistance and R plasmids of *Escherichia coli* strains isolated from pigs, slaughterers and breeders of pigs in Japan. *Antimicrob. Agents Chemother.* **19** : 1032–1036, 1981.
16) SATO, G., FURUTA, Y., KODAMA, H., IWAO, T. & OKA, M. : Enzootic occurrence of chloramphenicol-resistant *Salmonella typhimurium* var *copenhagen* in a calf population. *Am. J. Vet. Res.* **36** : 839–841, 1975.
17) SMITH, H. W. : The effect of the use of antibacterial drugs, particularly as feed additives, on the emergence of drug-resistant strains of bacteria in animals. *N. Z. Vet. J.* **15** : 153–166, 1967.