ANTIMICROBIAL RESISTANCE IN ESCHERICHIA COLI ISOLATES FROM FAECES
AND CARCASS SAMPLES OF SLAUGHTERED CATTLE, SWINE AND CHICKENS IN KENYA

Kikuvi, G. M[1]*, Ole-Mapenay I. M[2], Mitema, E. S[2] and Ombui J. N.[2]

1Department of Veterinary Services, Central Veterinary Investigation Laboratories, Kabete-Nairobi, Kenya.
2Faculty of Veterinary Medicine, Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya

Summary

Two hundred and thirty five Escherichia coli isolates from cattle, pigs and chickens were investigated for their resistance to seven antimicrobials by the disc diffusion method. Minimum inhibitory concentrations were determined for 154 isolates showing resistance to at least one of the antimicrobials tested. Resistance was found in 65.5% and multi-resistance (resistance to = 2 antibiotics) in 37.9% of the isolates. Resistance was highest in the isolates from chickens (74.0%), followed by pigs (64.8%) and cattle (61.3%). The most common resistance was to ampicillin, streptomycin, tetracycline, sulphamethoxazole/trimethoprim, and kanamycin (42.5-11.9%). Resistance to kanamycin, sulphamethoxazole/trimethoprim, and tetracycline was significantly lower in cattle (2.5-7.5%) than in the other species (12.0-40.0%) ($p < 0.01$). Resistance to streptomycin and ampicillin were significantly higher in cattle and pigs respectively ($p < 0.01$). Similar resistance rates were observed among the faecal (29.9%) and carcass swab (33.1%) isolates. Forty resistance patterns were recorded of which only 5 (12.5%) were common among the isolates studied.

This study shows that multi-drug resistant E. coli isolates are prevalent in cattle, pigs and chickens in Kenya and that a considerable proportion of E. coli isolates from fresh cattle and pig carcasses is resistant to a variety of antimicrobial agents. Differences in the rates and patterns of resistance were noted, perhaps reflecting differences in antibiotic use regimens among these species. It is recommended that the use of antimicrobials in food animals should follow prudent use guidelines to minimize the selection of resistant bacteria and that slaughter hygiene should be improved to minimize the risk of transfer of antimicrobial resistant bacteria to humans.

Introduction
The major influences on the amplification and spread of antimicrobial resistant bacteria are the use of antimicrobial agents in human medicine and their use in livestock for therapy, metaphylaxis, prophylaxis and growth promotion (1). Resistant bacteria from domestic animals can be transmitted to man indirectly via the food chain or directly from the animal (2), and potentially result in food-borne illness in humans that is less responsive to treatment with conventional antimicrobial drugs.

Food of animal origin may serve as a vehicle to transport resistant bacteria and resistance genes between animals and humans since contamination of carcasses with faecal flora inevitably occurs during slaughtering (3). In addition to the human health concerns, antimicrobial-resistant pathogens also pose a severe and costly animal health problem, as they prolong illness and decrease productivity through higher morbidity and mortality rates (4).
To generate baseline data to be used in future risk assessment of antimicrobial resistance, a number of surveillance systems on the local, continental and global scale have been initiated (5). Among the species proposed for surveillance is Escherichia coli. The prevalence of resistance in commensal E. coli is a good indicator for the selective pressure by antibiotics use and resistance problems to be expected in pathogenic bacteria. In food animals, a low prevalence and degree of antibiotic resistance in the intestinal flora should be considered as a distinguishing quality and safety mark (3). While antimicrobial resistance of commensal E. coli isolates of avian origin in Kenya has been reported (6,7), data on the prevalence and resistance patterns of E. coli from other food-producing animals are unavailable.

The aim of this study was to determine and compare the prevalence and patterns of antimicrobial resistance phenotypes among E. coli isolates from cattle, pigs and chickens in Kenya.

Material and Methods
Collection of samples
Fresh faecal and carcass swab samples were collected from individual animals from unrelated herds at the Dagoretti slaughterhouse complex (cattle) and Ndumbuini slaughterhouse (pigs) in Nairobi during June to December 2001. Cattle slaughtered at Dagoretti slaughterhouse complex originate from all parts of the country (8). Pigs are sent to the abattoir from farms in Kiambu and Nairobi districts which are among the main pig farming districts in Kenya. A single animal was selected at random as being representative of a herd and about 5 g of faeces aseptically removed from the large bowel after evisceration at the slaughtering line. The carcasses were sampled using sterile cotton wool swabs. The samples were immediately placed into Stuart´s transport medium (Oxoid, Basingstoke, United Kingdom), maintained on ice while being transported to the laboratory and processed on the same day. In addition, cloacal and pharyngeal swab samples collected from chickens at various markets in Nairobi were used for the study.

Isolation and identification of E. coli
The samples were inoculated into peptone water (Oxoid) and incubated at 37oC for 18 h. Subsequently; the cultures were streaked on Eosin Methylene Blue (EMB) agar (Oxoid) and incubated overnight at 37oC. Indole, methyl red, Voges-Proskauer reaction and Simons citrate (IMViC) tests were performed with the colonies that

showed growth characteristics of E. coli on EMB agar. Analytical profile index (API) 20E strips (Bio Merieux, Marcy-I'Etoile, France) were also used to confirm the identification of the isolates as E. coli. One isolate per sample was selected for resistance testing. The E. coli isolates selected for resistance testing were restreaked onto blood agar (Oxoid), incubated overnight at 37oC, and stored at 4oC until in-vitro susceptibility tests were performed. E. coli ATCC 25922 was used as a reference strain for quality control of the antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disc diffusion on Mueller-Hinton (MH) agar (Oxoid) according to the recommendations reported by the Clinical and Laboratory Standards Institute (CLSI; formerly known as the NCCLS). The antimicrobial agent discs used in this study were: ampicillin (10 g), tetracycline (30 g), streptomycin (10 g), kanamycin (30 g), gentamicin (10 g), sulphamethoxazole/trimethoprim (23.75/1.25 g) and chloramphenicol (30 g) (Himedia Laboratories Ltd, Mumbai, India). Minimum Inhibitory Concentration (MIC) values for the antimicrobials among the 154 E. coli isolates showing resistance on disc diffusion test were determined using the standard broth doubling dilution method on MH (Oxoid) medium according to the CLSI document M31-A2 (NCCLS, 2004(9)). The zone diameters around all the discs and MICs, except for streptomycin were interpreted according to the CLSI document M31-A2 (9), the breakpoints for the zone diameters and MICs used for streptomycin were those recommended by CLSI document M2-A6 (10) and the Danish Integrated Antimicrobial resistance Monitoring and Research Program (DANMAP, 2001)(11), respectively. The rates of resistance as well as MIC50 and MIC90 values were calculated and presented. Multi-drug resistance was defined as simultaneous resistance to at least two of the antimicrobials tested, with sulphamethoxazole plus trimethoprim considered as one unit since the testing was done in combination.

Statistical analysis

Chi-square test was used to compare the difference between the proportions of the isolates from cattle, pigs and chickens that were resistant to various antimicrobials. A value of $p < 0.05$ was considered as significant. The correlation between the standard broth dilution method and disc diffusion method was analysed by regression analysis.

Results

Bacterial isolates

A total of 235 E. coli isolates comprising of 80 isolates from cattle (carcass, n = 38 and faeces, n = 42), 105 from pigs (carcass, n = 52 and faeces, n = 53) and 50 from chickens (pharyngeal, n = 12 and cloacal swabs, n = 48) were studied.

Antimicrobial susceptibility testing

One hundred and fifty four (65.5%) of the E. coli isolates (pigs 28.9%, cattle 20.9% and chicken 15.7%) were resistant to at least one of the antimicrobial agents tested. Overall, 89 (37.9%) of the isolates were multi-drug resistant (resistant to =2 antibiotics). Resistance was highest in the chicken isolates (74.0%), followed by pigs (64.8%) and cattle (61.3%). Multi-drug resistance was significantly higher in the isolates from pigs (42.9%) and chickens (40.0%) than in those from cattle (30.0%) (p < 0.05). One isolate from pigs was resistant to all seven antibiotics tested.

The prevalence of resistance among the isolates from the three animal species sampled is represented in Figure 1. Resistance to kanamycin, tetracycline, and sulphamethoxazole/trimethoprim was significantly higher in the isolates from pigs and chickens (12.0-40.0%) than in those from cattle (2.5-7.5%). Isolates from cattle were significantly more resistant to streptomycin (43.7%) than those from pigs (25.7%) and chickens (34.0%) (p < 0.01). Resistance to ampicillin was significantly higher in isolates from pigs (50.5%) than in isolates from cattle (38.7%) or chickens (32.0%). Chloramphenicol resistance was significantly higher in the isolates from pigs (10.0%) than those from chickens (2.0%). There were no significant differences in the prevalence of resistance between the E. coli isolates isolated from faecal (30.0%, cattle and 29.7%, pigs) and carcass swabs (31.0%, cattle and 35.2%, pigs). The chicken isolates were not compared statistically because of the small number of isolates from the pharyngeal swabs.

Forty different resistance patterns were recorded. The most prevalent resistance patterns of each species are shown in Table 1. Only 5 (12.5%) of these resistance patterns were found to be shared by all three animal sources. The resistance spectra of the porcine isolates varied more than those from bovine or avian isolates. No significant differences were observed between the patterns of resistance among the carcass swab and faecal sample isolates from either cattle or pigs. The disc diffusion method results for the 154 isolates correlated well with the MIC determinations for all the agents tested (r = 0.949). The MIC of each antimicrobial agent varied widely with the isolate tested (Table 2). Some isolates showed exceptionally high MICs for various antimicrobials. These included ampicillin with 33 (21.4%), streptomycin with 22 (14.3%), chloramphenicol with 8 (5.2%) and tetracycline with 20 (13.0%) of the isolates showing MICs = 256 µg/ml. In addition, 42 (27.3%) of the isolates showed MICs = 1216/64 µg/ml for the sulphamethoxazole/trimethoprim combination.

Figure 1: Prevalence of antimicrobial resistance among E. coli isolated from cattle (n = 80), pigs (n = 105) and chickens (n = 50).
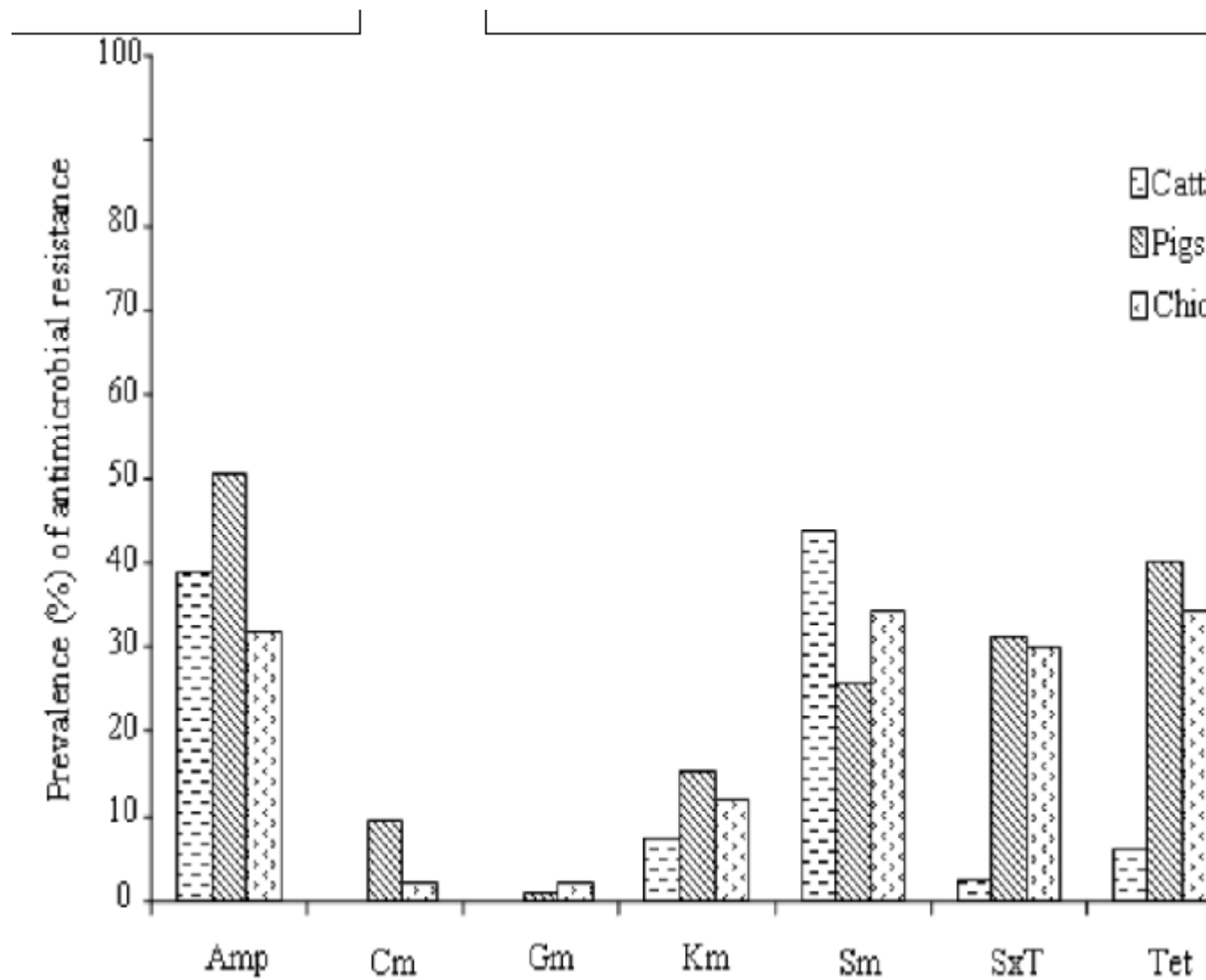
Antimicrobial agents: Amp, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; SxT, sulphamethoxazole/trimethoprim; Tet, tetracycline

Table 1: Antimicrobial resistance profiles of E. coli isolates from food-producing animals in Kenya

Resistance	Cattl	Pig	Chickens
Amp	1	1	6
Sn			4
Tet			4
SxT			3
AmpSm			1
AmpTet			2
KmSm			2
SxTTet			4
AmpKmSm			-
AmpKmTet			-
AmpSxTTet			-
TetSmSxT			-
AmpKmSxTTet			-
AmpSmSxTTet			2
AmpCmKmSmSxTT			-

Abbreviation: Amp, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; SxT, Sulphamethoxazole/trimethoprim; Tet, tetracycline.

Table 2: MIC distribution of 7 antimicrobials tested against 154 *E. coli* isolat

Antimicrobial agent	No. of isolates for whice MIC (5g/ml) is								
	≤0.25	0.5	1	2	4	8	16	32	64
Ampicillin	0	0	0	0	21	35	41	8	12
Chloramphenicol	0	0	0	12	40	89	5	0	0
Gentamicin	0	2	12	85	47	8	0	0	0
Kanamycin	0	0	0	0	26	81	85	12	0
Streptomycin	0	0	0	0	15	30	57	17	5
Tetracycline	0	2	4	34	57	19	2	2	3
Sulphmethoxazole/trimethoprim (19:1)[a]	95	3	8	0	4	0	0	2	42

Discussion

The potential for transfer of antimicrobial resistance from enteric zoonotic bacteria of food animals to the human population is a cause of concern (12). Contact with food animals or their excreta or consumption of foods of animal origin has been suggested to be the main route of dissemination of resistance from food-producing animals into human populations (2). The antimicrobial susceptibility data from the present study showed that food animal populations in Kenya harbour E. coli resistant to various antimicrobials commonly used in veterinary and human medicine. The variations in the MICs with the isolate tested may be accounted for by difference in the genes encoding resistance to the various antimicrobials since resistance phenotypes may arise from many different genetic determinants (13) and the distribution of MIC for antimicrobials like streptomycin has been reported to be greatly influenced by the genes encoding resistance (14).

Resistance was more commonly observed among chicken and swine isolates and multi-drug resistance was significantly higher in these isolates than those from cattle. The relatively intensive conditions under which pigs and chickens are housed may be associated with greater disease potential and therefore, a greater tendency for antibiotic use to control disease (12). The most common resistance among the isolates from the three animal species sampled were to ampicillin, streptomycin, tetracycline and sulphamethoxazole/trimethoprim. The resistance patterns most frequently observed in cattle were resistance to streptomycin and ampicillin in combination and streptomycin or ampicillin alone. Resistance to tetracycline and sulphamethoxazole/trimethoprim was most frequently seen among the chicken isolates.

The most frequent pattern in the multi-drug isolates from pigs, resistance to ampicillin, tetracycline, streptomycin, and sulphamethoxazole/trimethoprim, was also found among the E. coli isolates from chickens. Due to their relatively low cost and ready availability for sale 'over the counter', these drugs are widely used by farmers for therapeutic and prophylactic applications (15). Penicillins and tetracyclines are the most widely used antibiotics in humans and food animals in Kenya, respectively and extended-spectrum penicillins account for 67.5% of the penicillins in use (16) while tetracyclines account for nearly 55% of the antimicrobial use in food animals (17). Ampicillin is one of the most widely available orally administered antibiotics in humans in Kenya (18).

Isolates from pigs were significantly more often resistant to ampicillin and tetracycline than those from other animal species. A high prevalence of antimicrobial drug-resistant E. coli could also occur if the animals received high doses of these isolates from the environment (19). Ampicillin or tetracycline resistant E. coli from humans may reach pigs through feeding contaminated swill, which is a common practice by smallholders in Kenya. The majority of the pigs slaughtered at our sampling site came from these farmers. Isolates of E. coli from cattle had significantly lower rates of resistance to tetracycline, sulphamethoxazole/trimethoprim, or kanamycin than did isolates from pigs and chickens. While this may reflect lower usage of these antimicrobials in beef cattle (15), it may also be explained by the greater age of the cattle sampled, since adult cattle have been shown to harbour less resistant bacteria than calves (19).

Resistance to streptomycin was significantly higher in isolates from cattle (43.7%) than in isolates from both pigs (25.7%) and chickens (34.0%). Streptomycin accounts for more than 90% of the aminoglycoside use in food animals in Kenya (17). The selective pressure exerted by the use of streptomycin in streptomycin-penicillin combinations in intramammary and injectable preparations for the treatment of mastitis and other bacterial infections in cattle (17) might account for this finding. The injectable streptomycin-penicillin preparations are also used for treatment of bacterial infections in pigs. The relatively high incidence of resistance to streptomycin among the isolates from chickens may not be accounted for by its use in these species since oral formulations for mass medication are usually not available. Co-resistance with other unrelated compounds or horizontal transfer of resistance genes appears a likely explanation.

The levels of resistance to gentamicin and to chloramphenicol observed in this study were comparable to the level of resistance in other countries (12). Gentamicin, although a relatively old antimicrobial agent has had little use in animals (12) and in Kenya, no formulations are available for use in chickens. The resistance detected in E. coli isolates from chickens (2.0%) may have been caused by off-label use or the clonal spread of resistant isolates as suggested by Kijima-Tanaka et al. (20).

Approximately 4.7% of the E. coli isolates showed resistance to chloramphenicol, which was significantly higher in the isolates from pigs than those from chickens. In Kenya, as in the European Union and the United States of America chloramphenicol is not approved for use in food animals and its fluorinated analog, florfenicol has not been in use. Thus, the observed resistance is unlikely to be mediated by a gene encoding resistance to florfenicol (21). Other researchers have also reported chloramphenicol resistance among E. coli isolates from chickens and pigs in the absence of chloramphenicol use in these animal species and suggested co-resistance with other unrelated compounds as a possible explanation (12). Co-selection of chloramphenicol resistance during selective pressure imposed by the use of sulphonamides and streptomycin due to linkage of genes has also been reported (22,23). Furthermore, chloramphenicol resistance may be acquired via horizontal transmission of genes from other sources, such as water contaminated with human sewage or due to illegal use of chloramphenicol (24).

Overall, 40 different resistance patterns were recorded, of which, only five (12.5%) were found to be common among the isolates from all three species. Differences in production systems and antimicrobial usage patterns in the various populations may account for the differences in the resistance patterns observed among the E. coli isolates from the three animal sources. Additionally, these differences could also be related to the different antibiotic regimens used for the different antimicrobial agents and livestock species (25,5). There were no significant differences in the prevalence and patterns of resistance between the faecal and carcass swab isolates from either cattle or pigs. This may be due to the fact that slaughter is potentially the most important stage for bacterial contamination (12) and as a result resistant isolates from the gut may readily contaminate carcasses (26).

This study shows that multi-drug resistant E. coli isolates are prevalent in cattle, pigs and chickens and on fresh cattle and pig carcasses in Kenya. It is recommended that the use of antimicrobial agents in food animals should follow prudent use guidelines to minimize the selection and spread of resistant bacteria and that slaughter hygiene should be improved to minimize the risk of transfer of antimicrobial resistant bacteria

to humans.

References

1. Hart, W. S., Heuzenroeder, M. W. and Barton, M. D. Antimicrobial resistance in Campylobacter spp., Escherichia coli and Enterococci associated with pigs in Australia. J. Vet. Med. B 51: 216-221, 2004.

2. Helmuth, R. and Hensel, A.: Towards the rational use of antibiotics: results of the first international symposium on the risk analysis of antibiotic resistance. J. Vet. Med. B 51: 357-360, 2004.

3. van den Bogaard, A. E .J. M., London, N. and Stobberingh, E. E.: Antimicrobial resistance in pig faecal samples from The Netherlands (five abattoirs) and Sweden. J. Antimicrob. Chemother. 45: 663-671, 2000.

4. Bischoff, K. M., White, D. G., McDermott, P. F., Zhao, S., Gaines, S., Maurer, J. J. and Nisbet, D. J.: Characterization of chloramphenicol resistance in beta-hemolytic Escherichia coli associated with diarrhea in neonatal swine. J. Clin. Microbiol. 40: 389-394, 2002.

5. Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S. and Helmuth, R.: Phenotyphic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. J. Antimicrob Chemother. 52: 489-492, 2003.

6. Bebora, L. C., Yamamoto, W. K., Sang, J. O., Oundo, J., Khamala, A. Mwangudza, A. And Mukundi, P. W.: Plasmid profiles of Escherichia coli, Salmonella and Shigella organisms isolated in Kenya. East Afri. Med. J. 71: 619-623, 1994.

7. Kariuki, S., Gilks, C., Kimari, J., Obanda, A., Muyondi, J., Waiyaki, P. and Hart, C. A.: Genotypic analysis of Escherichia coli isolates isolated from children and chickens living in close contact. Appl. Environm. Microbiol. 65: 472-476, 1999.

8. Kithuka, J. M., Maingi, M., Njeruh, F. M. and Ombui, J. N.: The prevalence and economic importance of bovine fasciolosis in Kenya-an analysis of abattoir data. Onderstepoort J. Vet. Res. 69: 255-262, 2002.

9. NCCLS, 2004. National Committee for Clinical Laboratory Standards, Performance Standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals, informational supplement NCCLS Document M31-S1, vol. 24, No. 17 National Committee for Clinical Laboratory Wayne Pennsylvania, USA.

10. NCCLS, 1997. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing, sixth edition. Approved standard. NCCLS document M2-A6, supplement M100-S7, vol. 17. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA.

11. DANMAP, 2001. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans ISSN 1600-2032.

12. Bywater, R., Deluyker, H., Deroover, E., Anno de Jong, Marion, A., McConville, M., Rowan, T., Shryock, T., Shuster, D., Thomas, V., Valle, M. and Walters, J.: A European survey of the antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. J. Antimicrob. Chemother. 54: 744-754, 2004.

13. Lanz, R., Kuhnert, P. and Boerlin, P.: Antimicrobial resistance and resistance gene determinants in clinical Escherichia coli from different animal species in Switzerland. Vet. Microbiol. 91: 73-84, 2003.

14. Sunde, M. and Norstrom, M.: The genetic background for streptomycin resistance in Escherichia coli influences the distribution of MICs. J. Antimicrob. Chemother. 56: 87-90, 2005.

15. Kariuki, S., Revathi, G., Gakuya, F., Yamo, V., Muyodi, J. and Hart, A.: Lack of clonal relationship between non-typhi Salmonella strains types from humans and those from animals living in close contact. FEMS Immunol. Med. Microbiol. 33: 165-171, 2002.

16. Mitema, E. S. and Kikuvi, G. M.: Surveillance of the overall use of antimicrobial drugs in humans over a 5 year period (1997-2001) in Kenya. J. Antimicrob. Chemother. 54: 966-967, 2004.

17. Mitema, E. S., Kikuvi, G. M., Wegener, H. C. and Stohr, K.: An assessment of the antimicrobial comsumption in food producing animals in Kenya. J. Vet. Pharmacol. Therap. 24: 385-390, 2001.

18. Gakuya, F. M., Kyule, M., Gathura, P. B. and Kariuki, S.: Antimicrobial susceptibility and plasmids from Escherichia coli isolated from rats. East Afri. Med. J. 78: 518-522, 2001.

19. Khachatryan, A. R., Hancock, D., Besser, T. E. and Call, D. R.: Role of calf-adapted Escherichia coli in maintenance of antimicrobial drug resistance in dairy calves. Appl. Environm. Microbiol. 70: 752-757, 2004.

20. Kijima-Tanaka, M., Ishihara, K., Morioka, A., Kojima, A., Ohzono, T., Ogikubo, K., Takahashi, T. and Tamura, Y.: A national surveillance of antimicrobial resistance in Escherichia coli isolated from food-producing animals in Japan. J. Antimicrob. Chemother. 51: 447-451, 2003.

21. White, D. G., Hudson, C., Mauer, J. J., Ayers, S., Zhao, S., Lee, M. D., Bolton, L., Foley, T. and Sherwood, J.: Characterization of chloramphenicol and florfenicol resistance in Escherichia coli associated with bovine diarrhea. J. Clin. Microbiol. 38: 4593-4598, 2000.

22. Kehrenberg, C. and Schwarz, S.: Occurrence and linkage of genes coding for resistance to sulfonamides, streptomycin and chloramphenicol in bacteria of the genera Pasteurella and Mannheimia. FEMS Microbiol. Lett. 205: 283-290, 2001.

23. Bischoff, K. M., White, D. G., Hume, M. E., Poole, T. L. and Nisbet, D. J.: The chloramphenicol resistance gene cmlA is disseminated on transferable plasmids that confer multiple-drug resistance in swine Escherichia coli. FEMS Microbiol. Lett. 243: 285-291, 2005.

24. van Donkersgoed, J., Manninen, K., Potter, A., McEwen, S., Bohaychuk, A., Klashinsky, S., Deckert, A. and Irwin, R.: Antimicrobial susceptibility of hazard analysis critical control point Escherichai coli isolates from federally inspected beef processing plants in Alberta, Saskatchewan, and Ontario. Can. Vet. J. 44: 723–728, 2003.

25. Schwarz, S. and Chaslus-Dancla, E.: Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet. Res. 32: 201-225, 2001.

26. Aslam, M., Nattress, F., Greer, G., Yost, C., Gill, C. and McMullen, L.: Origin of contamination and genetic diversity of Escherichia coli in beef cattle. Appl. Environ. Microbiol. 69: 2794-2799, 2003.