East African Medical Journal Vol. 78 No. 10 October 2001
ANTIMICROBIAL SUSCEPTIBILITY AND PLASMIDS FROM *ESCHERICHIA COLI* ISOLATED FROM RATS
F.M. Gakuya, BVM, MSc, Field Veterinarian, Kenya Wildlife Services, M.N. Kyule, BVM, MSc, PhD, Senior Lecturer, P.B. Gathura, BVM, MSc, PhD, Senior Lecturer, Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi and S. Kariuki, BVM, MSc, PhD, Senior Research Officer, Centre for Microbiology Research, Kenya Medical Research Institute, P.O. Box 43640, Nairobi.

Request for reprints to: Dr F.M. Gakuya, Veterinary Unit, Kenya Wildlife Service, P.O. Box 40241, Nairobi.

# ANTIMICROBIAL SUSCEPTIBILITY AND PLASMIDS FROM *ESCHERICHIA COLI* ISOLATED FROM RATS

F.M. GAKUYA, M.N. KYULE, P.B. GATHURA and S. KARIUKI

## ABSTRACT

***Objectives:*** **To determine if antimicrobial resistance occurs in *E. coli* isolated from rats and if this resistance is transferable via plasmids.**
***Methods:*** **Sixty *Escherichia coli* isolates obtained from 215 rats trapped in and around Nairobi, Kenya, were analysed for antimicrobial susceptibility to eleven commonly used antimicrobials. Plasmid DNA analysis and *in-vitro* conjugation tests were conducted on twenty two resistant isolates.**
***Results:*** **A total of 12 (20%) E. *coli* were resistant to at least one of the commonly used drugs including ampicillin, sulphamethoxazole and streptomycin. Eight (13.3%) isolates were multi-drug resistant. The drug resistant *E. coli* fell into five plasmid profile groups with plasmids ranging between two and 98 megadaltons (Mda). Resistance to ampicillin was transferable.**
***Conclusion:*** **The results of this study show that rats from the study area may form an important reservoir for drug-resistant *E. coli* that could pose a public health hazard.**

## INTRODUCTION

There has been controversy over the natural ecology of *E. coli* and its infectious plasmids. Whether *E. coli* isolates and their plasmids are derived from humans and transmitted to animals or vice-versa is still unclear. It is thought by some that animals may serve as reservoirs for *E. coli* strains found in humans and that the frequency of transmission to humans of *E. coli* containing antibiotic resistance plasmids could be as high as 78% within two days of exposure(1).

Although use of antimicrobials for treatment and prophylaxis in human and veterinary medicine has resulted in the improvement of health(2), their indiscriminate use has provided a selective advantage to resistant bacteria and accelerated spread of the genetic elements coding for resistance(3). The extensive use of antibiotics for treatment of bacterial infections in humans and animals selects for resistant micro-organisms, which may in turn transfer resistance to other bacteria thereby enhancing their spread(4). The transfer of resistant bacteria has been shown to occur among different animal species, between humans, and from animals to humans and vice versa(5,6). Antibiotic resistant *E. coli* may be passed from animals to humans through contact with faecal material or faecally contaminated food sources. Normal *E. coli* flora acquire resistance plasmids from ingested resistant *E. coli* strains which may disseminate to pathogenic bacteria(2).

Antibiotic resistance in animals may have great impact on human health, especially in an environment where human and animals share the same ecosystem(7). For example, rats may contaminate water sources and invade houses where they contaminate foods(8), and subsequently act as an important vehicle of transmission of antibiotic resistant *E. coli* and their plasmids.

The aim of the present study was to investigate the importance of *E. coli* isolated from rats as reservoirs for transferable antimicrobial drug resistance.

## MATERIALS AND METHODS

*Study sites:* Rats were trapped in the densely populated area (slums) of Kibera and the less densely populated areas of Kabete and Kawangware in Nairobi, Kenya. The rats were classified as either domestic or wild according to the colour of their coat(9) and from the history of the residents as to where they trapped them (inside or outside the house). They were later transported to the laboratory.

*Collection and laboratory processing of specimens:* In the laboratory, the rats were sacrificed using chloroform (Oxoid, Unipath Ltd, Basingstoke, England). Dissection of the abdomen was performed and faecal material was collected by opening the intestines of each rat. Intestinal mucosal scrappings were also collected using a sterile blunt scalpel blade.

The faecal and intestinal mucosal specimens were inoculated into peptone water (Oxoid) and incubated at 37°C for 18 hours for enrichment. In order to select for potentially pathogenic bacteria, each specimen was sub-cultured into Rapparports vassiliaidis soy peptone (RVS) broth (Oxoid) and incubated overnight at 42°C. A loopful of each specimen was then sub-cultured onto MacConkey agar and Salmonella-Shigella agar plates (Oxoid) and incubated overnight at 37°C. Identification of *E. coli* was confirmed using biochemical tests on analytical profile index (API) 20E strips (Bio Merieux, Marcy-l'Etoile, France). All *E. coli* isolates were stored at -80°C until analysed.

*Antimicrobial susceptibility testing: Escherichia coli* isolates were tested for their susceptibility to eleven commonly used antimicrobials using the National Committee for Clinical Laboratory Standards (NCCLS)(10) on Isosensitest agar plates

(Oxoid). These antimicrobials and their disc strengths were: ampicillin (10 μg), co-amoxyclav (20:10 μg), tetracycline (10 μg), ciprofloxacin (5 μg), gentamicin (10 μg), trimethoprim (5 μg), sulphamethoxazole (10 μg), cefuroxime (30 μg), ceftazidime (30 μg), nalidixic acid (30 μg) and streptomycin (10 μg). A separate plate was inoculated with E. *coli* ATCC 25922 as a control. Results were interpreted according to criteria set by NCCLS(10).

Susceptibility to ten antimicrobials was also tested by minimum inhibitory concentration test (MIC). The inoculum of each *E. coli* isolate was adjusted to a concentration of about $10^6$ cfu/ml by comparing its turbidity to that of barium chloride 0.5 McFarland standard. Doubling dilutions of each antimicrobial agent were prepared in sterile distilled water from commercial tablets of known potency and concentration (Mast pharmaceuticals, Meyseyside, UK). The inoculum was plated onto Isosensitest agar containing antibiotics using a multiple inoculator. A control agar plate (without antibiotics) was inoculated last to ensure that viable bacteria were present throughout the experiment. *Escherichia coli* strain ATCC 25922 with known MIC values of each antimicrobial agent was included in each test as a control for antimicrobial potency. The MICs were interpreted according to criteria set by NCCLS(10). β-lactamase production by ampicillin resistant *E. coli* was performed using paper strips coated with the chromogenic cephalosporin, nitrocephin.

*Plasmid studies:* Plasmid DNA was isolated using a commercial plasmid preparation kit (Hybaid Limited, Middlesex, UK) based on a rapid alkaline lysis procedure according to the manufacturer's instructions. After extraction plasmid DNA was resuspended in 50 μl of sterile distilled water and analysed by electrophoresis on 1% agarose gel on horizontal tanks containing 0.5X TBE (0.1M Tris, 0.1M boric acid, 0.2M EDTA) buffer. *Escherichia coli* strains 39R861 and V517 containing plasmids of known molecular sizes (98, 42, 24, 4.6 and 35.8, 4.8, 3.7, 3.4, 2.6, 2.0, 1.8, 1.4, respectively) were included in each of the agarose gels used in electrophoresis.

After electrophoresis the gel was viewed on a ultraviolet transilluminator (VVP INC., San Gabriel, California, USA) and pictures taken using a Polaroid MP-3 Camera (Polaroid, Cambridge, Ma., USA). Molecular sizes for test plasmid DNA were calculated from a standard curve obtained from the natural log of the molecular sizes (Mda) against the migration distance in millimetres of the molecular size standards.

*Mating tests: In-vitro* conjugation tests on transferable antimicrobial resistance was performed using the method of Walia *et al*(11) with modifications. Single discrete colonies of each donor bacteria strain and recipient *E. coli* K12 (F-, $Nal^R$) were sub-cultured into 5ml tryptic soy broth and incubated at 37°C overnight. The donor and the recipient bacterial broth cultures were then diluted 1:10 in fresh tryptic soy broth and allowed to multiply to the logarithmic phase by incubating them at 37°C for four hours. The recipient and donor bacterial broths were mixed at a ratio of 1:2 respectively and incubated at 37°C overnight.

To select transconjugants, 3 μl-samples were drawn from the overnight culture and plated onto isosensitest agar plates containing 16 μg/ml nalidixic acid + 8 μg/ml tetracycline, 16 μg/ml nalidixic acid + 16 μg/ml streptomycin and 16 μg/ml nalidixic acid + 16 μg/ml co-trimoxazole, using a multipoint inoculator. For β-lactam antibiotics (ampicillin and co-amoxyclav), *E. coli* cells were washed with sterile normal saline to remove leached β-lactamase enzyme from the solution.

*Statistical analysis:* Chi-square tests with Yate's correction was used to compare proportions of *E. coli* isolates obtained from rats trapped in different areas and the proportions of *E. coli* isolates obtained from rats categorised as wild or domestic.

## RESULTS

*Bacterial isolates:* A total of 131 *E. coli* isolates were obtained from 215 rats studied. Fifty eight *E. coli* isolates were obtained from rats from Kabete, 73 from Kibera while 93 were from domestic rats and 38 from wild rats. A total of 60 (45.8%) representative *E. coli* were selected for further study.

*Susceptibility testing:* Eight (13.3%) of the 60 *E. coli* isolates were fully sensitive to all eleven antimicrobials tested. All isolates were susceptible to gentamicin, nalidixic acid, cefuroxime, ceftazidime and ciprofloxacin. Twenty (33.3%) of the isolates were resistant to at least one or more antimicrobial agents namely ampicillin, streptomycin, sulphamethoxazole, co-amoxyclav, trimethoprim and tetracycline. Eight (13.3%) of the isolates were multidrug resistant (that is, resistant to two or more antimicrobials) (Table 1). One (12.5%) of the eight isolates showing multi-drug resistance was isolated from wild rats. Table 2 shows the antimicrobial susceptibility profile of the 60 *E. coli* isolates studied.

**Table 1**

*Multi-drug resistant E. coli isolates from rats trapped from Kabete, Kibera and Kawangware areas in Kenya, 1998*

| Type of rat | No. of resistant *E. coli* | Resistance pattern |
|---|---|---|
| Domestic | 1 | Amp, Sulpha, Tet, Trim |
| Domestic | 1 | Strept, Sulpha, Trim |
| Domestic | 1 | Amp, Strept. |
| Domestic | 1 | Amp, Amc. |
| Domestic | 1 | Strep, Sulpha. |
| Wild | 1 | Amp, Tet. |
| Domestic | 1 | Amp, Sulpha. |
| Domestic | 1 | Sulpha, Trim. |

Key: Amp=Ampicillin, Amc=Co-amoxyclav, Strept=Streptomycin, Sulpha=Sulphamethoxazole, Tet=Tetracycline, Trim=Trimethoprim

**Table 2**

*Antimicrobial susceptibility profile of E. coli isolates from rats trapped from Kabete, Kibera and Kawangware areas in Kenya, 1998*

| Antimicrobial agent | No. (%) *E.coli* N=60 | | |
|---|---|---|---|
| | Resistant | Intermediate | Susceptible |
| Ampicillin | 12 (20.0) | 32 (53.3) | 16 (26.7) |
| Co-amoxyclav | 1 (1.7) | 5 (8.3) | 54 (90.0) |
| Streptomycin | 8 (13.3) | 40 (66.7) | 12 (20.0) |
| Sulphamethoxazole | 15 (25.0) | 22 (36.7) | 23 (38.3) |
| Tetracycline | 2 (3.3) | 23 (38.3) | 35 (58.3) |
| Trimethoprim | 3 (5.0) | 7 (11.7) | 50 (83.3) |
| Cefuroxime | 0 (0.0) | 9 (15.0) | 51 (85.0) |
| Ceftazidime | 0 (0.0) | 8 (13.3) | 52 (86.7) |
| Nalidixic acid | 0 (0.0) | 7 (11.7) | 53 (88.3) |
| Gentamicin | 0 (0.0) | 8 (13.3) | 52 (86.) |
| Ciprofloxacin | 0 (0.0) | 2 (3.3) | 58 (6.7) |

MICs were determined for ten antimicrobials (Table 3). Resistance was observed for ampicillin (23.3%), streptomycin (15%), co-trimoxazole (6.6%), tetracycline (3.3%) and co-amoxyclav (1.7%). Some isolates showed

**Table 3**

*Minimum inhibitory concentrations (MICs) of 10 antimicrobials tested against 60 E. coli isolates from rats trapped from Kabete, Kibera and Kawangware areas in Kenya, 1998.*

| Antimicrobial | MIC range (µg\ml) | Mode | $MIC_{50}$ | $MIC_{90}$ | * No. of resistant isolates | % resistant |
|---|---|---|---|---|---|---|
| Ampicillin | 8-128 | <8 | 16 | 64 | 14 | 23.3 |
| Streptomycin | 8-128 | <8 | 8 | 32 | 9 | 15.0 |
| Co-trimoxazole | 4-64 | <4 | <4 | 16 | 4 | 6.6 |
| Tetracycline | 4-64 | <4 | <4 | 4 | 2 | 3.3 |
| Co-amoxyclav | 4-64 | <2 | <2 | 4 | 1 | 1.7 |
| Gentamicin | 2-32 | <2 | <2 | 8 | 0 | 0.0 |
| Ceftazidime | 2-32 | <2 | <2 | 2 | 0 | 0.0 |
| Cefuroxime | 2-32 | <2 | <2 | 16 | 0 | 0.0 |
| Nalidixic acid | 2-32 | <2 | <2 | 4 | 0 | 0.0 |
| Ciprofloxacin | 1-16 | <1 | <1 | 1 | 0 | 0.0 |

Key: * The number of resistant *E. coli* was determined using NCCLS (1997) cut-off values

exceptionally high MICs for various antimicrobials. These included, ampicillin with five (8.3%) and one (1.7%) of isolates showing MIC≥64 and MIC≥128 µg/ml respectively, streptomycin with two (3.3%) and one (1.7%) of the isolates showing MIC≥64 and MIC≥128 respectively and co-trimoxazole with two (3.3%) of isolates showing MIC≥64 µg/ml. *E. coli* from rats from Kibera area were more resistant to antimicrobials than *E. coli* from rats from Kabete (p=0.043). In general, no statistical difference (p=0.2884) was found in resistance to antimicrobials for *E.* coli isolates from wild and domestic rats.

*β-lactamase production:* A total of fifteen ampicillin resistant *E. coli* isolates were studied for production of β-lactamases. Fourteen (93.3%) out of the fifteen isolates produced β-lactamases.

*Plasmid studies:* Sixteen (72.7%) of the twenty two *E. coli* isolates resistant to one or more antimicrobials carried plasmids. The molecular sizes of these plasmids ranged from 2 to 98 Mda. Six (27.3%) isolates did not carry any plasmids. The isolates were categorised into five plasmid profile groups according to the number of plasmids they carried with those without any plasmid forming one plasmid profile group (Table 4). Two (25%) of the eight multidrug resistant isolates had both the 90-100 and 55-65 Mda plasmids while four (50%) isolates had either the 90-100 or 55-65 Mda plasmids. Two (25%) isolates did not have any plasmids.

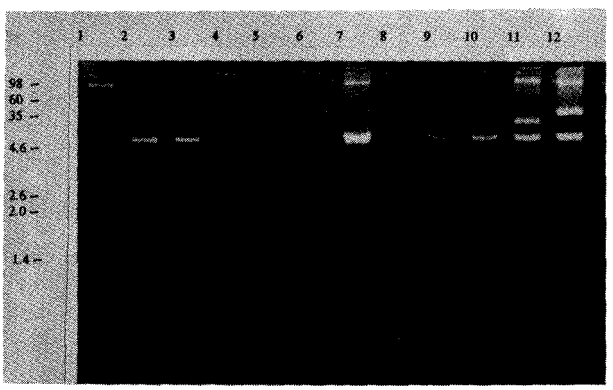
**Table 4**

*Plasmid profile groups of antimicrobial resistant E. coli isolates from rats trapped from Kabete, Kibera and Kawangware areas in Kenya, 1998*

| Plasmid profile group | No. of plasmids carried | Molecular sizes of plasmids (Mda) | No. of isolates |
|---|---|---|---|
| 1 | 0 | - | 6 |
| 2 | 1 | 95, 90, 60, 2 | 6 |
| 3 | 2 | 98, 96, 95, 60, 56, 59, 42, 2 | 5 |
| 4 | 3 | 96, 62, 56, 50, 42, 40, 18, 8, 4, 2 | 4 |
| 5 | 4 | 95, 56, 40, 4 | 1 |

**Figure 1**

*Molecular weights in megadaltons of plasmids of reference drug-resistant E. coli. Plasmids of E. coli V517 and 39R861 are in lane 1 and 12 respectively and those of plasmids of E. coli isolates from rats in lane 2 to 11*




*Transferable resistance:* In conjugation experiments, five (22.7%) of the 22 antimicrobial resistant *E. coli* isolates transferred ampicillin resistance to *E. coli* K12. Other antimicrobials tested did not transfer resistance to *E. coli* K12. The number of plasmids in three transconjugants was one or two with molecular sizes ranging from 4 to 98 Mda. In two transconjugants with plasmids it was not possible to identify which plasmids were transferred.

## DISCUSSION

Antimicrobial susceptibility data from the present study showed that rats harbour *E. coli* resistant to various antimicrobials commonly used in both humans and animals.

Although there is controversy over the natural ecology of *E. coli* and its infectious plasmids(1), rats could act as one important source of transmission of *E. coli* and its plasmids to humans and other animals.

In this study, resistance was observed for ampicillin, sulphamethoxazole, trimethoprim, cotrimoxazole, streptomycin and tetracycline. No resistance was observed for gentamicin, nalidixic acid, ceftazidime and ciprofloxacin. These results agree with those from other authors(12, 13) who investigated antimicrobial resistance of normal gut microflora in chickens and pigs. In this study the drug resistance in *E. coli* isolates could have arisen from the fact that rats get into contact with these antimicrobials through various sources in the environment, for example food, water and sewer systems.

Eight (13.3%) *E. coli* isolates from rats were fully susceptible to all antimicrobials tested while eight (13.3%) showed multi-drug resistance mainly to ampicillin, sulphamethoxazole and streptomycin. The highest resistance was observed for sulphamethoxazole followed by that of ampicillin and streptomycin. These results agree with those of other authors(13,14,15) who reported similar resistance trends in E. *coli* isolates from chickens, pigs and milk. In contrast, these investigators reported high level of tetracycline resistance, which was not observed in the present study. It is important to note that ampicillin, streptomycin and sulphamethoxazole (mostly in combination with trimethoprim as co-trimoxazole) and tetracycline are used as "first" line agents for treatment of bacterial infections in humans. This could lead to very high residue effect in faeces and other wastes of humans which can be passed to the rats hence leading to acquisition of resistance due to antibiotic selection pressure in resident *E. coli* microflora of the intestines of the rats. These drugs are also used in veterinary medicine and therefore their residues can pass through faeces or fecally-contaminated water or feeds from treated animals or animals fed on antibiotic supplemented feeds.

*E. coli* isolates from rats from Kibera area were more resistant to antimicrobials than *E. coli* from rats from Kabete. Kibera is a densely populated area (slum) with poor drainage and open sewer systems where rats frequent to feed. Kabete is less densely populated with better sewer systems and relatively well managed dumping sites hence contacts of rats with human waste was minimal. This led to the low frequency of antimicrobial resistant *E. coli* isolated from rats trapped in Kabete area.

Fourteen (93.3%) of the fifteen *E. coli* isolates resistant to ampicillin produced β-lactamases. This agrees with the results observed by other authors(16,17) who reported that 90% of resistance to β-lactamases is through production of β-lactams. Resistance to ampicillin presents a major problem since it is one of the most widely available orally administered antibiotics. It is used in most hospitals for the treatment of enterobacterial infections and pneumonia in children. Therefore, ampicillin resistant *E. coli* isolated from rats is of major public health concern.

Considering that in Kenya, *E. coli* is an important cause of bacteraemia in nosocomial infection(18) and a significant public health problem, and that antibiotics are widely used in clinical practice, the need to avert the spread of resistance is important. It seems rats can act as reservoirs of genetic pools of antimicrobial resistance genes which could be transferred to humans. On the other hand, humans may act as the reservoirs of genetic pools of antimicrobial resistance genes, which could be transferred to rats. These rats can thus act as the foci for multiplication of these genes with subsequent transmissions to humans and other animals posing a major threat in treatment of diseases in humans and other animals.

The number of plasmids per *E. coli* isolate ranged from 0 to 4. These results compare well with those of other authors(15,19) who reported similar ranges in the number of plasmids in studies of E. *coli* isolates from cow milk and from children. However, resistance to various antimicrobial agents was not closely associated with the presence of plasmids since resistance was found in isolates with no plasmids as well as those that had plasmids. Nevertheless, large plasmids of 90-100 Mda and 55-65 Mda were more associated with the resistance as they occurred in 75% of the antimicrobial resistant *E. coli* isolates. Kariuki *et al*(14) also reported that a plasmid of 90-100 Mda was associated with antimicrobial resistant *E. coli* isolates from chicken and children who lived in close proximity. In another study, Bebora *et al*(19) reported an association of the 60 Mda plasmid with drug resistant *E. coli* from chickens.

Resistance was only transferred in five (22.7%) of ampicillin resistant *E. coli* isolates to *E. coli* K12. The ability of multidrug resistant *E. coli* isolates to transfer resistance to *E. coli* K12 has been reported by Niljesten *et al*(20) to range from 26% to 50% in isolates from human and about 50% to 76% in isolates from pigs and by O'Brein *et al*(21) to be 24% in isolates from poultry. Although the results of this study agree with the findings by Niljesten *et al*(20) for human *E. coli* isolates and O'Brein *et al*(21) in poultry, they do not agree with ranges obtained in pig isolates. Furthermore, resistance was transferrable for only one antimicrobial namely ampicillin, as opposed to their findings where resistance was transferrable to one, two or more antimicrobials. Large plasmids of 90-100 MDa and 55-65 MDa were transferred in three (60%) of the five isolates hence it can be concluded that these plasmids were responsible for transfer of ampicillin resistance.

In conclusion we demonstrated that rats in the wild may have been directly or indirectly exposed to materials containing antimicrobial residues. In addition this study showed that rats carry antimicrobial resistant *E. coli* and their plasmids pose a public health hazard.

ACKNOWLEDGEMENTS

To the Director, Kenya Medical Research Institute, for allowing us to use their laboratory facilities.

## REFERENCES


1. Levy, S.B., Marshall, B., Schluederberg, S., Rowse, D. and Davies, J High frequency of antimicrobial resistance in human fecal flora. *J. Antimicrob. Chemother* 1988; **32**:1801-1806.
2. Espinasse, J. Responsible use of antimicrobials in Veterinary Medicine. Perspective in France. *Vet. Microbiol.* 1993; **35**:289-301.
3. Mazodier, P. and Davies, J. Gene transfer between distantly related bacteria. *Annual Rev. Gen.* 1991; **25**:147-171.
4. Levy, S.B., Fitzgerald, G.B., and Macone, A.B. Spread of antibiotic resistance plasmids from chicken to chicken and chicken to man. *Nature* 1976; **260**:40-42.
5. Marshall, B., Petrowski, D. and Levy, S.B. Inter- and intra pecies spread of *E. coli* in a farm environment in absence of antibiotic usage. *Proc. Nat. Acad. Sci. (USA)* 1990; **87**:6609-6613.
6. Rollard, R.M., Hausfater, G., Marshall, B. and Levy, S.B. Antibiotic-resistant bacteria in wild primates: Increased prevalence in baboons feeding on human refuse. *Appl. Environ. Microbiol.* 1985; **49**:791-794.
7. Kruse, H., and Sorum, H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins. *Appl. Environ. Microbiol.* 1994; **60**:4015-4021.
8. Centre for Disease Control. *Salmonella* isolates from the United States, 1984-1986. CDC Surveillance 1988; **37**: 25-31.
9. Kingdom, J. Taxonomy of rats. In J Kingdom (ed.), East African Mammal, an atlas of evolution in Africa 1974; pp 577-594. Academic Press, London.
10. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards 1997, Wayne, Pa.
11. Walia, S.K., Madhavan, T., Chagh, T.D. and Sharma, K.B. Characterisation of self-transimissible plasmids determining lactose fermentation and multiple antibiotic resistance in clinical strains of *Klebsiella pneumoniae*. *Eur. J. Clin. Microbiol. Infect. Dis.* 1987; **7**: 279-284.
12. Kariuki, S.M. Epidemiology and Genetics of antimicrobial resistance *Salmonella* spp and *Escherichia coli* isolated from clinical and environmental sources in Kenya. PhD thesis, University of Liverpool, UK, 1996.
13. Niljesten, R., London, N., Vau den Bogaard, A. and Stobberingh, E. Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. *J. Antimicrob. Chemother.* 1996a; **37**:1131-1140.
14. Kariuki, S., Gilks, C., Kimari, J., Obanda, A., Muyodi, J., Waiyaki, P. and Hart, C.A. Genotypic analysis of *Escherichia coli* strains isolated from children and chickens living in close contact. *Appl. Environ. Microbiol.* 1999; **65**:472-476.
15. Ombui, J.N., Macharia, J.K. and Nduhiu, G. Frequency of antimicrobial resistance and plasmid profiles of *Escherichia coli* strains isolated from milk. *East Afr. Med. J.* 1995; **72**: 228-230.
16. Ling, T.K.W., Lyon, D.J., Cheng, A.F.B. and French, G.L. In-vitro antimicrobial susceptibility and β-lactamases of ampicillin-resistant *Escherichia coli* in Hong Kong. *J. Antimicrob. Chemother.* 1994; **18**: 189-197.
17. Sanders, C.C. β-lactamases of Gram-negative bacteria: new challenges for new drugs. *Clin. Infect. Dis.* 1992; **14**:1085-1099.
18. Gilks, C.F., Brindle, R.J., Otieno, L.S., Simani, P.M., Newnham, R.S., Bhatt, S.M., Lule, G.N., Okello, G.B.A., Watkins, W.M., Waiyaki, P.G., Were, J.B.O. and Warell, D.A. Life threatening bacteraemia in HIV-1 seropositive adults admitted to hospitals in Nairobi, Kenya. *Lancet* 1990; **336**:545-549.
19. Bebora, L.C., Oundo J.O. and Yamamoto H. Resistance of *E. coli* strains recovered from chickens to antimicrobials with special reference to trimethoprim-sulphamethoxazole (septrin). *East Afr. Med. J.* 1994; **71**:624-627.
20. Niljesten, R., London, N., Vau den Bogaard, A. and Stobberingh E. In-vitro transfer of antibiotic resistance between fecal *Escherichia coli* strains isolated from pig farmers and pigs. *J. Antimicrob. Chemother.* 1996b; **37**:1141-1154.
21. O'Brien, T.F., DiGiorgio, J., Parsonett, K.C., Kass, E.H. and Hopkins, J.D. Plasmid diversity in Escherichia coli isolated from poultry and poultry processors. *Vet. Microbiol.* 1993; **35**:243-255.