PharmacologyOnLine December 30, 2015

Archives • 2015 • vol.3 • 44-52

ANTIBIOTIC RESISTANT ESCHERICHIA COLI IN FEACAL OF CAPTIVE BABOONS NOT NORMALLY EXPOSED TO ANTIBIOTIC

Mureithi, D.K. [1*]; Mitema, E.S.[2]; Mapenay, I.M.[2]; Ozwara, H.S.[3]; Jung'a, J.O.[4]

[1] Department of Animal Health and Production, Mount Kenya University, P O Box 342-01000 Thika, Kenya.
[2]Department of Public Health Pharmacology and Toxicology, University of Nairobi, PO Box 29053 Nairobi, Kenya
[3]Department of Tropical and Infectious Diseases, Institute of Primate Research, PO Box 24481, Nairobi, Kenya
[4]Department of Animal Production, University of Nairobi, PO Box 29053 Nairobi, Kenya

*dmureithi@mku.ac.ke

Abstract

Escherichia coli are common inhabitant of intestinal tract of many animals and humans. These intestinal bacteria including antibiotic-resistant bacteria can be easily transferred between animals and humans especially when there is close contact. Most studies on commensal antibiotic-resistant bacteria have been limited to food animals and seldom in non human primates. To understand the possible risks to humans in close contact with captive baboons, we analyzed the phenotype and genetic characteristics of antimicrobial resistance in ninety seven *Escherichia coli* isolates recovered from 100 faecal samples of two groups of captive baboons at the Institute of Primate Research, Kenya. Susceptibility to 16 antibiotics was studied in these isolates, and the most common resistance observed in *E.coli* isolated from both group of baboons was to ampicillin (34.4-36.1%), sulphamethoxazole (33-36.1%), amoxyclav (26.2-30.6%), piperacillin (22.2-23%), tetracycline (19.7-22.2%), streptomycin (11.1-21.3%), and co-trimoxazole (9.8-25%). The percentage of resistance to chloramphenicol, ceftazidime, ceftriaxone, gentamicin, amikacin and ofloxacin was below 8.2%, and no resistant isolates were detected for meropenem and ciprofloxacin. Multi-drug resistance was found in case of 7-8 antibiotics for all strains tested. The $bla_{TEM,}$ bla_{SHV} and bla_{CTX-M} genes were detected in 21, 19 and 5 of 37 ampicillin-resistant isolates respectively, the *aadA1* or *aadA2* genes in 5 of 20 streptomycin-resistant isolates and *cmlA* in 5 of 8 chloramphenicol-resistant isolates. It may be concluded that captive baboons may be a potential reservoir for zonotic transmission of multidrug resistance gene to humans and therefore minimal contact with these animals should be maintained.

Keywords: Antimicrobial resistance, *Escherichia coli*, CTX-M, Baboons.

Introduction

The role of commensal bacteria in the spread of antibiotic resistance is being recognized as a vital component in understanding how to preserve the therapeutic usefulness of antibiotics. *Escherichia coli*, a common inhabitant of gastrointestinal tract of human and the majority of animals is considered as practical "indicator bacteria" that could be used to track the evolution of antimicrobial resistance in different ecosystems. However, it has also emerged as important causes of nosocomial and community acquired infections [5,19].

In fact, antibiotic-resistant intestinal bacteria, at least in the minority populations of enterics and enterococci, have been found widely in environments where antibiotics are used. Antibiotic-resistant bacteria have also been found in settings where antibiotic exposure is expected to be rare or nonexistent. Surveys of antibiotic-resistant bacteria in wild animals have detected resistant bacteria in intestinal contents [4, 9]. These studies, however, have also been limited to the numerically minor bacterial populations [13]. Proximity to human activities influences the antibiotic resistance profiles of the gut bacteria of wild mammals, which live in densely populated microbial habitats in which antibiotics select for resistance [3]. About 13.3% of E.coli isolates from domestic and wild rats captured in peri-urban areas of Kenya were fully sensitive to all the eleven antimicrobial tested [12]. However, in Finland the faecal enterobacteria of wild elk, deer and voles showed almost no resistance [20]. Other studies have reported that African baboons and apes that are in contact with humans harbour more antibiotic-resistant enteric bacteria than those that dwell in areas that are remote from human activity [21, 23].

Therefore, these reports might suggest that human activities influence antibiotic resistance profiles in bacterial communities in wild animals, although other factors that affect the frequency of antibiotic resistance cannot be eliminated. Whilst several studies in different animals including baboons have analysed *E. coli* for their susceptibility to antimicrobial agents and genetic determinants [24, 11, 14] zoonotic components of antimicrobial resistance varies between countries [10] and studies of *Enterobacteriaceae* of non human primates origin in Kenya are limited. Therefore, this study was undertaken to investigate the role played by non human primates in the transmission of antimicrobial resistance in Kenya..

Materials and methods

Animals

The two group of captive olive baboons (*Papio*amina and redish in the back. It possess two masculine inflorescences with 2 cm long bracts, 6 – 8 cm long *anubis)* population in the Institute of Primate Research (IPR) of Kenya served as the subject for the present study. The first group, group I consisted of 20 adult male baboons and 16 female baboons which were captured from Aberdare National park (Located approximately 180 km north of Nairobi) and transported to Institute of Primate Research. This group of baboons had been in captivity for a period of less than one month. For the first two weeks they were housed in group cages and later in individual cages. The second group, group II consisted of 64 adult male baboons weighing 15- 30kg that had lived at the Institute of Primate Research for a period of between one year and 5 years. They were housed in individual cages. Both groups of baboon had very minimal contact with people unless during feeding and general cleaning which was done in the morning. No animal received antibiotic treatment prior to our sampling. The concrete floors of the cage area were regularly cleaned with hoses. All primates received comparable diet and they were fed a diet of Purina monkey chow (no less than 5% protein), fruit, and water.

Samples and bacterial isolates

A total of 100 feacal samples (36 of group I and 64 of group II) collected from healthy captive baboons following approval from Institutional Review Committee (IRC) reference number IRC/06/09 were tested in this study. Feacal samples were collected immediately after passage as baboons move away from their own feces by climbing onto the cages. The fresh feacal bolus was then sampled from its center by using a sterile swab which 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.

Isolation and identification of Escherichia coli

Faecal swab samples were pre-enriched in buffered peptone water (Oxoid, Basingstoke, England) and incubated for 3-6 hours at 37^{o}C. A loopful aliquot of the pre-enrichment broth was streaked on MacConkey agar (Oxoid Ltd, Basingstoke Hampshire, England) and incubated for 24 h at 37^{o}C. Three colonies per sample, with typical *E. coli* morphology, were selected and identified by classical biochemical methods (gram, indole, methyl red-voges proskauer

(MRVP), and citrate), and by the API 20E system (BioMe´rieux) test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by the agar disk diffusion method as recommended by the Clinical and Laboratory Standards Institute [7]. A total of 16 antimicrobial agents were tested, these were; ampicillin (10µg), piperacillin (100µg), amoxyclav (30µg), ceftriaxone (30µg), ceftazidime (30µg), meropenem (10µg), gentamicin (10µg), amikacin (30µg), Kanamycin (30µg), streptomycin (10µg), tetracycline (30µg), co-trimoxazole (25µg), sulfamethoxazole (25µg), ciprofloxacin (5µg), ofloxacin (5µg) and chloramphenicol (30µg). (Himedia Laboratories Ltd, Mumbai, India). *E. coli* ATCC 25922 was used as a reference organism for growth of bacteria and potency of antibiotics.

Bacteria DNA preparation and PCR assays

DNA of *E.coli* isolates was extracted by boiling method as previously described [1]. Specific polymerase chain reaction (PCR) assays were used for the detection of genes for ampicillin resistance (bla_{TEM}, bla_{SHV} and bla_{CTX-M}), chloramphenicol resistance (*cmlA*), and streptomycin resistance (*aadA1* and *aadA2*) as described previously [16,9,8] with some minor modification using specific primers (Table 1). The PCR products were detected by electrophoresis in 1.5% agarose gels. The migration distances of DNA bands were compared with those of TrackIt™ 1 Kb DNA ladder (Invitrogen, Life Technologies) to estimate the PCR products length. Positive and negative controls from the bacterial collection of the University of Nairobi, department of public health, pharmacology and toxicology, Kenya, were used in all assays..

Results

Prevalence of Escherichia coli

A total of 97 *E. coli* isolates were recovered from the 100 faecal samples of baboons collected and analysed in this study. Of the ninety seven *E.coli* isolates, thirty six were from group I baboons while sixty one *E.coli* isolates were from group II baboons. No *E. coli* isolates were recovered in three of the faecal samples obtained of group II baboons.

Antimicrobial susceptibility

The resistance to 16 antimicrobial agents for *E. coli* isolates from both groups of baboons is shown in Table 2. Most common resistance observed in *E.coli* isolated from both group of baboons was observed in ampicillin (34.4-36.1%), sulphamethoxazole (33-36.1%), amoxyclav (26.2-30.6%), piperacillin 22.2-23%), tetracycline (19.7-22.2%), streptomycin (11.1-21.3%), and co-trimoxazole (9.8-25%). Resistance to chloramphenicol and ceftazidime was observed in 8.2% of the isolates. No *E.coli* isolates from group I baboons showed resistance to ceftriaxone, gentamicin, amikacin and ofloxacin. However, one (1.6%) isolate from group II baboons showed resistance against these four antimicrobial agents. No resistant isolates from both groups of baboons were detected for meropenem and ciprofloxacin. Of the nine isolates resistant to ceftazidime and ceftriaxone, five of these isolates harbored the gene CTX-M. Resistance to co-trimoxazole was significantly higher ($p \leq 0.05$) in group I baboons as compared to group II baboons isolates. The phenotypes of resistance exhibited by the 97 *E. coli* isolates from both groups of baboons are presented in Table 3. The percentage of strains showing multidrug resistance was 38.9 % and 41% of *E. coli* isolates from group I baboons and group II baboons, respectively. Although sulphamethoxazole resistance was the most frequently observed among *E. coli* isolates (4.9%) from group II baboons, combined resistance to ampicillin-piperacillin-amoxyclav-streptomycin-tetracycline-co-trimoxazole-sulphamethoxazole was the most common among isolates (8.3%) from group I baboons. No significant difference was observed in the patterns of multidrug resistance between the isolates from group I and group II baboons.Twenty eight (75.7%) of the thirty seven ampicillin *E.coli* isolates were positive for at least one of the three β-lactamase genes tested.

Characterization of resistance gene

Using specific primers, PCR was carried out on the genomic DNA of thirty seven ampicillin resistant *E. coli* isolates for the presence of genes encoding TEM, SHV and CTX-M β-lactamases. (Table 4). Twenty eight (75.7%) of the thirty seven ampicillin *E.coli* isolates were positive for at least one of the three β-lactamase genes tested. The majority of the strains showed (21 of 37) positive amplification for bla_{TEM}. This was followed by bla_{SHV} (positive in 19 of 37) and bla_{CTX-M} (5 strains). Of the thirty seven ampicillin resistant *E.coli* isolates, one isolates showed resistance to ceftriaxone and seven to cefotaxime. Five of these *E.coli* isolates harboured the gene encoding CTX-M β-lactamase. The *cmlA* gene, which is a non-enzymatic chloramphenicol resistance gene, was detected in 5 of 8 chloramphenicol resistant *E.coli* isolate. The *aadA1* or *aadA2* gene, encoding an aminoglycoside adenylyltranferase that modifies streptomycin, was detected in 5 of the 20

streptomycin resistant isolates of this study.

Discussion

In general, *E. coli* isolated from both groups of baboons showed low percentages of resistance to chloramphenicol, kanamycin, gentamicin, which compares with previous reports in food animals in Kenya [15]. Chloramphenical use in food animal has been banned in Kenya; however there still exist restricted use mainly in topical application for treatment of ophthalmic condition in dogs. The most common resistance observed in this study was to ampicillin, sulphamethoxazole followed by amoxyclav, piperacillin tetracycycline, streptomycin and co-trimoxazole. This finding is in agreement with the results of previous studies, [5] which have shown a common occurrence of resistance to these antimicrobials in *E. coli* isolates from healthy children. The high frequency of resistance to these antimicrobials in baboons may be associated with the contact of these animals with human during feeding and cleaning. Other authors has also suggested a possible close correlation between the level of antibiotic resistance in bacteria from animals and the level of contact of these animals with humans, suggesting that the current prevalence of antimicrobial resistance found in fecal animal bacteria may be of anthropogenic nature [8]. Although limited data are available for comparison, bacterial isolates from group II baboons were more resistant than those from group I baboons against most antimicrobials tested in this study. Similarly, the frequency of resistance was higher in isolates from baboons feeding on human refuse compared to those living in undisturbed live in the wild in an older study done in Kenya which unfortunately did not use standard susceptibility testing method [22]. Since the isolates included in this study originated from baboons captured from various location throughout the country, they may be considered to be representative and epidemiologically unrelated. However, group I baboons tested in this study could not be simply assumed to be wild baboons, because they had stayed in captivity for a period of less than a month being fed on a diet of Purina monkey chow (no less than 5% protein), fruit, and water. Therefore the direct effect of diet could not be eliminated. Studies should be carried out in the future to obtain more data on antimicrobial resistance among both pathogenic and indicator bacteria from wild baboons. When compared to other livestock animals in Kenya, the prevalence of resistance observed in this study was generally similar to those reported in healthy cattle but lower than in healthy pigs and poultry [15]. Especially, resistance against antimicrobials commonly used as feed additives or used for a long time in livestock animals such as tetracycline, ampicillin, and streptomycin was lower in both groups of baboons than in pigs and poultry: 19.7-22.2%, 34.4-36.1%, and 11.1-21.3%, in baboons, whereas 40%, 50.5%, 25.7% in pigs and 34% 32%, 34% in poultry, respectively. However, *E. coli* isolates from both groups of baboons showed higher resistance against chloramphenicol (8.2%), which it use in food animal has been banned in Kenya. Nonetheless, resistance against chloramphenicol (8.2%) kanamycin (1%), and co-trimoxazole (15.5%) was still much lower in baboons, compared with 20%, 12%, and 30% in poultry, respectively [15]. The low level of resistance in *E.coli* isolates in our study to chloramphenicol, ceftazidime, kanamycin, amikacin, gentamycin and ceftriaxone are in line with the observation by [18] that resistance in newer antibiotic is emerging. There was no resistance observed in *E.coli* isolates for ciprofloxacin and meropenem. These results agree with the finding by [12] in a study of antimicrobial resistance in *E.coli* isolates from wild and domestic rat. Probably this could be due to the fact that these two antibiotic are used a second line of treatment in Kenya and in most cases they are usually not available. Compared to other food producing animals in other part of the world like Bagradesh which reported a much higher frequency of resistance of 82% to ciprofloxacin [2], this study report complete susceptibility of *E.coli* isolates. Studies in Korea of *E.coli* isolates from dogs have also reported frequencies of up to 16% to cipfloxacin [18]. Unexpectedly, resistance to co-trimoxazole was significantly higher ($p \leq 0.05$) in group I baboons as compared to group II baboons. Although group I baboons were in captivity for a period of less than a month resistance could have been acquired by interaction of resistance commensal bacteria from the environment since these baboons were captured from Aberdare national park where there is interaction with food producing animals and pastoralist who may have been exposed to antimicrobial agent. In this study, patterns of multiresistance in *E. coli* isolate from both group I baboons and group II baboons were similar. Also, one isolate from group II baboons showed resistance to 8 out of 16 antimicrobials tested, whereas three isolate from group I baboons was resistant to 7 antimicrobials. A previous study reported that the prevalence of multiresistance in *E. coli* isolates from baboons feeding on human refuse was

significantly higher than those observed in strains originating from baboons in the wild [21]. The author also documented that the reason for the elevated resistance prevalence in strains from baboons feeding on human refuse is due to close contact with human which could make this resistance to be of anthropogenic nature. When wild baboons are captured from the wild and brought to the Institute of Primate Research, they are first kept in group cages and later in individual cages. During the holding period in cages, wild baboons could have been exposed to bacteria from cohabitated baboons or from cleaners, who may have contained resistance genes that could be transmitted horizontally among baboons in the same place. Resistance was encoded by genes that are widespread in *Enterobactericeae* and are known to be commonly located on transposons, which are mobile DNA elements that play an important role in transmission and dissemination of antimicrobial determinants. Ampicillin resistance in *E. coli* isolates observed in this study was largely associated with TEM and SHV ß-lactamase genes, with only five isolate positive for CTX-M ß-lactamase genes. This agrees with other reports that TEM and SHV ß-lactamase genes are the most prevalent in ampicillin resistant *E. coli* of animal origin, as well as being commonly reported in human *E. coli* isolates of hospital origin [6]. Further, this study identified 9 isolates as resistant to cephalosporins (i.e. potential ESBL producers), of which five were positive for CTX-M ß-lactamase genes. Extended spectrum beta-lactamase (ESBL) resistance genes have previously been reported in wild animals and pets by [9]. ESBL-targeted drugs are being used more frequently, but may result in mutations of TEM and SHVβ-lactamase genes, as well as the widely prevalent *ctx-m* types [19]. For the potential ESBL producers more identification and confirmation is required and further genotypic analysis in needed. The present observation showed that *E. coli* resistance genes from baboons are similar to those found in other animals and humans; however these need further investigation, specifically by sequencing the TEM, SHV and CTX-M β-lactamase PCR products. It is important to indicate that the *E. coli* isolates showed in general lower percentages of resistance to aminoglycosides (with the exception of streptomycin) and as referred by others, the *aadA1* and *aadA2* genes were detected in 5 (25%) of 20 streptomycin resistant phenotype. The primers used in this study were able to detect either of the two *aadA* genes. These genes were only detected in group II baboons' isolates perhaps suggesting that group I baboons had not yet acquired the resistance mechanism to streptomycin since they had been in captivity for a short period. Although we were not able to amplify the gene *aadA1* or *aadA2* in the remaining 15 streptomycin resistant isolates, other mechanism of streptomycin resistance, such as the production of APH (3")-1 or APH (6)-1 phosphoryltranferase [25] cannot be excluded. All the chloramphenicol resistant isolates were of the MDR phenotype, suggesting that resistance to chloramphenicol is likely to be part of a multiple resistance system. The non-enzymatic chloramphenicol resistance gene (*cmlA*) which also confers resistance to florfenicol was identified by PCR in 5 (62.5%) of the 8 resistant phenotype. While no chloramphenicol mechanism was identified in the three remaining resistant isolates, suggesting that other mechanism of chloramphenicol resistance such as the chloramphenicol acetyltranferase (*cat*) responsible for enzymatic inactivation of the drug or *flo* genes that encodes efflux pump may be involved. The use of chloramphenicol in Kenya veterinary medicine in food animal has been banned however restricted use mainly in topical application as a treatment for ophthalmic conditions in dogs and cat, and is hardly ever used systemically. Chloramphenicol resistance was almost exclusively found in group II baboons-derived samples indicating that, chloramphenicol resistance has most probably been co-selected via linked trimethoprim and ampicillin resistance genes. The resistance genes could also have been of anthropogenic nature due to existence of close contact of these animals with humans. It may be concluded that captive baboons may be a potential reservoir for zonotic transmission of multidrug resistance gene to humans and therefore minimal contact with these animals should be maintained to prevent possible horizontal transfer of resistant commensal bacteria to humans.

Acknowledgments

The authors are thankful to the Institute of Primate Research for facilitating sample collection. We are grateful to James Macharia, Alfred Mainga and J.G Nduhiu for their assistance in the laboratory work.

References

1. Abdelrahman, I. O., Elbagir, N. M., Osman, A. M. et al., PCR detection of *E.coli* in chicken feacal samples. Internation. J Mol Med Adva Sci 2008;4:82-85.
2. Akond, M.A., Hassan, S.M.R., Alam, S., et al., Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Ameri J Environ Sci 2009;5:47-52.
3. Allen, H.K., Donato, H., Wang, H., et al., Call of the wild: antibiotic resistance genes in natural environments.

Nature Rev Microbiol 2010;8:254-258.
4. Anderson, J.F., Parrish, T.D., Akhtar, M., et al., Antibiotic resistance of Enterococci in American bison (Bison bison) from a nature preserve compared to that of Enterococci in pastured cattle. Appl Environ Microbiol 2008;74:1726–1730.
5. Barreto, A., Guimarães, B., Radhouani, H., et al., Detection of antibiotic resistant *E. coli* and *Enterococcus* spp. in stool of healthy growing children in Portugal. J Basic Microbiol 2009;49:503–512.
6. Brin˜as, L., Zarazaga, M., Sa´enz, Y., et al., β-lactamases in ampicillin-resistant Escherichia coli isolates from foods, humans and healthy animals. Antimicrob Agents Chemother 2002;46:3156–3163.
7. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing. 18th Informational Supplement. CLSI Document M100-S18 Wayne, PA 2008.
8. Costa, D., Poeta, P., Saenz, Y., et al., Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. Vet Microbiol 2008;127:97-105.
9. Costa, D., Poeta, P., Saenz, Y., et al., Mechanisms of antibiotic resistance in Escherichia coli isolates recovered from wild animals. Microb Drug Resist 2008;14:71–77.
10. De Jong, A., Bywater, R., Butty, P., et al., A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. J Antimicrob Chemother 2009;63:733-744.
11. Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., ey al., Impact of hospitalization and antimicrobial drug administration on antimicrobial susceptibility patterns of commensal Escherichia coli isolated from the feces of horses. J Am Vet Med Assoc 2006;228:1909-1917.
12. Gakuya, F.M, Kyule, M.N, Gathura, P.B, et al., Antimicrobial susceptibility and plasmids from *Escherichia coli* isolated from rats. East Afri Med J 2001;78:518-522.
13. Jeters, R.T., Rivera, A.J., Boucek, L.M., et al., Antibiotic resistance genes in the vaginal microbiota of primates not normally exposed to antibiotics. Microb Drug Resist 2009;15:309-315.
14. Kadlec, K., Schwarz, S., Analysis and distribution of class 1 and class 2 integrons and associated gene cassettes among *Escherichia coli* isolates from swine, horses, cats and dogs collected in the BfT-Germ Vet monitoring study. J Antimicrob Chemother 2008;62:469-473.
15. Kikuvi, G.M., Schwarz, S., Ombui, J.N., et al., Streptomycin and chloramphenical resistance genes in Escherichia coli isolates from cattle, pigs and chicken in Kenya. Microb Drug Resist 2007;13:63-69.
16. Kiratisin,P., Apisarnthanarak, A., Laesripa, C., et al., Molecular characterization and epidemiology of extended β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing health care associated infecton in Thailand, where the CTM-M family is endemic. Antimicrobialagents chemoth *2008;*52(8):2818-2824
17. Nam, H., Lee, H., Byun, J., et al., Prevalence of antimicrobial resistance in fecal escherichia coli isolates from stray pet dogs and hospitalized pet dogs in Korea. Microb Drug Resist 2010;16:75-79.
18. Nys, S., Okeke, I.N., Kariuki, S., et al., Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries. J Antimicrob Chemother 2004;54:952–955.
19. Osterblad, M., Norrdahl, K., Korpimaki, E., et al., Antibiotic resistance. How wild are wild mammals? Nature 2001;409:37–38.
20. Paterson, D.L., Bonomo, R.A., Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-686.
21. Rolland, R. M., Hausfater, G., Marshall, B., et al., Antibiotic-resistant bacteria in wild primates: Increased prevalence in baboons feeding on human refuse. Appl Environ Microbiol 1985;49:791–794.
22. Routman, E., Miller, R.D., Phillips-Conroy, J., et al., Antibiotic resistance and population structure in *Escherichia coli* from free-ranging African yellow baboons. Appl Environ Microbiol 1985;50:749–754.
23. Rwego, I. B., Isabirye-Basuta, G., Gillespie, T. Ret al., Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 2008;22:1600–1607.
24. Saennz, Y., Brinas, L., Dominquez, E., et al., Mechanisms of resistance in multiple-antibiotic resistant *Esherichia coli* strains of human, animal, and food origins. Antimicrob. Agents Chemother 2004;48:3996-4001.
25. Vakulenko., Mobashery, S., Versatility of aminoglycosides and prospects for their future Clin Microbiol Rev 2003;430–450.

Table 1. Nucleotide sequence and annealing temperature of the primers used in the PCR reactions carried out in this study for detection of antimicrobial resistance genes.

Primers	Oligonucleotide sequence (5-3)	Annealing temp. ^{0}C	Amplicon Size (bp)	Reference
TEM-F	TCCGCTCATGAGACAATAACC	50	931	[16]
TEM-R	TTGGTCTGACAGTTACCAATGC			
SHV-F	TGGTTATGCGTTATATTCGCC	55	868	[16]
SHV-R	GGTTAGCGTTGCCAGTGCT			
CTX-M-F	TCTTCCAGAATAAGGAATCCC	55	909	[16]
CTX-M-R	CCGTTTCCGCTATTACAAAC			
AadA-F	GCAGCGCAATGACATTCTTG	60	282	[9]
AadA-R	ATCCTTCGGCGCGATTTTG			
cmlA-F	CCGCCACGGTGTTGTTGTTATC	45	698	[15]
cmlA-R	CACCTTGCCTGCCCATCATTAG			

Table 2. Frequency of antimicrobial susceptibility among ninety seven Escherichia coli isolates

Antimicrobial agents	Resistance % (no of resistant isolates)		
	Group I baboons (n=36)	Group II baboons (n=61)	Total (n=97)
Ampicillin	13 (36.1)	21 (34.4)	34 (35.1)
Piperacillin	8 (22.2)	14 (23)	22(22.7)
Amoxyclav	11 (30.6)	16 (26.2)	27 (27.8)
Ceftriaxone	0 (0)	1 (1.6)	1 (1)
Ceftazidime	1(2.8)	7 (11.5)	8 (8.2)
Meropenem	0 (0)	0 (0)	0 (0)
Gentamicin	0 (0)	1 (1.6)	1 (1)
Amikacin	0 (0)	1 (1.6)	1 (1)
Kanamycin	2 (5.6)	0 (0)	2 (2.1)
Streptomycin	4 (11.1)	13 (21.3)	17 (17.5)
Tetracycline	8 (22.2)	12 (19.7)	20 (20.6)
Co-trimoxazole	9 (25.0)	6 (9.8)	15 (15.5)
Sulphamethoxazole	12 (33.0)	22 (36.1)	34 (35.1)
Ciprofloxacin	0 (0)	0 (0)	0 (0)
Ofloxacin	0 (0)	1 (1.6)	1 (1)
Chloramphenicol	2 (5.6)	6 (9.8)	8 (8.2)

Table 3. Phenotypes of resistance detected among the *E. coli* isolates recovered from baboons

Group I baboons			Group II baboons	
No. of antimicrobials	No. of isolate (%)	Most frequent pattern (%)	No. of isolate (%)	Most frequent pattern (%)
Susceptible	16(50.8%)	-	30 (49.2%)	-
1	2 (5.6%)	AMC	1 (1.6%)	AMC
1	1 (2.8%)	AMP	1(1.6%)	AMP
1	1(2.8%)	TET	3 (4.9%)	SMX
1			1 (1.6%)	CAZ
2	1(2.8%)	TET-SMX	1 (1.6%)	PIP-OFL
2	1(2.8%)	SXT-SMX	1 (1.6%)	AMC-CTR
2	1(2.8%)	AMP-CAZ	1 (1.6%)	AMC-SMX
2			1 (1.6%)	CAZ-CHL
3	1(2.8%)	AMP-AMC-CHL	1 (1.6%)	AMP-CAZ-SMX
3	1(2.8%)	AMP-TET-SMX	1 (1.6%)	AMC-CAZ-SMX
3			1 (1.6%)	AMP-AMC-CAZ
4	1(2.8%)	AMP-PIP-AMC-TET-SMX	1 (1.6%)	AMP-STR-SXT-SMX
4	2 (5.6%)	AMP-PIP-AMC-SXT-SMX	1 (1.6%)	AMP-AMC-TET-CHL
4			1 (1.6%)	AMP-PIP-STR-SXT
4			2 (3.3%)	AMP-PIP-TET-SMX
5			1 (1.6%)	AMP-AMC-AMK-SXT-SMX
5			1 (1.6%)	AMP-PIP-AMC-GEN-SMX
5			1 (1.6%)	AMP-AMC-STR-TET-SMX
5			1 (1.6%)	AMP-PIP-AMC-STR-SMX
6	1(2.8%)	AMP-PIP-AMC-KAN-SXT-SMX	2 (3.3%)	AMP-PIP-AMC-STR-TET-SMX
6	1(2.8%)	AMP-KAN-STR-SXT-SMX-CHL	1 (1.6%)	AMP-PIP-AMC-KAN-TET-SMX
6	1(2.8%)	AMP-PIP-AMC-TET-SXT-SMX	1 (1.6%)	AMP-PIP-AMC-STR-SMX-CHL
6			1 (1.6%)	AMP-PIP-CAZ-STR-TET-SMX
6			1 (1.6%)	AMP-STR-TET-SXT-SMX-CHL
7	3 (8.3%)	AMP-PIP-AMC-STR-TET-SXT-SMX	1 (1.6%)	AMP-PIP-AMC-STR-TET-SMX-CHL
7			1 (1.6%)	AMP-PIP-KAN-TET-SXT-SMX-CHL
8			1 (1.6%)	AMP-PIP-AMC-CAZ-STR-TET-SXT-SMX

Table 4. Resistance genes detected among antimicrobial resistant *E.coli* isolates from baboons origins

	Group II baboons				Group I baboons			
Phenotype of resistance		Genes detected				Genes detected		
	Number of isolates with this phenotype	Genes	Number of isolates	Percentage of resistant gene detected	Number of isolates with this phenotype	Genes	Number of isolates	Percentage of resistant gene detected
Ampicillin	23	*bla* $_{TEM}$	13	56.5%	14	*bla* $_{TEM}$	8	57.1%
		bla $_{SHV}$	11	47.8%		*bla* $_{SHV}$	8	57.1%
		bla $_{CTX-M}$	4	17.4%		*bla* $_{CTX-M}$	1	7.1%
streptomycin	16	*aadA1 or aadA2*	5	31.3%	4	*aadA1 or aadA2*	0	0%
chloramphenicol	6	*cmlA*	4	66.7%	2	*cmlA*	1	50%