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Antimicrobial Susceptibility Patterns of *Escherichia coli* Isolated from Olive Baboon (*Papio anubis*) Gut

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Abstract **Background**: Antimicrobial resistance is widely acknowledged as a global health problem that has resulted in devastating emerging and re-emerging conditions which are difficult to manage due to limited or unavailable intervention options. It is deepened by the fact that genes encoding for antimicrobial resistance can be transferred horizontally by mobile genetic elements. *Escherichia coli* is primarily a gut microbial flora in warm-blooded animals including non-human primates that can acquire any of these gene elements from other resistant bacterial strains resulting in their transmission between humans and animals. This study aimed to determine antimicrobial susceptibility of *E. coli* against commonly used agents as well as production of extended spectrum β-lactamases. **Methods**: *E. coli* was isolated from stool samples that were collected from sixty-two captive and sixty-two wild baboons using culture-based methods. The isolates were subjected to fourteen antimicrobial agents followed by characterization of three putative resistance genes; $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} using polymerase chain reaction. **Results**: *E. coli* isolates from both groups of animals were resistant to all antimicrobial agents except Ciprofloxacin. Prevalence of Ampicillin resistance was high in *E. coli* isolated from both captive (32.3%) and wild (35.5%) baboons. There was higher prevalence of ESBLs in *E. coli* isolated from wild (17.7%) than captive (14.5%) baboons. **Conclusion**: As reservoirs of ESBL producing *E. coli* type, baboons could play a potential role in antibiotic resistant plasmids transmission to the environment and other animals including humans.

Keywords: *Escherichia coli, ESBL, antimicrobial, wild, captive*

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1. Introduction

Globally, there is a growing concern over antimicrobial resistance (AMR) which has translated into high morbidity and mortality in both humans and animals [1]. Over the past decade there has been a rapid increase in development of AMR by previously susceptible bacteria against different agents by varied mechanisms [2]. Large amounts of antibiotics used for therapy have resulted in the selection of pathogenic bacteria resistant to multiple drugs. Complications that arise from antibiotics resistant pathogens increases the severity of the infections that require sophisticated management including prolonged chemotherapy and even hospitalization [3,4,5].

Escherichia coli, a Gram-negative bacteria is a known gut commensal of majority of warm blooded animals including non-human primates (NHPs) [6,7,8]. This diverse organism not only plays a role in the maintenance of gut health by helping to prevent the establishment of pathogenic bacteria in the gastrointestinal tract (GIT), but can also exist in a number of pathogenic forms that cause diarrheal illness, life threatening intestinal and extraintestinal infections worldwide [9]. Since this bacterium is equally exposed to antibiotics used for treatment of other infections, it can be used as an important indicator for dissemination of AMR [10]. Presence of *E. coli* in livestock that are closely in contact with humans plus their interactions with the wild animal populations including NHPs through shared habitats increases the risk of spreading AMR to a vast range of susceptible hosts even when wildlife has not been exposed to antimicrobial therapies [1,10,11]. In addition, forest fragmentation has increased the interactions between humans and other animals that shed *E. coli* from their GITs and can acquire the bacterium from any of the available hosts whenever they come into contact with their feces [12,13]. Both bacterial pathogens and commensals in the infected sites of animals including humans are exposed to same groups of antimicrobial agents during chemotherapy [14]. The antibiotic pressure on the target bacterial pathogens and normal microbial flora results in

development of resistance against these antimicrobial agents and high risk of subsequent transfer [15].

The β-lactam antibiotics have been used successfully to treat infections caused by pathogenic *E. coli*. However, currently, their utility is being challenged severely by a large number of hydrolytic enzymes– the β-lactamases expressed by bacteria. *E. coli* have developed multidrug resistance (MDR) mechanisms such as efflux of administered drug, inactivation of the agent by bacterial enzymes and alteration of the antimicrobial target in order to survive in these toxic environments that are constantly bombarded by antimicrobial agents [14,16]. These β-lactam antibiotics which includes penicillins, monobactams, carbapenems, and cephalosporins, possess a β-lactam ring in their molecular structure and has been one of the most important group of antimicrobial agents for treating bacterial infections in animals. They act by inhibiting bacterial cell wall biosynthesis by forming adduct with transpeptidase domain of penicillin binding proteins (PBPs) thereby inactivating enzymes involved in transglycosylation and transpeptidation with subsequent death of the target microorganism [17,18]. Since β-lactams target peptidoglycan that is only found in bacterial cell wall but absent in animals, they are less toxic to host tissues unlike other antimicrobial agents [18]. Despite their safety and efficacy, bacteria such as *E. coli* have developed mechanisms of evading destruction. The most common of these mechanisms is AMR by production of plasmid encoded β-lactamases that hydrolyze β-lactam ring of the antimicrobials resulting in their inactivation. The menace is further compounded by the highly flexible genome of *E. coli*, and propensity of resistance dissemination through horizontal gene transfer and clonal spread [19].

Extended spectrum β-lactamases (ESBLs) confer resistance against a wide range of β-lactam antimicrobials but are inhibited by Clavulanic acid and Tazobactam [11,20,21]. The most common ESBL enzymes are Temoniera (TEM), Cefotaximases (CTX-M) and sulfhydryl variable (SHV). They are encoded for by highly transmissible mobile genetic elements like transposons, integrons, gene cassettes and plasmids [19,22,23] resulting in the global spread of AMR in both hospital and community settings [24,25,26]. bla_{TEM} β-lactamases are widely distributed in *E. coli* and other Gram-negative bacteria. Frequent isolation of *E. coli* harbouring $bla_{\text{TEM-1}}$ from human gut is not uncommon and over 200 emerging variants of bla_{TEM} with varying efficiencies of oxyamino-cephalosporins hydrolysis have been characterized [27,28].

$bla_{\text{CTX-M}}$ β-lactamases capable of hydrolysing Cefotaxime and Ceftazidime are broadly classified into five groups according to their amino acids sequences [29,30] but other variants have been identified comprising of over 200 enzymes. $bla_{\text{CTX-M}}$ is the most prevalent ESBL among Enterobacteriaceae that has been frequently detected in *E. coli* causing both nosocomial and community acquired infections [31]. $bla_{\text{CTX-M}}$ enzymes producing bacteria is rapidly increasing in both human clinical and community settings since 2000 [29,32]. Emergence and global spread of *E. coli* producing $bla_{\text{CTX-M-15}}$ that is incriminated to cause both nosocomial and extraintestinal infections has been reported [33,34]. ESBL-producing *E. coli* often display resistance to non-β-lactam antibiotics and chemotherapeutics. This results from the fact that genes coding for ESBLs and those conferring resistance to other antimicrobial drugs often reside within the same conjugative plasmids [35].

The constant exposure of *E. coli* to antimicrobial agents makes it an efficient indicator of evolution, development and spread of antibiotic resistance besides the risk of being a major cause of nosocomial infections that are difficult to treat [36]. Presence of AMR determinants in *E. coli* from olive baboons and/or other NHPs could be a major threat to its rapid spread to humans that get into contact due to settlement and other anthropogenic activities [8,12] with subsequent development and/or dissemination of community acquired AMR. Carriage of ESBLs by both pathogenic and non-pathogenic *E. coli* is well documented in humans [20,37], but in NHPs which are phylogenetically related to humans, the data is limited. These groups of animals are known to share same habitat and thus the possibility for a bidirectional transmission of antibiotic resistant bacteria including *E. coli.* This can result in emergence or re-emergence of infections that are difficult to treat due to limited and/or inaccessible treatment options especially in low income countries leading to high morbidity and mortality [38,39]. The aim of the present study was to determine the antimicrobial susceptibilities of ESBL producing *E. coli* strains colonizing the GIT of captive and wild baboons. In addition, the genes responsible for causing resistance were investigated.

2. Materials and Methods

Animals: Two groups of animals consisting of 62 captive and 62 wild baboons were used in this study. The captive baboons were housed in standard animal facility within the Institute of Primate Research (IPR), Nairobi, Kenya, which is a World Health Collaborating (WHO) collaborating Centre and is accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). They are fed on commercial monkey chow (Unga Farm Care Limited, Nairobi Kenya) supplemented with fruits, vegetables and water *ad libitum*. The wild baboons sampled were from troops found in Mpalla ranch conservancy located at Laikipia County, Kenya. The research in this study complied with protocols approved through IPR's Institutional Scientific and Ethics committee (ISERC) based on National and International guidelines for animal care and use before sampling was conducted.

Fecal sample collection: Samples of both captive and wild baboons were collected from freshly voided feces using sterile cotton tipped applicator swab moistened in normal saline, with care to collect from the top of the sample to avoid ground contamination. All fecal swabs were aseptically inoculated into Stuart transport media (Oxoid, Basingstoke UK), placed on ice, transported to the laboratory and subsequently processed.

Bacterial isolates: The swabs were inoculated onto Xylose-lysine deoxycholate (XLD, Oxoid, Basingstoke UK) agar plates for isolation of putative *E. coli*. Pure isolates were obtained by re-plating lactose fermenting colonies onto another XLD agar plate. The identification of the suspected *E. coli* was confirmed by biochemical tests using analytical profile index system for enterobacteriaceae (api 20E, BioMeriux® SA, Marcy l'Etoile, France) as guided by manufacturer's instructions.

Antimicrobial susceptibility testing: *E. coli* isolates were screened for AMR using Kirby-Bauer agar disk diffusion method on Mueller-Hinton agar (Becton Dickinson and Co. Sparks NV, USA) according to Clinical and Laboratory Standards Institute (CLSI) recommendations [40]. All antimicrobial disks were sourced from Becton Dickinson and Co. Disks containing the following commonly used antimicrobial agents were used: Ampicillin (AM) 10 µg, Chloramphenicol (C) 30 µg, Tetracycline (TE) 30 µg, Gentamycin (GM) 10 µg, Streptomycin (S) 10 µg, Trimethoprim/Sulphomethoxazole (SXT) 25 µg, Norfloxacin (NOR) 10 µg, Ciprofloxacin (CIP) 5 µg, Cefaclor (CEC) 30 µg, Ceftriazone (CRO) 30 µg, Cefotaxime (CTX) 30 µg, Cefuroxime (CXM) 30 µg, Cefepime (FEP) 30 µg, and Amoxicillin/Clavulanic acid (AMC) 20/10 µg. *E. coli* ATCC 25922 was used as the quality reference strain.

Phenotypic detection of ESBL genes: Phenotypic screening of ESBLs was performed by double disk synergy test followed by genotypic detection using polymerase chain reaction (PCR). Four disks of antimicrobial agents; CAZ 30 µg, FEP 30 µg, CTX 30 µg and CRO 30 µg (Liofilchem s.r.l Zona Industriale, 64026, Roseto degli Abruzzi (Te) Italy) were placed onto isolated *E. coli* inoculum on Mueller-Hinton agar (Becton Dickinson and Co. Sparks NV, USA) to surround a centrally positioned AMC 30 µg (Becton Dickinson and Co. Sparks NV, USA) 30 mm apart from each cephalosporin. These plates were incubated for 24 hours then examined for enhanced zone(s) of inhibition between AMC 30 µg in the middle and any of the four cephalosporins. Enhanced zone of inhibition indicated synergistic activity and production of ESBL. *E. coli* isolates that were positive for double disk synergy test were further tested against Ceftazidime/Clavulanic acid (CAL) 40 µg and CAZ 30 µg (Becton Dickinson and Co. Sparks NV, USA) alone for confirmation. The strains that had a zone of inhibition around CAL which exceeded that of CAZ alone by 5 mm were considered to produce ESBL enzymes [41].

DNA extraction and PCR analysis: For DNA extraction, a suspension was prepared from overnight Mueller-Hinton (Oxoid, Basingstoke UK) cultures in 500 µl Nuclease free water. The suspension was boiled at 95^{o}C for 10 minutes using a heating block then cooled on ice before centrifugation at 12 000 ×g for 5 minutes. The supernatant containing DNA was transferred into a sterile epperndorf tube and stored at -20^{o}C until needed for PCR assays [42].

Genotypic detection of ESBL was conducted by PCR that targeted three putative genes; *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ (Table 1). PCR for detecting each gene was conducted using 5 µl of *E. coli* DNA template, 1 µl of each 10 pM primer (Integrated DNA Technologies Inc., Illinois, USA), 12.5 µl DreamTaq PCR mastermix 2x (Thermo Scientific, Lithuania, UK) then topped up with nuclease free water to obtain a final reaction volume of 25 µl [43,44]. All the reactions were performed in a thermocycler (SimpliAmp™ Thermocycler, Applied Biosystems, ThermoFisher Scientific, Singapore) using thermocycling conditions shown in Table 1. *Klebsiella pneumoniae* ATCC 700603 was used as a positive control while *E.* coli ATCC 25922 was the non-ESBL producing control in the entire experiment. Amplified PCR products were subjected to electrophoresis on 1.5% agarose gel then visualized using ultra-violet (UV) transilluminator documentation system (UVP Bio-Doc It™ Imaging System, Upland, CA, USA).

3. Results

3.1. Antimicrobial Susceptibility Testing

All *E. coli* isolates were subjected to fourteen antimicrobial agents in order to determine their susceptibility profiles (Table 2). Based on the disk diffusion results, the isolates from both groups of animals were most commonly resistant to AM (32.3%) captive and (35.5%) wild baboons. *E. coli* isolated from feces of captive baboons showed higher prevalence of resistance to SXT (37.1%), AMC (25.8%) and S (11.7%) than those from their wild counterparts. Except for CIP that showed no resistance from any of the *E. coli* in both groups of animals, the remaining antibiotics tested had lower occurrence of resistance among the isolates tested (Table 2).

3.2. Determination of ESBLs

Screening for presence of ESBL genes was performed by double disk synergy test with AMC at the middle of the test plate surrounded by third generation cephalosporins (CAZ, CRO, CTX and FEP). A 'ghost' zone between the AMC and any of the cephalosporins indicated that the *E. coli* isolate was an ESBL producer (Figure 1). Putative ESBL producing *E. coli* isolates were then subjected to PCR using three primers that target the most ubiquitous genes; *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ (Table 1). PCR products were analysed using 1.5% agarose gel in tris-acetate ethylenediaminetetracetic acid (TAE) buffer and images captured from the UV transilluminator documentation system (Figure 3 - Figure 5).

Table 1. Primer sequences and PCR conditions

Primers	Oligonucleotide sequence (5'-3')	Thermocycling conditions	Reference	Product Size (bp)
bla$_{TEM}$	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	1 cycle of 5 min at 96°C, 35 cycles of 1 min at 96°C, 1 min at 58°C 1 min at 72°C, 1 cycle of 10 min at 72°C	[45]	867
bla$_{SHV}$	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC	1 cycle of 5 min at 96°C, 35 cycles 1 min at 96°C, 1 min at 60°C, 1 min at 72°C, 1 cycle of 10 min at 72°C	[45]	867
bla$_{CTX-M}$	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	1 cycle of 7 min at 94°C, 35 cycles 50 sec at 94°C, 40 sec at 50°C, 1 min at 72°C, 1 cycle of 5 min at 72°C	[46]	593

Table 2. Comparison of antimicrobial susceptibility patterns of *E. coli* in Captive and Wild baboon fecal samples

Antimicrobial agent	Number (%) of resistant isolate	
	Captive (n=62)	Wild (n=62)
Ampicillin	20 (32.3%)	22 (35.5%)
Chloramphenicol	3 (4.8%)	1 (1.6%)
Tetracycline	16 (25.8%)	16 (25.8%)
Gentamycin	1 (1.6%)	1 (1.6%)
Streptomycin	7 (11.3%)	1 (1.6%)
Trimethoprim/Sulphomethoxazole	23 (37.1%)	11 (17.7%)
Norfloxacin	1 (1.6%)	1(1.6%)
Ciprofloxacin	0 (0.0%)	0 (0.0%)
Cefaclor	1 (1.6%)	4 (6.5%)
Ceftriazone	3 (4.8%)	8 (12.9%)
Cefotaxime	7 (11.3%)	11 (18.3%)
Cefuroxime	5 (8.1%)	17 (27.4%)
Cefepime	3 (4.8%)	3 (4.8%)
Amoxicillin clavulanic acid	16 (25.8%)	4 (6.5%)

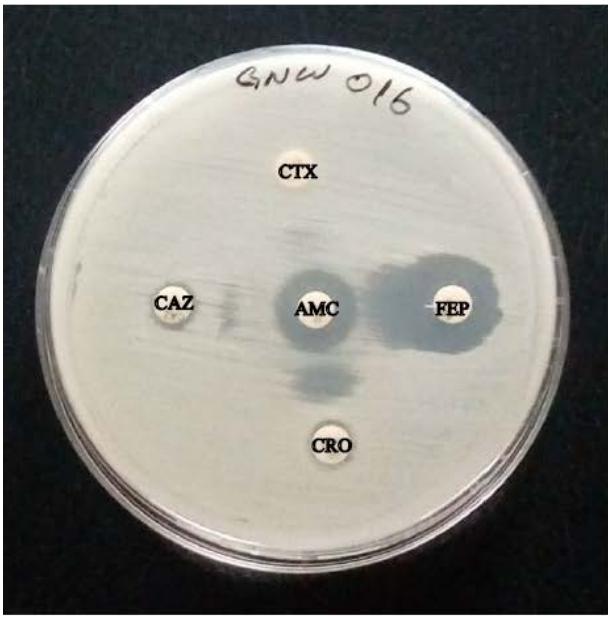

Figure 1. *E. coli* isolate showing enhanced zone of inhibition between AMC at the centre and FEP, CRO and CAZ

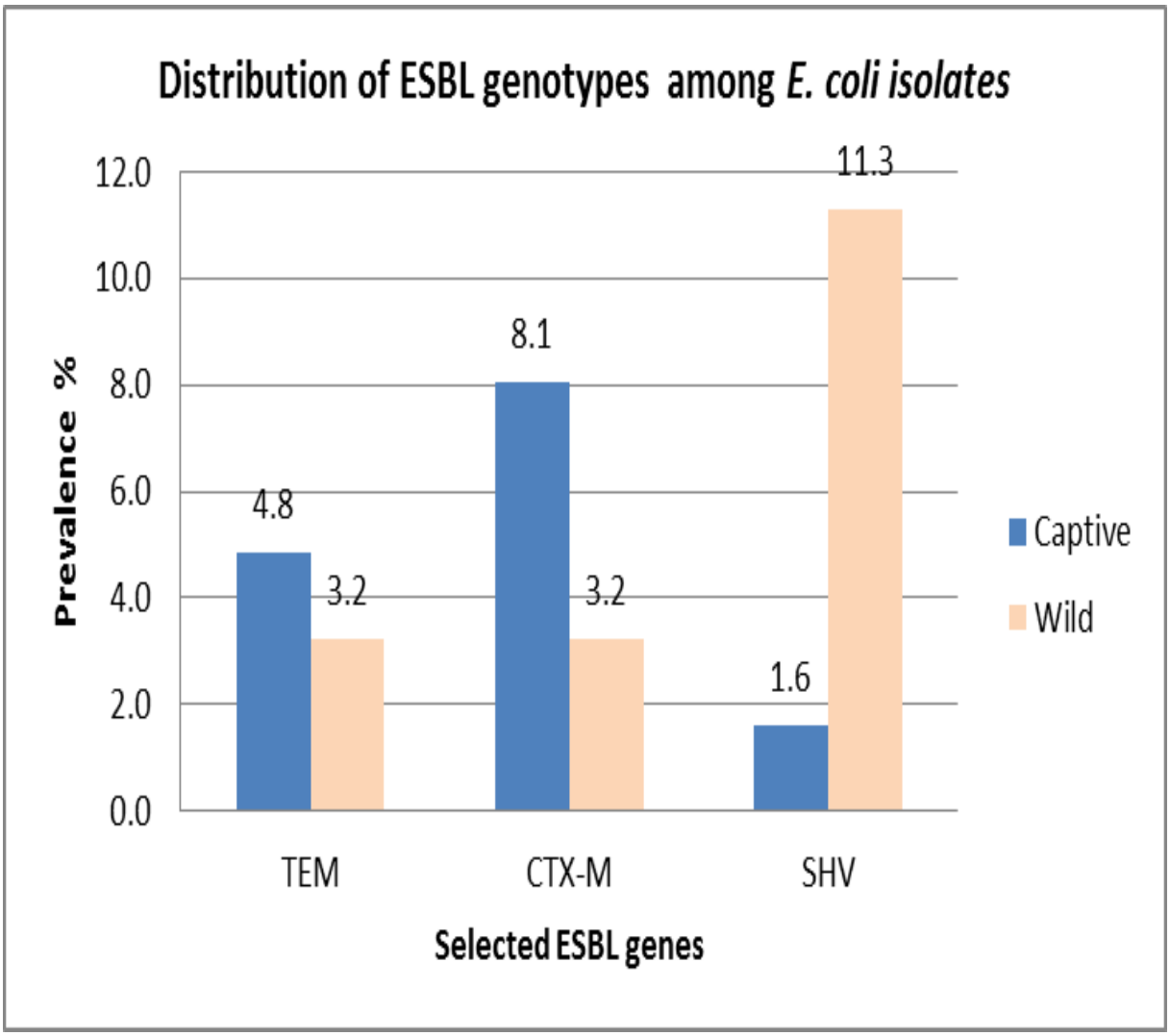

Figure 2. Comparison of ESBLs in *E. coli* isolated from Captive and Wild baboon fecal samples

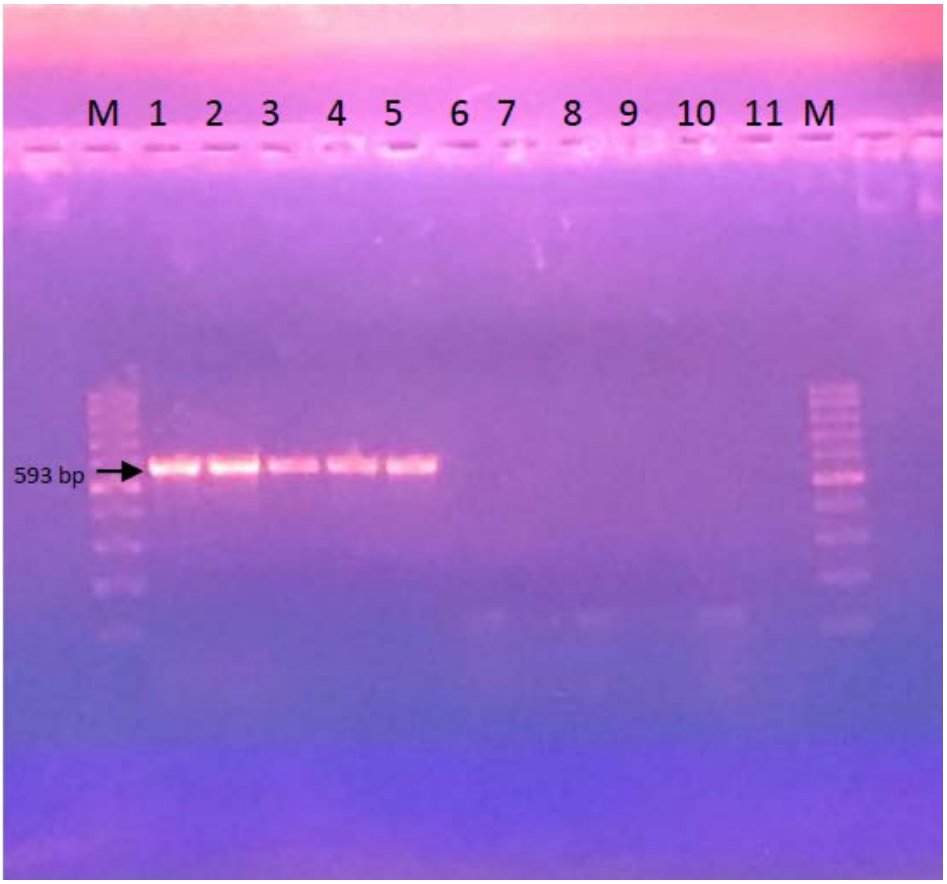

Figure 3. Agarose gel electrophoresis showing *bla*$_{CTX-M}$ amplification. M-100bp molecular marker, 1-positive control (*K. pneumoniae* ATCC 700603), 2,3,4,5-samples with positive amplification, 6,7,8,9,10-samples with negative amplification and 11-Negative control (*E. coli* ATCC 29522)

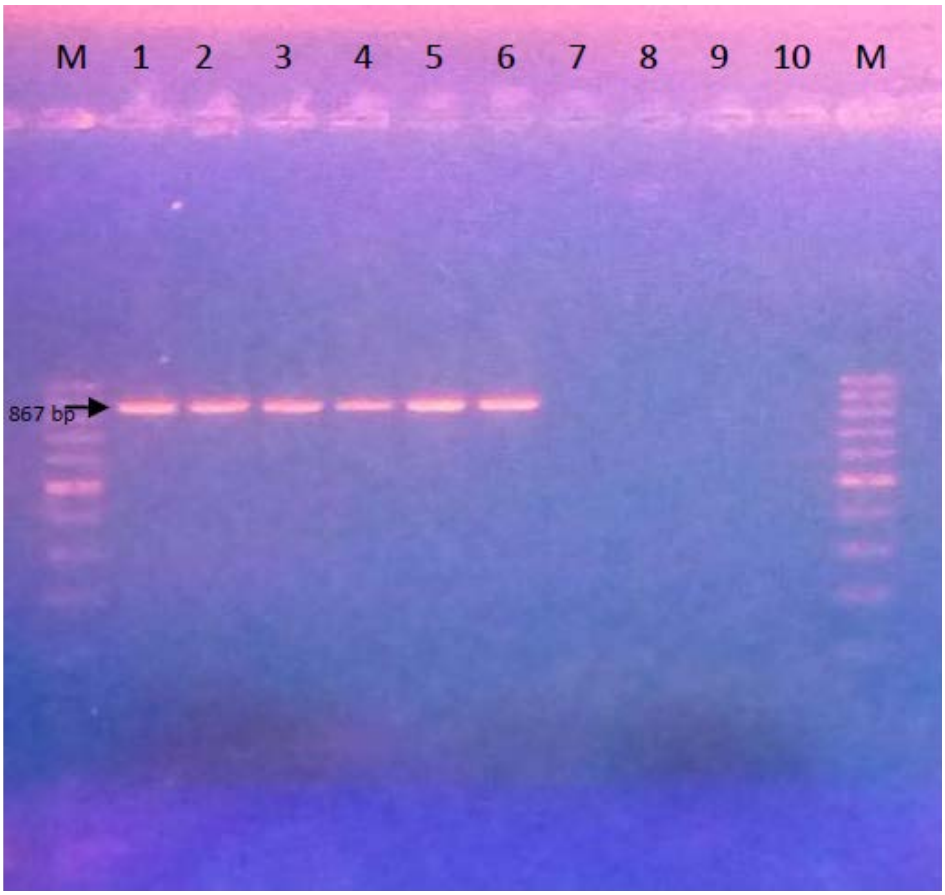

Figure 4. Agarose gel electrophoresis showing *bla*$_{TEM}$ amplification. M-100bp molecular marker, 1-positive control (*K. pneumonia* ATCC 700603), 2,3,4,5,6-samples with positive amplification, 7,8,9-samples with negative amplification and 10-Negative control (*E. coli* ATCC 25922)

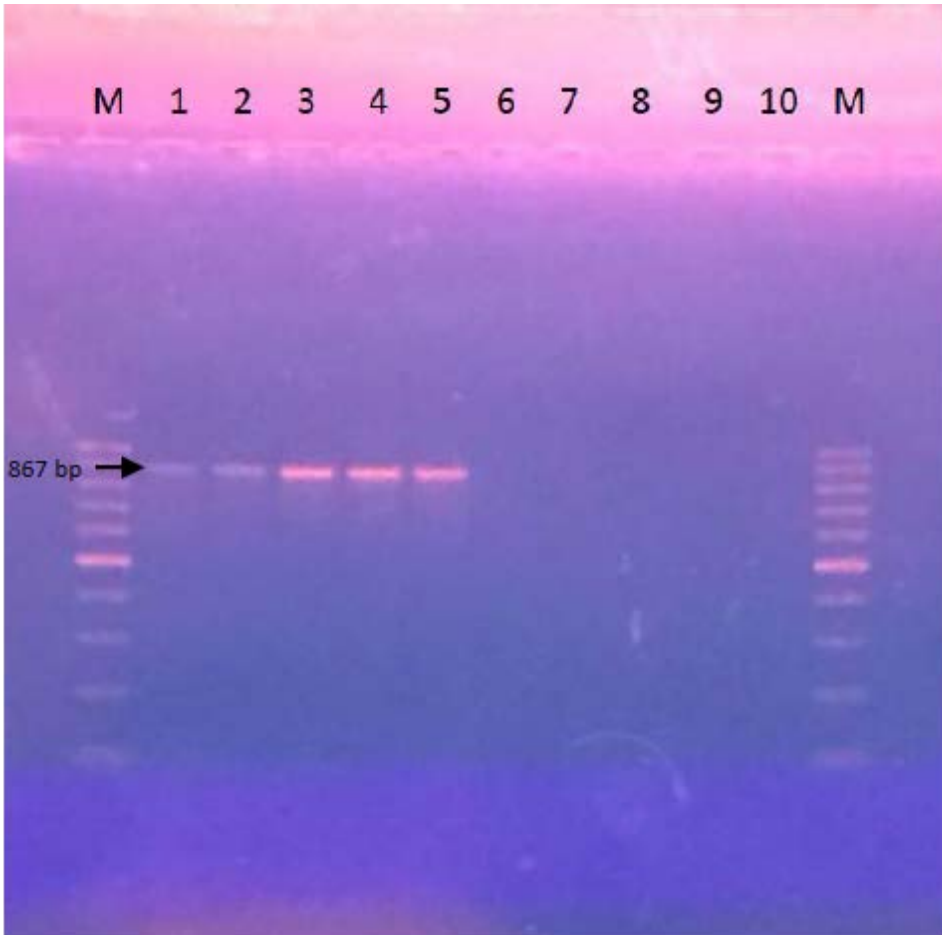

Figure 5. Agarose gel electrophoresis showing *bla*$_{SHV}$ amplification. M-100bp molecular marker, 1-positive control (*K. pneumonia* ATCC 700603), 2,3,4,5-samples with positive amplification, 6,7,8,9-samples with negative amplification and 10-Negative control (ATCC 25922).

4. Discussion

Globally, AMR has become a significant threat to the prevention and treatment of bacterial infections [14,47]. One of the mechanisms of development of antibiotics resistance by different groups of microorganisms including *E. coli* is production of ESBL enzymes that are capable of hydrolysing a range of β-lactam antimicrobial agents [21]. ESBLs that include *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ have been detected in *E. coli* isolated from clinical specimens in different parts of the world with reported upsurge of multidrug resistance that complicate morbidity caused by this bacterium [44]. Our study sampled fecal samples of 124 olive baboons clustered into two equal groups of 62 in captivity and wild. Baboons sampled from the wild were free-ranging within Mpalla ranch located in Laikipia County, Kenya. The captive baboons were socially housed within IPR with stringent husbandry conditions whereas the wild troops due to their free-ranging nature are frequently in close contact with humans and livestock within the region. The present study provides vital data on antimicrobial susceptibility profiles, resistance patterns and presence of selected ESBL genes using *E. coli* as an indicator organism. The fact that these genes can be born on plasmids and mobile genetic elements makes them highly transmissible horizontally resulting into 'super' pathogens [48]. Our study demonstrated that *E. coli* isolates from both captive and wild olive baboons were resistant to thirteen out of the fourteen antimicrobial agents that we used (Table 2). The current study showed high levels of antibiotic resistance from both captive and wild baboons against AM, TE and SXT. These results are consistent with the findings of a previous study that was conducted on animals not previously exposure to antimicrobial chemotherapy [49]. *E. coli* Isolated from captive baboons displayed high resistance against SXT (37.1%), AMC (25.8%) and S (11.3%) compared to those from the wild. Captive baboons are routinely exposed to antimicrobial agent used in chemotherapy as an essential husbandry procedure. Surprisingly, *E. coli* isolated from the wild were more resistant to cephalosporins; CEC, CRO, CTX and CXM in addition to AM (Table 2).

A study conducted on free-ranging yellow baboon troops from Amboseli National Park in Kenya revealed high prevalence of AMR against TE (94.1%), Kanamycin (70.6%), AM (47.1%) and Cephalothin (17.6%), but third generation cephalosporins and ESBLs were not included [50]. This high prevalence of antibiotics resistance observed in baboons from Amboseli National Park was attributed to foraging on food wastes and other refuse that could be implicated as the source of resistant non-pathogenic bacteria including *E. coli [50]*. This also explains our findings since the wild baboons sampled in this study closely interact with humans and different types of waste within the ranch. This could be reinforced by the fact that vehicles of transmission of *E. coli* comprise of human and NHPs faecal material in areas where there is close contact during human activities in the animals infested areas and contaminated water bodies [12]. Transmission of bacteria including *E. coli*, between

different susceptible hosts like humans, livestock, NHPs and other wild animals due to forest fragmentation [51] increases probability of transfer of mobile genetic elements encoding for AMR resulting in infections whose interventions are scarcely available or lacking. The interspecies transmission is accelerated by the ecologic overlap created by the fragments and anthropogenic activities in the affected regions with these resistant microbes spreading to the community settings [13]. Public health problem of AMR is emphasized by the high prevalence against SXT (80.6-95.2%), AM (77.4-95.2%), TE (57.1-81.0%), C (14.3-35.7%), GM (6.4%) and CIP (3.2%) that has been reported among pathogenic *E. coli* isolated from humans from different parts of Kenya [52].

The PCR conducted on *E. coli* isolated from both groups of baboons' detected carriage of three resistance genes; $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} (Figure 2 - Figure 5). The isolates from wild baboons displayed higher prevalence of ESBLs (17.7%) compared to the captive (14.5%). However, prevalence of $bla_{\text{CTX-M}}$ (8.1%) and bla_{TEM} (4.8%) appeared higher in *E. coli* isolates from captive baboons than the wild in which only 3.2% was observed from either of the two genes. bla_{SHV} was the most dominant among *E. coli* isolates from the wild baboons (11.3%) compared to those from captive animals. These genes have been detected in *E. coli* isolates from different human clinical specimens [19]. AMR that was observed among the wild population could be attributed to habitat contamination by human or domestic animal waste. Close interaction between animals including baboons and humans in conflict increases the risk of transmission of microbes like *E. coli* that have been subjected to constant antimicrobial pressure through livestock farming, poor human waste disposal and polluted environment [53]. Commensals from healthy individuals including *E. coli* are reservoirs for antibiotics' resistance genetic material that can be readily acquired by pathogens [4]. Besides being part of the essential gut microbial flora, *E. coli* is responsible for paediatric septicaemia and community-acquired sepsis in sub-Saharan Africa [54] adding to the burden of morbidity which when coupled with AMR as revealed in this study deteriorates to a grave public health problem.

The conjugational transfer of plasmid-mediated ESBLs occurs efficiently in the intestinal tract, where enteric rods, in particular *E. coli*, often act as a reservoir of self-transmissible resistance markers that can be exchanged between species of the *Enterobacteriaceae* family [20]. Successful management of infections caused by such resistant strains requires an understanding of the diversity of β-lactamases, their unambiguous detection, and molecular mechanisms underlying their expression and spread with regard to the most relevant information about individual bacterial species [19]. As colonized baboons may become a potential but under-estimated reservoir of β-lactamases-encoding genes in the environment, further investigation to determine the variants of the three ESBL is warranted.

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Statement of Competing Interest

The authors do not have any competing interests.

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