

Antimicrobials have been in use for decades in disease management, but emergence of resistant pathogens such as *Escherichia coli* have rendered its future of concern to public health. Increased human-wildlife interaction is associated with emergence of devastating human diseases and resistance genes are increasingly being isolated. Of importance is the increased bacterial infection that requires prolonged treatment periods as seen in Lambwe Valley which points to antibacterial resistance among the community members of Lambwe Valley. However, major gaps exist on whether human and intensively managed wild animals such as black rhinoceros harbors bacterial strains with similar antibacterial resistance profiles. The present study aimed at investigating antimicrobial resistance patterns and genetic variation in *E. coli* isolates from human and black rhinoceros in Lambwe Valley. The specific objectives were to determine differences in antimicrobial resistance to seven commonly used antimicrobials and to characterize resistance genes in *E. coli* isolates from human and the black rhinoceros in Lambwe Valley. A cross-sectional study design was adopted in collecting 184 fecal samples from humans. The study employed a simple random technique in collecting human samples. In addition, 16 fecal samples from black rhinoceros were collected. Antimicrobial susceptibility was determined by disk diffusion method. Polymerase Chain Reaction was used for molecular characterization of antimicrobial resistance genes. *Escherichia coli* were isolated in all human samples and in 15 of the black rhinoceros samples. The prevalence of antimicrobial resistance in *E. coli* isolates from black rhinoceros to erythromycin was 86.7%, gentamicin was 80.0%, ampicillin was 73.3%, tetracycline was 40.0%, amoxicillin/clavulanic acid was 60.0%, cotrimoxazole was 33.3% and ceftriaxone was 13.3%, but 86.7% of the isolates were susceptible to chloramphenicol. The level of resistance of *E. coli* isolates from human to amoxicillin/clavulanic acid was 85.3%, cotrimoxazole was 83.1%, gentamicin was 28.3%, erythromycin was 76.1%, ampicillin was 75.0%, tetracycline was 64.7%, ceftriaxone was 58.3% and chloramphenicol was 29.9%. Human and black rhinoceros isolates showed significant similarity in resistance to ampicillin ($z=0.143$, $p=0.889$), tetracycline ($z=1.901$, $p=0.057$), chloramphenicol ($z=1.923$, $p=0.055$) and erythromycin ($z=0.935$, $p=0.3524$). However resistance to cotrimoxazole ($z=4.587$, $p=0.000$), ceftriaxone ($z=3.310$, $p=0.001$), amoxicillin/clavulanic acid ($z=0.935$, $p=0.352$) and gentamycin ($z=4.125$, $p=0.000$) was significantly different with gentamycin resistance being higher in black rhinoceros while cotrimoxazole, ceftriaxone and amoxicillin/clavulanic acid resistance was higher in human. Multi-drug resistance was 69.0% in humans and 43.4% in black rhinoceros with resistance phenotype being ampicillin, cotrimoxazole, chloramphenicol, tetracycline, amoxicillin/clavulanic acid and erythromycin. PCR analyses of selected samples indicated presence of *bla*_{TEM}, *tetA*, *tetB*, *df*_{rA1} and *sul*_I genes in isolates from humans and black rhinoceros. The observed similarity in phenotypic and genotypic antimicrobial resistance profiles between human and black rhinoceros isolates suggests that antimicrobial resistance is no longer confined to humans, but is a wider environmental issue raising grave concern to public health. It also suggests that human and wildlife are experiencing high rate of resistance genes cross-transfer. Hence there is need for multi-sectorial coordinated action plan on surveillance of antimicrobial resistance by incorporating public health, livestock and wildlife sectors.