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Deema Elchoufi April 14, 2014

Prevalence of antibiotic resistance in humans, wildlife, and livestock in and around Gombe National Park, Tanzania

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Abstract

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Purpose: Habitat overlap increases the risk of bacterial exchange between humans and wildlife, threatening human health and wildlife conservation. Antibiotic resistance (AR), like disease, can spread between wildlife, livestock, and humans, serving as a possible proxy for disease spillover. In Gombe National Park, Tanzania, little is known about the prevalence of AR in the ecosystem. This project examined the prevalence of sulfonamide resistance genes in humans, livestock, chimpanzees (*Pan troglodytes schweinfurthii*), and baboons (*Pabio anubis*). Further, we examined risk factors for resistance acquisition in the Gombe ecosystem, hypothesizing that the Mitumba chimpanzee community would have higher AR prevalence than the Kasekela community due to its proximity to Mwamgongo, the village bordering the park.

Methods: From March 2010 to February 2011, fecal samples were collected from humans (n=178), livestock (n=98), and wildlife (n=131) from Mitumba and Kasekela (park communities) and Mwamgongo (village outside the park). At time of collection, human subjects were surveyed for socio-demographic (sex, age, profession, etc.) and health data (diarrheal illness, medication usage, water usage, etc.). DNA extracted from samples was screened for AR genes *sul1* and *sul2*. Previously collected data on prevalence of SIV and *Cryptospiridium* were used as possible risk factors for the chimpanzee analyses. Chi-square tests of independence, Fisher's Exact Tests, and McNemar's Tests were used to measure associations between risk factors and *sul* positivity.

Results: Kasekela had the highest prevalence of AR genes for human and chimpanzees (93.2% and 28.8%, respectively). All wildlife and livestock had some level of resistance (26.2% for chimpanzees, 36.2% for baboons, 77.8% for dogs, 7.1% for sheep, 12% for goats). Humans residing in Kasekela were four times more likely to have AR (OR=4.010) than Mitumba. Positivity for *sul1* related to positivity for *sul2* in humans ($p<0.001$) and chimps ($p=0.0253$). There was a strong statistical significance for chimpanzees having SIV and AR genes ($p=0.020$).

Conclusion: Humans seem to be the reservoir for AR genes to wildlife, regardless of human density. The established prevalence of AR genes in the wildlife is a call for concern. Limiting human interactions with wildlife and presence in the park is needed to reduce transmission risk.

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Background

Antibiotic Resistance and Wildlife

Antibiotic resistance (AR) is a growing threat worldwide, as antibiotics that could once cure almost any infection are rapidly becoming obsolete. Only a few years after penicillin was first discovered, penicillin resistant strains of bacteria began to emerge (Rammelkamp and Maxon 1942). Since then, antibiotic use continues to grow globally, becoming the single most important reason for increased antibiotic resistance (Byarugaba 2004). Antibiotic use provides the strongest selective pressure on bacteria for antibiotic resistance. The widespread sale of counterfeit and over the counter antibiotics further worsen the problem, as unmonitored distribution of antibiotics results in inappropriate use, becoming the norm in resource-limited settings (Bennish and Khan 2010). In such countries, suboptimal use of antibiotics for disease prevention and treatment, antibiotic use in livestock and agriculture, and poor government oversight of antibiotic distribution (Byarugaba 2010) are widespread. This lack of regulation is not only ubiquitous in health care settings, but also in the community (Mitema 2010). As private shops and markets become the local dispensers (Bennish and Khan 2010), the risk of AR developing grows exponentially. Further, in today's globalized society, resistant bacterial strains can spread through air travel and the distribution of AR in food animals and their products. As disease-causing agents can move freely in diverse environments, antibiotic resistance can also move among humans, animals, and even plants (Senok 2012). Thus, to view antibiotic resistance as a regional—or even human—issue is to have a limited scope of the magnitude of the problem. To fully understand the weight of the issue, we must use a global lens, with all life as actors.

Our work seeks to understand antibiotic resistance with such a lens, particularly focusing on AR in wildlife and in ecosystems as a whole. A number of papers have examined the impact of AR in wildlife, but few have done so in ape populations (Goldberg, Gillespie et al. 2007, Rwego, Isabirye-Basuta et al. 2008, Benavides, Godreuil et al. 2012). In one study, gorilla, human, and livestock bacterial isolates collected from in and around Bwindi Impenetrable National Park, Uganda harbored varying levels of resistance to at least one antibiotic used by local people (Rwego, Isabirye-Basuta et al. 2008). Further, they found that as habitat overlap with humans decreased, AR in wildlife also declined, suggesting that habitat overlap between species affects the dynamics of gastrointestinal bacterial transmission. In a study examining patterns of gastrointestinal bacterial exchange, chimpanzees in Kibale National Park were found to harbor bacteria genetically more similar to humans working in chimpanzee-directed research and tourism than humans in the local population (Goldberg, Gillespie et al. 2007). These studies show that human interaction and habitat overlap with wildlife not only facilitate bacterial transmission but also AR transmission.

Gombe National Park

In Gombe National Park, Tanzania, where death from disease is the leading cause of mortality in chimpanzees (*Pan troglodytes schweinfurthii*) (Williams, Lonsdorf et al. 2008), bacterial exchange between humans and chimpanzees is a matter of conservation. As the park continues to lose habitat due to human encroachment and as the human-chimpanzee interface grows, the potential for spillover of disease increases. The number of major epidemics in Gombe from polio to respiratory illnesses (Williams, Lonsdorf et al. 2008) stand as a testament to this. As populations become smaller and/or more isolated, the risk of disease increases substantially (Lonsdorf, Travis et al. 2006), with most outbreaks believed to be caused by close contact with

humans (Hill, Boesch et al. 2001, Walsh, Abernethy et al. 2003). Park managers and researchers suspect that disease from humans poses a large risk to the sustainability of the Gombe chimpanzees. The two chimpanzee populations—Mitumba, an edge community in close proximity to a growing village, and Kasekela, a centrally located community within Gombe National Reserve—encounter varying degrees of human contact. The differences in habitat overlap with humans create potentially different opportunities for disease transmission. Further, because Gombe faces similar AR issues as other resource-limited areas, antibiotic resistance transmission to these two chimpanzee communities is of huge concern. With no oversight of antibiotic sale and persistent suboptimal use of antibiotics, a selective pressure for resistance genes in the Gombe system increases. The use of antibiotics on livestock exacerbates the problem, as non-human primates such as baboons and chimpanzees may interact with these animals, or their habitats, increasing the potential for exchange of these genes.

Since antibiotics are not regularly administered to the chimpanzees and other non-human primates at Gombe, AR genes in the wildlife population may be evidence of bacterial spillover from the human population. Thus, the aims of this study are to test for the presence of AR genes in the Gombe system and elucidate modes of transmission, in particular, between chimpanzees and humans. Transmission of these genes through feces, soil, and water is of special concern as the interface between chimpanzees and humans continues to widen. Many studies have shown that *E. coli* from normal gut flora is a major reservoir for resistance genes (van den Bogaard and Stobberingh 2000). In a study examining AR in gut commensal microflora, conjugation experiments demonstrated that resistance to several classes of antibiotics could be transferred en bloc to commensal microflora. Mobile DNA elements such as plasmids may be the source of these multidrug resistance phenotypes (Bartoloni, Benedetti et al. 2006). Since *E. coli* can

survive in extra-intestinal environments, it can further acquire MDR phenotypes from soil and water bacteria.

Sulfonamide Antimicrobials

For this study, genes coding for resistance to sulfonamide antimicrobials were used to detect antibiotic resistance from total fecal DNA samples. The widespread use of sulfonamide antimicrobials in health-care and community settings made the *sul1* and *sul2* genes strong candidates for detection.

Sulfonamides are a class of bacteriostatic antimicrobials that act against bacteria via competitive inhibition (Byarugaba 2010). Sulfonamides target the folic acid pathway of bacteria, selecting for the enzyme dihydropteroate synthase (DHPS). Most bacteria lack the ability to uptake folic acid and thus have to produce it. Because sulfonamides are structurally analogous to the *p*-aminobenzoic acid (PABA) substrate, they competitively inhibit this crucial step in folic acid synthesis. Bacteria containing the *sul1* or *sul2* genes overcome this mechanism, as *sul1* and *sul2* encode forms of DHPS that is not inhibited by sulfonamides (Enne, Livermore et al. 2001). These genes are typically located on small nonconjugative plasmids or large transmissible multi-resistance plasmids (Byarugaba 2010). Because of their dwindling efficacy, sulfonamide antibiotics are being phased out in developed countries though continuing to be used widely in developing countries.

Understanding the mechanism of various antibiotics allows for understanding the mechanisms of resistance and transmission of resistance genes to antibiotic-susceptible bacteria. Objectives of this research include quantifying the level of resistance in human, livestock, and non-human primate populations; examining overlap in resistance genes across groups; examining the significance of comorbidities; and using the resistance genes as potential markers for disease

spillover. We hypothesize that the humans would have the highest prevalence of resistance genes of any group. Further, because the Mitumba chimpanzee community resides closest to the large and densely populated village of Mwamgongo, we predict that Mitumba chimpanzees will have a higher prevalence of antibiotic resistance compared to the Kasekela population.

Methods

Study Area

The study site, Gombe National Park, Tanzania (4°41'59.97"S, 29°36'59.96"E), is a 35 km^2 forest reserve residing at 1500 m above sea-level (Wallis and Lee 1999) Lake Tanganyika forms the western border of the park and villages border it to the north and south. The southern border of the park is 15 km north of the town of Kigoma. The park is limited to researchers, ecotourists, park management staff, local field assistants, and their families. People living outside the park frequently use the same resources as the wildlife living in the forest. For example, the lake is used for bathing, washing clothes, and cooking utensils. Baboons and chimpanzees can be found drinking from the same lake (Wallis and Lee 1999). The park border is permeable, as wildlife venture out of the forest and villagers and their animals enter the periphery of the park. Nonhuman primates also emerge from the forest and overlap with human living spaces. Chimpanzees have been reported raiding crop fields in the village of Mwamgongo (Parsons, personal communication).

Three chimpanzee communities inhabit Gombe National Park: Mitumba, Kasekela, and Kalande. Having been studied for over decades, there is almost entire lifespan data on the habituated adult chimpanzees (Lonsdorf, Travis et al. 2006). Of the three communities in the park, Kasekela and Mitumba have been habituated. The two groups have varying levels of

human encroachment and interaction. The Mitumba group inhabits the northern part of the forest, sharing the park border with Mwamgongo. The permeable park border allows for baboons and chimpanzees to leave the park and for humans and their animals to enter the park. With a narrow habitat range (Figure 1) and flanked by a highly dense human population to the north and researchers residing within the park (Gillespie, Lonsdorf et al. 2010), the Mitumba community is at particular risk for spillover and AR gene acquisition. The Kasekela group is located in a less disturbed forest setting, in the center of the park (Williams, Lonsdorf et al. 2008). A small camp of researchers, members of the Tanzanian Park Authority, and their families live within Kasekela and interact with the Kasekela chimpanzees. Because of Gombe's status as the smallest wildlife reserve in Tanzania, the chimpanzee communities are at an elevated risk for disease (Lonsdorf et al., 2006). The habitat ranges of the two communities overlap slightly, providing minimal opportunity for contact between the two. The unhabituated Kalande group overlaps minimally with Kasekela, but little data is available on this southernmost community.

Fecal Sample Collection

Fecal samples were collected between March 2010 and February 2011, during both dry (July 1- August 15) and wet (November 1- December 15) seasons. Fecal specimens were collected from human subjects after informed consent given. Livestock samples were concurrently collected from domesticated animals (*Canis lupus, Capra hircus,* and *Ovis aries*) owned by Mwamgongo residents. Baboons (*Pabio anubis*) were opportunistically sampled during the wet and dry seasons. Chimpanzees were sampled non-invasively at quarterly intervals from known individuals during periods of routine observational follows. All fecal specimens were freshly voided and aseptically moved to a plastic vial with a 2.5% potassium dichromate

solution (Fisher Scientific, Pittsburgh, PA). Wildlife samples required special care to avoid collecting soil, foliage, or water contaminants. Samples were then sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) and stored at 4^{o}C.

Survey Instrument

At the same time of fecal sample collections, humans were surveyed for data on demography, gastrointestinal symptoms, medication usage, and water usage. Trained local enumerators gave surveys in the native language of Swahili, minimizing response bias. Through surveys of the study population and interviews with the Mwamgongo Health Clinic, antibiotics used, their cost, frequency of use, and reasons of use were determined. Co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, was frequently used in both hospital and village settings for diarrhea. For its widespread use in gastrointestinal infections, we chose to use resistance genes to this drug as a marker for bacterial spillover.

Nucleic Acid Extraction

Total nucleic acid extractions were done on all the fecal samples with the FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH). Two hundred microliters of the fecal sample was placed inside Lysing Matrix E Tubes and then washed twice with 800 µl of de-ionized water to remove the 2.5% potassium dichromate. Washing involved rinsing with de-ionized water, re-suspending, centrifuging for 6 min at 14,000 rcf, and then discarding of supernatant. After washing, the tubes were filled with 978 µl of sodium phosphate buffer and 122 µl of MT Buffer and re-suspended before placing in Fast Prep® Cell Disrupter Model FP120A Instrument for 30 seconds at a speed of 5.5. The lysing tubes were then centrifuged for

30 seconds at 14,000 rcf and the supernatant transferred to clean 1.5 ml microcentrifuge tubes. To each tube, 250 µl of PPS (Protein Precipitation Solution) solution was added to each tube and then inverted by hand 10 times. To form a pellet of remaining solid fecal matter, each sample was centrifuged at 14,000 rcf for 5 min. The supernatant, now containing the nucleic acid, was transferred to a 15 ml falcon. To this was added one milliliter of Binding Matrix Suspension. To allow the nucleic acid to bind to the matrix, the falcon tubes were inverted by hand for 2 minutes and placed on the bench to settle for five minutes. The matrix-nucleic acid mixture was continually added to the SPIN Filter Tubes and centrifuged at 13,400 rcf for 1.5 minutes until the entire matrix was caught in the filter. The filter was then cleaned with 500 µl of SEWS-M (Salt/Ethanol Wash Solution) by centrifuging through the matrix at 13,400 rcf for 2 minutes. After air drying for 5 minutes, 100 µl of DNase/Pyrogen Free Water was gently stirred into the matrix and centrifuged at 13,400 rcf for 1 minute. The filter was then discarded and the final nucleic acid, in the remaining liquid in the catch tube, was stored in -20º C freezer for working use and with archive storage at -80º C.

Polymerase Chain Reaction and Gel Electrophoresis

Once extracted, DNA was screened using multiplex polymerase chain reaction (PCR) (Chen, Zhao et al. 2004). Four primers were used (Table 1). In each PCR run, water was used as the negative control, *E. coli* strain DH0032 was used as the positive control for *sul1,* and *E. coli* strain DH3507 was the positive control for *sul2*. Each PCR reaction was carried out in 1.5-ml microcentrifuge tubes, consisting of a 23 µl reaction mixture. This mixture included 17.25 µl sterile distilled water, 2.5 µl of 10X PCR Buffer, 2.5 µl of deoxynucleoside triphosphates

(2.5mM [each] dATP, dTTP, dCTP, dGTP), 0.5 µl of 25µM primer mix, and 0.25 µl *Taq* polymerase.

Twelve microliters of PCR products were gel electrophoresed using 1.5% SeaKem® LE Agarose [Cat#50004 (Lonza, Rockland, ME)]. Gels were stained with 3 µl ethidium bromide in the gel itself and 2 µl in the running buffer. Gel images were captured under UV exposure.

Data Analysis

All statistical analysis was done with JMP®, Version 9. SAS Institute Inc., Cary, NC, 1989-2007. A chi-square test of independence was used to compare *sul* positivity across groups within a species and with other risk factors including sex, occupation classification (humans), and seasonality. When sample size was too small, a Fisher's Exact Test was used. Odds ratios and 95% confidence intervals were calculated for all cross-tabulations. All comorbidities were analyzed with a McNemar's test.

Other wildlife infection data, such as SIV and *Cryptospiridium,* came from the work of other researchers of the Jane Goodall Institute. These data were used to examine risk factors for AR acquisition.

Results

Descriptive Statistics of All Individuals

The overall prevalence of genes (*sul1* and/or *sul2*) that encode for sulfamethoxazole resistance was: humans (84.2%), chimpanzees (26.2%), baboons (36.2%), dog (77.8%), sheep (7.1%), and goat (12%). Among individual groups, 93.2% of Kasekela humans, 77.4% of Mitumba humans, and 80.7% of Mwamgongo humans had either or both genes coding for

sulfamethoxazole resistance. Further, 28.8% of Kasekela chimpanzees and 20% of Mitumba chimpanzees had either one or both *sul* genes. We observed differences in the frequency of *sul* genes detected from these groups (Table 2). In each group, *sul2* was more prevalent than *sul1*.

In the chimpanzee population, there is a prevalence of 8.3% for the *sul1* gene and 20.2% for the *sul2* gene, with most individuals being from the Kasekela group. Mitumba showed the lowest prevalence rates, as prevalence within the group for *sul1* was 8% and 16% for *sul2*. Kasekela chimpanzees mirrored a similar trend: among Kasekela chimpanzees, 13.6% of individuals had *sul1* and 28.8% had *sul2*.

Further, the human population showed a much higher prevalence in both genes, (44.9%) for the *sul1* gene and 84.8% in the *sul2* gene. Within the Kasekela group, 44.1% of individuals had the *sul1* gene, and 96.6% had the *sul2* gene, the highest prevalence rates of any group. Mitumba followed closely with 54.8% of individuals in the community having the *sul1* gene, and 77.4% had the *sul2* gene. Mwamgongo had prevalence rates of 42.0% for *sul1* and 79.5% for *sul2*.

Analysis of Risk Factors

Using a chi-square test of independence, no statistically significant correlation was shown between humans harboring *sul* positivity and living in their respective locations ($p=0.0634$, $R^2=0.0398$). When analyzed further using odds ratios, the odds for those living in Kasekela being *sul* positive were significantly higher than for those living in Mitumba (OR=4.01, 95% CI=1.073-14.99%). Analysis of the presence of *sul2* versus location revealed the same result as for *sul* positivity, suggesting that *sul2* is responsible for most of the trend seen in *sul* positivity

(p=0.0634, R^2 =0.0398). For humans, there was no statistical significance for individuals to have *sul1* depending on their location (p=0.52, Chi-square test) (Table 3).

The chimpanzees showed similar trends, with no statistically significant correlation between being positive for *sul* genes and their respective groups (p=0.5879, R^2 =0.0076, Fisher's Exact 2-tail Test). In contrast to the humans, *sul* positivity was not as dependent upon harboring the *sul2* gene, as a Fisher's Exact Test revealed a 2-tail p-value of 0.3728 and an R^2 value of 0.0191 (Table 3).

A McNemar's Test revealed a strong statistical significance in the difference between *sul2* and *sul1* prevalence in humans (p<0.001). Individuals positive for *sul2* were more likely to also be positive for *sul1* (84.3%) than those positive for *sul1* were to have *sul2* (45.5%). The same trend was observed in chimpanzees (p=0.0253, 20.2% for those with *sul2* to also have *sul1*, 8.33% for those with *sul1* to have *sul2*). The baboons showed no statistical significance between the two genes (p=0.071).

In examining other potential factors that could select for antibiotic resistance in chimpanzees, a McNemar's Test revealed a statistically significant correlation between *sul* positivity and SIV status (p=0.0201, R^2 =0.0764). In calculating odds ratios for risk factors for human subjects, values for *sul2* across all risk factors were exactly the same as those for *sul* positivity (Table 3). The same trend did not hold for chimpanzee subjects (Table 4).

Discussion

We examined the prevalence of AR genes in the Gombe ecosystem in humans, their livestock, baboons, and chimpanzees. Further, we sought to test the following hypothesis: if wildlife had a higher degree of contact with humans, then there would be a higher prevalence of

antibiotic resistance in their respective groups. Our focus on sulfonamide resistance genes was based on widespread usage within the human community and clinical setting (Parsons, personal communication). Sulfonamide antibiotics have been shown to lose their efficacy after a few years of widespread use (Enne, Livermore et al. 2001) through the development of resistance genes. Therefore, we expected high levels of AR genes within the human community. We predicted that, if they were present in the wildlife population, they would be potential indicators of bacterial spillover among species.

Though there is clear evidence of high *sul* prevalence (84.2%) in the human community and a strong presence of AR genes in wildlife and animals, there seems to be no statistically significant correlation between proximity to densely populated human areas and higher prevalence of *sul* genes in chimpanzees. Mitumba, though closer to the permeable park border, showed less prevalence than the Kasekela community, which experiences human contact but not from densely populated areas. Though contrary to our hypothesis, this finding presents important and interesting questions to consider in understanding the distribution and spread of AR genes in Gombe. Results showing the high levels of resistance in the Kasekela system versus the Mitumba system, the high prevalence of AR in wildlife, the significantly higher levels of *sul2* versus *sul1*, and the linkage between SIV status and *sul* positivity demonstrate the complexity of the Gombe system and beg for further research and consideration.

Antibiotic Resistance in Kasekela

The higher prevalence of *sul* positivity in the Kasekela camp may have several explanations. We propose and explore a few possibilities, first focusing on the composition of the human community within the Kasekela camp. Though Kasekela (n~100) is far less dense

than Mwamgongo, consisting of researchers and members of the Tanzanian National Park Authority, individuals living in Kasekela are far wealthier than the Mwamgongo residents (Gillespie, communication). With more money to purchase antibiotics, Kasekela residents may be prone to higher antibiotic use, thus account for higher levels of AR than those in Mitumba or Mwamgongo. Researchers may be taking antibiotics whenever they become sick, to quickly recover and return to work as sick researchers are not allowed to go into the field (Parsons, personal communication). The higher prevalence of *sul* in Kasekela chimpanzees may come from interaction with this specific population of humans. If researchers are interacting most with the chimpanzees, they may be potential reservoirs of AR genes to Kasekela chimpanzees. Further, Kasekela chimpanzees are far more habituated to people and thus feel safe in the human environment. They may roam the camp or steal food, coming in contact with humans and their environment (Gillespie, personal communication). By coming in contact with human feces, chimpanzees can acquire either sulfonamide antibiotics or plasmids containing resistance genes in the stool. These explanations would be consistent with findings from Rwego et al. who found that local antibiotic use was responsible for the types and prevalence of AR genes in the gorilla population in Bwindi Impenetrable National Park, Uganda.

Antibiotic Resistance in Wildlife

The high levels of resistance in wildlife may have numerous reasons behind them. Interactions between Kasekela humans and chimpanzees have already been discussed as possibly contributing to the observed prevalence in chimpanzees. The prevalence in Mitumba, though, may have other explanations than Kasekela. Despite the high density of Mwamgongo and the presence of researchers in the Mitumba chimpanzee habitat range, these individuals had a

slightly lower prevalence (20%) of genes than the Kasekela community (28.8%). Since high human density does not seem to be as strong a risk factor for wildlife having AR, it may be that human interaction at all is the strongest risk factor.

Another possibility is that another mode of transmission, besides direct contact with humans, could be driving resistance in wildlife populations. A study done in Gabon found no evidence of transmission of AR *E. coli* strains from humans to gorillas (Benavides, Godreuil et al. 2012). By analyzing the phylogenetic relationship of *E. coli* strains, they determined that *E. coli* isolates from humans and domestic animals were significantly different from isolates from gorillas and other wildlife. This finding suggests horizontal gene transfer or naturally acquired resistance to be at work (Benavides, Godreuil et al. 2012) instead of bacterial transmission from humans. Resistance strains or resistance genes could be acquired from other environmental factors, such as soil and water. Because sulfonamide antibiotics have a high excretion rate in humans and animals (Thiele-Bruhn and Beck 2005), they may reside or accumulate in the environment as the parent compound in certain types of soil for months (Hamscher, Pawelzick et al. 2005). Further, because of their ionizible, polar properties, they can accumulate in the aquatic environment (Blasioli, Martucci et al. 2014). Because humans, baboons, chimpanzees, and livestock share the same stream that flows in Lake Tanganyika (Wallis and Lee 1999), the possibility of encountering sulfonamides in the environment may be high. Further, latrines have been found within 20 m of the lake, increasing the possibility of polluting lake water (Wallis and Lee 1999). If feces are polluting water sources used by humans, wildlife, and livestock alike, antibiotics and antibiotic resistant bacteria may be accumulating in the environment. Further research is needed to test water sources for antibiotic concentrations and AR genes in the water.

The high prevalence of *sul* genes in the dog population (77.7%) may also inform the resistance observed in wildlife. Dogs are not prevented from crossing into the park. When they defecate in the forest, they may be bringing *sul* genes into close proximity with chimpanzees or baboons, possibly explaining the presence of resistance in the wildlife. Baboons (prevalence of 36.2%) may also be actors in spreading resistance genes, as they are known to come out of the forest and raid trash and huts. Trash pits are typically buried less than 2 m below the surface, thereby being easily accessible to raiding baboons and/or other forest animals (Wallis and Lee 1999). Their high overlap with the human population poses further risks of bringing disease and resistance genes into the forest.

Differences in Sul1 *vs.* Sul2

Across all species, there was a higher prevalence of the *sul2* gene. Further, results showed that being positive for *sul2* coincided with also being positive for *sul1*. Both genes of resistance code for a similar mechanism, but have distinct modes of transmission. The *sul1* gene is typically linked to other resistance genes in class 1 integrons. The *sul2* gene is located on small nonconjugative plasmids or large transmissible multi-resistance plasmids (Byarugaba 2010). Other studies have seen similar trends with *sul2* prevalence higher than *sul1*. Namely, Enne et al. found that even after sulfonamide use had been drastically decreased in the UK, several years later, there was no significant decline in resistance genes in the community, with *sul2* genes significantly more common than *sul1* genes. One potential explanation involves the linkage of *sul2* to other resistance determinants. Because *sul2* is located on large plasmids containing many resistance genes, positive selection pressure may also exist for other antibiotic resistance genes, thus selecting for *sul2* as well (Enne, Livermore et al. 2001).

SIV Status and Sul *Positivity*

The significant correlation between SIV positivity and *sul* positivity poses interesting questions, as well. SIV status may predispose chimpanzees to gut bacteria or enteric disease that harbor higher AR content. One study demonstrated that the gut microbiome of Gombe chimpanzees were significantly altered after SIV infection, by tracking animals before and after infection (Moeller, Shilts et al. 2013). Gut microbiota of SIV-infected chimpanzees occupied a larger area of compositional space that was never achieved by gut communities of uninfected individuals. The suppression of the immune system relieved constraints on the growth of the gut microbiome (Moeller, Shilts et al. 2013). The larger space for gut bacterial colonization may allow a niche for AR strains to thrive. Besides the aforementioned study, no other research seems to have been conducted in this field. Provided the vast history and data present with the Gombe ecosystem, opportunities to study the effect of SIV on gastrointestinal bacteria, and more specifically, on resistance, are vast but, until now, relatively unexplored.

Conclusions

As chimpanzee populations become more forced to overlap with humans through habitat reduction, the risk for disease increases (Williams et al., 2008). Though antibiotic resistance genes from areas of high human density have not shown to be significantly associated with the presence of these genes in wildlife, humans are still the most likely reservoir for exposing wildlife to drug-resistant bacteria and to antibiotics themselves. The established presence of AR genes in the wildlife is of great concern. Efforts to limit the human population within the park and implementing more stringent regulations to reduce transmission risks (Bennish and Khan

2010, Moeller, Shilts et al. 2013)are needed. Further research is also needed to understand the impact AR is having on wildlife and the relationship between SIV infection and antibiotic resistance.

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Tables and Figures

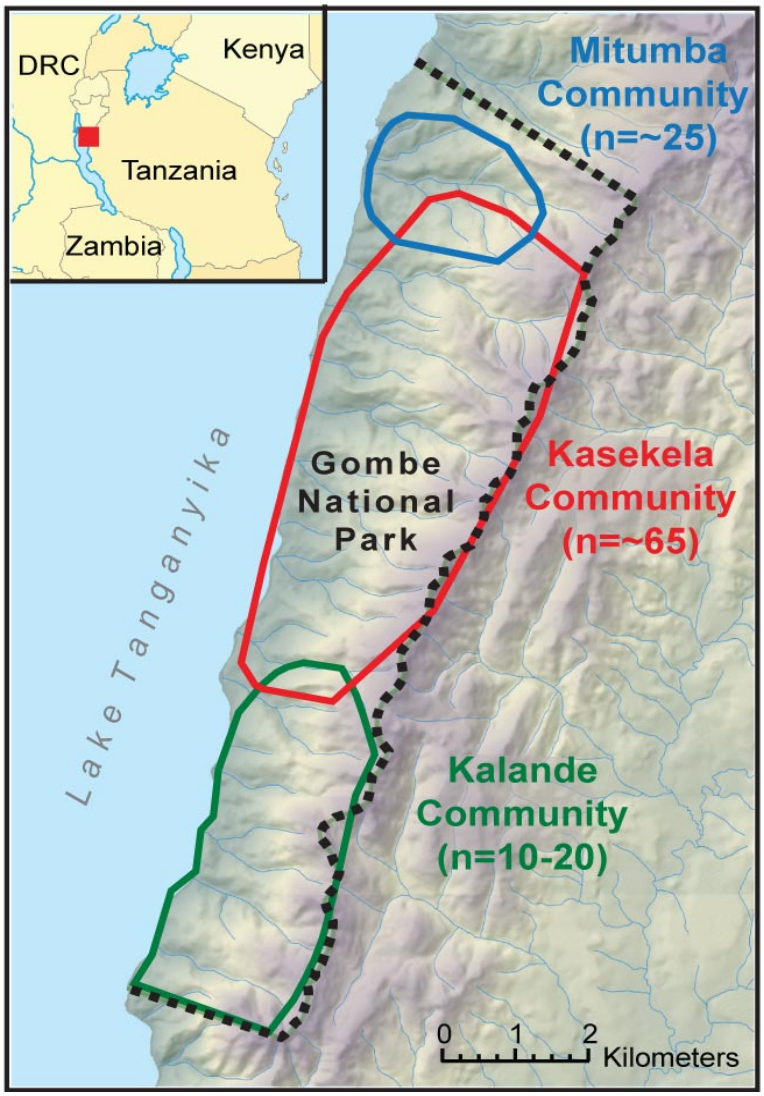

Figure 1. Map of Gombe Stream National Park in Tanzania. Mitumba chimpanzee community and Kasekela chimpanzee communities are the two habituated groups within the park. Because of Mitumba's proximity to the forest edge, the habitat of these chimpanzees has higher overlap with humans. The larger Kasekela community is more centrally located and thus does not overlap as much with humans (Source: Travis et al. presentation).

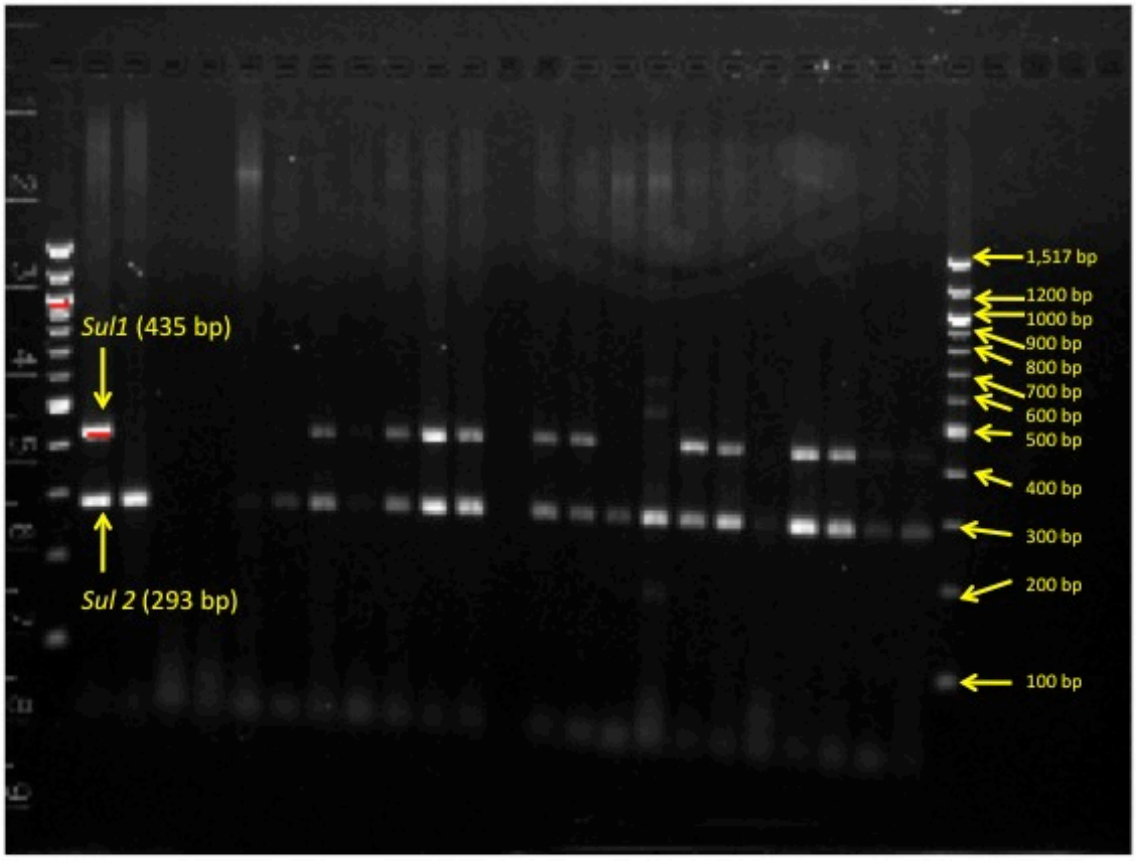

Figure 2. Gel image showing bands for *sul1* and *sul2* in human samples. 100 kb ladder (right most well) used to estimate size of bands.

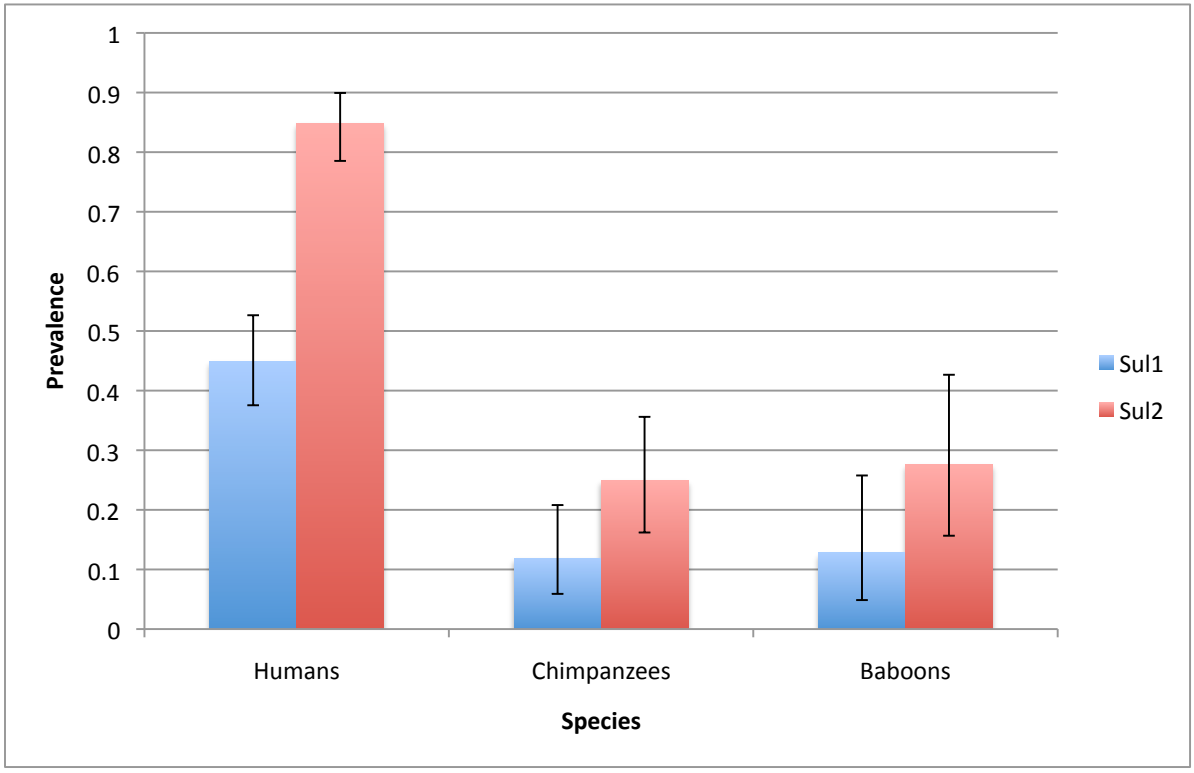

Figure 3. Prevalence of *sul1* and *sul2* genes for humans, chimpanzees, and baboons. All species show a higher prevalence of *sul2*, with a significant difference between *sul1* and *sul2* for humans.

Table 1. Summary of primers used in multiplex PCR in screening for sulfonamide resistance.

Gene	Primer	Expected Amplicon Size
*sul*1	Forward: 5'-TCA CCG AGG ACT CCT TCT TC-3'	331 bp
*sul*1	Reverse: 5'-CAG TCC GCC TCA GCA ATA TC-3'	331 bp
*sul*2	Forward:5'-CCT GTT TCG TCC GAC ACA GA-3'	435 bp
*sul*2	Reverse: 5'-GAA GCG CAG CCG CAA TTC AT-3'	435 bp

Table 2. Prevalence of *sul1* and *sul2* detected by species and location in and around Gombe National Park, Tanzania.

Host	Positive/Total	*Sul genes* detected (n)	Prevalence (95% CI)
Humans			
Mwamgongo Village	71/88	*sul1* (37), *sul2* (70)	0.807 (0.709-0.883)
Kasekela	55/59	*sul1* (26), *sul2* (55)	0.932 (0.835-0.981)
Mitumba	24/31	*sul1* (17), *sul2* (24)	0.774 (0.589-0.904)
Livestock			
Dog	7/9	*sul1* (4), *sul2* (7)	0.786 (0.4-.972)
Goat	9/75	*sul1* (2), *sul2* (8)	0.12 (0.056-0.216)
Sheep	1/14	*sul1* (1), *sul2* (1)	0.071 (0.00181-0.339)
Baboons			
Baboon	17/47	*sul1* (6), *sul2* (13)	0.362 (0.227-0.515)
Chimpanzee			
Kasekela	17/59	*sul1* (8), *sul2* (17)	0.288 (0.178-0.421)
Mitumba	5/25	*sul1* (2), *sul2* (4)	0.2 (0.068-0.407)

Table 3. Risk factors for sulfonamide resistance in people living in or around Gombe National Park, Tanzania

		sul positive				*sul1* positive				*sul2* positive			
			95% CI				95% CI				95% CI		
Variable	**n**	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***
Seasonality: dry vs wet season	178	0.577	0.255	1.305	0.201	0.565	0.301	1.061	0.085	0.577	0.255	1.305	0.201
Location (Mwamgongo vs Mitumba)	119	0.821	0.304	2.219	0.795	1.598	0.701	3.641	0.299	0.821	0.304	2.219	0.795
Location (Mitumba vs Kasekela)	**90**	**4.010**	**1.073**	**14.994**	**0.043**	0.649	0.271	1.556	0.379	**4.010**	**1.073**	**14.994**	**0.043**
Location (Kasekela vs Mwamgongo)	147	3.292	1.048	10.324	0.052	1.037	0.533	2.016	1.000	3.292	1.048	10.324	0.052
Mwamgongo vs park resident	178	1.720	0.755	3.917	0.221	1.204	0.667	2.173	0.551	1.720	0.755	3.917	0.221
Sex (female vs. male)	170	0.945	0.420	2.126	1.000	0.992	0.542	1.814	1.000	0.945	0.420	2.126	1.000

Table 4. Risk factors for sulfonamide resistance in chimpanzees in Gombe National Park, Tanzania

		sul positive				*sul1* positive				*sul2* positive			
Variable	**n**[a]	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***	**OR**	**Lower**	**Upper**	***p***
Location (Kasekela vs Mitumba)	84	1.619	0.523	5.014	0.588	1.065	0.192	5.893	1.000	2.281	0.593	8.776	0.373
Sex (female vs. male)	84	1.080	0.406	2.872	1.000	0.949	0.199	4.528	1.000	0.863	0.293	2.540	1.000
Positive for SIV	**68**	**6.667**	**1.394**	**31.881**	**0.020**	1.571	0.160	15.460	0.543	5.667	1.195	26.870	0.166