Gastrointestinal Microbial Flora in Wild and Captive Olive Baboons (Papio anubis)

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Abstract Background: Vertebrate gut microbiota plays essential roles in host biology, including immune regulation, energy acquisition, vitamin synthesis and disease risk. There are however several other pathogenic microorganisms found in the gut and are transmissible by fecal oral route. About 60% of all human diseases and approximately 75% of emerging infectious diseases are zoonotic. Due to an observed increase in conflicts and interactions between human and nonhuman primates, both are at risk of pathogen transfer and infection. Methods: This study was conducted on 50 captive baboons and 67 wild baboons. Stool samples were collected and cultured and species identification of each isolate was done by the use of Analytical Profile Indexing tool. Results: Species of Gram-positive cocci, Gram-positive and Gram-negative rods were identified, with more isolates being obtained from wild than captive baboon fecal samples. Unlike the Gram-negative rods, the captive baboons harbored more Gram-positive cocci and Gram-positive rods than the wild baboons. Escherichia coli was the most dominant isolate and was collected in more than 50% of the samples from both groups of animals. Of the Gram-positive cocci and Gram-positive rods, Aerococcus viridans, Bacillus cereus and Bacillus firmus were found to be the most common isolates in both groups of animals. Conclusion: Though the wild and captive baboons harbor different gastrointestinal bacteria, similarities do occur. The wild baboons have a richer microbial diversity as compared to the captive baboons.

Keywords: gastrointestinal microbes, emerging infectious diseases, nonhuman primates, zoonotic

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1. Introduction

All higher animals are associated with a diverse microbial community that is composed mainly of bacteria. Relation between the gut microbiota and health is being increasingly recognized as it describes interactions between microorganisms with each other, their environment, as well as all other forms of life [1]. The gut microbiota influences essential functions including digestion, energy metabolism and inflammation by modulating multiple endocrine, neural and immune pathways of the host, which altogether have an enormous impact on the nutritional and health status of the host [1,2]. Establishing and maintaining beneficial interactions between the host and its associated microbiota are key requirements for host health [3]. Beneficial intestinal microbes carry out critical functions for their hosts and are collectively considered an “essential organ”. Intestinal bacteria provide nutrients to the host through synthesizing essential amino acids and vitamins such as vitamin B9 and B12, while others promote epithelial cell development and thereby aid innate immunity in humans [5]. Recent studies have reported that the microbiota also influences the development and homeostasis of other host tissues, including the bone. The microbiota also benefits from this mutualistic association, as the mammalian intestine is a nutrient-rich environment that is maintained at a constant temperature. However, it is also a dynamic habitat that undergoes constant and rapid changes in its physiological parameters owing to variations including host diet, lifestyle, hygiene or use of antibiotics, all of which affect gut microbial composition [3]. In man, inter-individual variation in gut microbial composition has more often been linked to major health concerns, among them obesity, diabetes, cancer, heart disease, and autoimmune disorders [7,8].

Emerging infectious diseases (EIDs) pose a significant threat to global health security. The origin of human EIDs has been traced from multiple sources, including host shifts from animal reservoirs (zoonoses), evolution of existing organisms, and recurrence due to antimicrobial resistance, that are increasing in number, and are globally...
distributed [9]. In Kenya baboon-human interactions and conflicts are especially common in many parts of the country. The conflict is mostly due to baboon crop raiding reputation [10,11] which has also been reported in other parts of Africa [12]. Various enteric pathogens have been isolated from restricted baboons under research. Among them Salmonella, Shigella, Campylobacter and Helicobacter species. [13,21]. According to World Health Organization (WHO), past experience shows that outbreak of diseases caused by these microorganisms could not only potentially cause large numbers of human deaths as they spread, but also have huge social and economic impact in today’s interconnected world. During the past three decades, more than 30 new microorganisms have been identified worldwide and many of them have originated at the level of human-animal interface [26]. The ability to foresee when, where, and within which species pathogens are most likely to emerge is hence ever more urgent.

Understanding the course of disease emergence is critical for mounting strategies to minimize risk and cut down on the high cost of managing outbreaks once a disease has emerged. Population, environmental, and behavioral changes that increase contact with wildlife intensify emergence of these pathogens. Human intrusion into formerly undisturbed areas increases remote area accessibility and avails more vectors and reservoirs of infection to new hosts. Several pandemics and epidemics have been linked to this practice of bush meat hunting, preparation and consumption [16]. Several infection transmission occurrences from human to nonhuman primate (NHP) populations have been either suspected or established. Among them are human respiratory syncytial virus and metapneumovirus in chimpanzees in Côte d’Ivoire and intestinal pathogens Giardia and Escherichia coli in mountain gorillas and chimpanzees in western Uganda [34]. Even though local populations or researchers have been the sources of these infections, tourists pose an uncalculated risk to wildlife, which subsequently has the likelihood of producing overwhelming health and economic outcomes [18].

Human and NHP endogenous intestinal flora are not fully understood. Culture-based studies have been able to conclude that initial colonization of microbiota within the gut of humans is through the vagina and feces of mothers to infants during childbirth (9). It has also been suggested that vertical transmission of important commensal gastrointestinal tract (GIT) bacteria occurs in wild ape populations between mother and offspring [10]. Describing nature of relationships between intestinal flora and other pathogenic bacteria is of great importance as this information can be used to possibly help combat or prevent pathogen transmission between humans and NHPs. Baboons are genetically closely related to humans [11], yet knowledge of the composition of gut microbial ecosystem is limited. Characterization of the intestinal bacteria of this species is of primary interest for it has health implications for both the NHPs and humans. In addition, it can potentially offer pertinent information regarding bacterial, and possibly pathogen transmission events. In the face of rising cases of zoonotic, anthropozoonotic and EIDs, information on the gut bacteria of baboons will not only help in understanding the dynamics of such diseases, but also risk reduction in relation to exposure, transmission, emergence of new and re-emergence of old infections. Our goal was to evaluate the gut bacteria profile of both captive and wild baboons.

2. Materials and Methods

2.1. Ethical Considerations

All experimental protocols and procedures of the study were reviewed and approved by the Institutions Scientific and Ethical Research Committee (ISERC) of the Institute of Primate Research (IPR) (ref no: ISERC/11/16) on the use of baboon as model of study for biomedical science in accordance with the international guidelines on animal care, handling and use for biomedical research.

2.2. Animals

The samples were obtained from two groups of animals comprising of 117 Olive baboons (Papio anubis) from Kenya, East Africa. Of these, 50 had lived in captivity for a period of between one and five years. The captive baboons were housed in group cages at IPR, Nairobi, Kenya, which is a WHO collaborating Centre and is accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The baboons were fed on commercial monkey cubes (Unga Feeds Ltd, Nairobi, Kenya) supplemented with fruits, vegetables and water ad libitum. These animals had very minimal contact with humans unless during feeding and general cleaning which was done in the daily in the morning. None of the animals had received antibiotic treatment three months prior to sampling. The other 67 samples were obtained four different troops of free ranging wild baboons in Northern part of Laikipia County, which is located in Central region of Kenya. Economic activity of this county mainly entails tourism and agriculture. Agricultural activities consist cultivation of food crops, greenhouse horticulture and ranching. Large-scale ranches such as Solio, Borana and Oljogi ranch occupy 37% of Laikipia, with 32% under pastoralist grazing use and 21% under small holder farmers. Pure wildlife-based tourism and mixed ranch tourism is practiced in both the large ranches and smaller land holdings. Such activities have seen this area experience intensified integration of human and wildlife habitats leading to the increased possibility of conflict between the two.

2.3. Sample Collection and Processing

Sampling was carried out after obtaining approval of study protocol by ISERC. Purposive sampling technique was used and fecal sample of each animal was collected once. Each fecal bolus was sampled from the center by using sterile cotton swab to avoid contamination [19]. All samples collected from the animals were placed in Stuart’s transport medium (Oxoid, Basingstoke, UK) and transported to the laboratory for processing. The swabs were removed from the transport medium and used to inoculate blood agar for aerobic and anaerobic culture, Xylose Lysine-deoxycholate agar, MacConkey agar and Mannitol Salt agar. These were incubated as previously described [20]. All culture media were used from Oxoid, Basingstoke, UK. Anaerobic atmosphere was achieved by
use of gas generating kit (Oxoid, Basingstoke, UK) in anaerobic jars. Initial identification of the isolates was based on oxygen requirement, colony morphology and Gram stain appearance. The unique isolates were sub-cultured on suitable culture media until pure colonies were obtained. Gram staining was done to determine Gram’s reaction of the pure isolates as previously described [20]. For biochemical identification, all tests were performed using reagents and methods provided by the manufacturer in the Analytical Profile Index (api®) kits (Biomerieux®, SA 69280; Marcy L’Etoile, France). The kits used included api 20 Strep for most streptococci and closely related organism, api 20E for Enterobactereacea, Vibrio spp and other non-fastidious Gram-negative rods, api Coryne for coryneform bacteria, api Staph for staphylococci and related genera, api 50CH for genus Lactobacillus and related genera and api 50 CHB/E medium for Bacillus and related genera. Analysis was carried out using APIWEB™ stand-alone V1.2.1 identification software (Biomerieux® SA 69280).

3. Results

3.1. Gram Stain Assessment

Each Gram’s stained smear of the fecal swab isolates was assessed and evaluated under oil immersion ×100 objective lens magnification. On microscopy, Gram positive cocci of Staphylococci (Figure 1-a) and Streptococci (Figure 1-b) were observed from fecal samples obtained from both wild and captive animals. In addition, Gram positive rods including pleomorphic Corynebacterium species (Figure 1-c), capsulated Leuconostoc species (Figure 1-d), and drum stick shaped Clostridium species with terminal spores (Figure 1-e) and Bacillus species with endospores (Figure 1-f) were observed. Gram negative rods observed included Escherichia coli (Figure 1-g), capsulated Klebsiella pneumoniae (Figure 1-h) and short thick rods of Raoultella planticola (Figure 1-i).
Figure 1. Microscopic examination of bacteria isolated from baboon gut. (a) Staphylococci (b) Streptococci consisting of short chains of *Aerococcus viridans*. (c) *Corynebacterium renale* with pleomorphic characteristic (d) Capsulated *Leuconostoc lactis* rods (e) Drumstick shaped *Clostridium bifermentans* with terminal spores (f) *Bacillus firmus* with endospores (g) Gram negative rods of *Escherichia coli* (h) Gram-negative capsulated rods of *Klebsiella pneumoniae* (i) Short thick Gram-negative rods of *Raoultella planticola*

3.2. Bacterial Culture

Numerous cultivable bacteria were present in the fecal sample swabs of 117 olive baboons and the number that grew on the plates were highly variable. The analysis of the isolates identified Gram-positive cocci (Table 1), Gram-positive rods (Table 2) and Gram-negative rods (Table 3). Of the 25 species of Gram-positive cocci identified, *Aerococcus viridans* was dominant and was found in 33 (21.7%) and 24 (23.1%) of the isolates from the wild and captive animals respectively. Nine *Staphylococci* were identified and fecal samples from the wild animals were found to have the highest number of
and was present in more than 50% of the isolates from both groups. Of the 28 species, only six, E. coli, Citrobacter koseri, were common to both groups. All choleraesuis wild animals (Table 3). rods bacteria were isolated from samples obtained from wild animals only. The highest number of Gram-negative Pseudomonas spp isolates were from samples from the wild and captive animals respectively. Seven Clostridia were isolated of which only one, C. beijerincki, was common to both groups. Likewise of the four Corynebacteria isolated, only one, C. renale was common to both groups. Five Lactobacilli were isolated and four were common to both groups (Table 2). The captive animals comprised the highest number of Gram-positive rods (Table 2).

For the enteric bacteria, E. coli was the most common and was present in more than 50% of the isolates from both groups. Of the 28 species, only six, E. coli, Citrobacter koseri Enterobacter cloacae, Salmonella choleraesuis, Raoulella ornithinolytica, and Pantoea spp were common to both groups. All Klebsiella and Pseudomonas spp isolates were from samples from the wild animals only. The highest number of Gram-negative rods bacteria were isolated from samples obtained from wild animals (Table 3).

Table 1. Comparison of Gram Positive Cocci Isolates in Wild and Captive Baboons

| ISOLATE NAME          | No (%) ISOLATE |
|-----------------------|----------------|
| Aerococcus spp        | WILD 15(15.7)  |
| A. viridans           | WILD 34(21.7)  |
| Staphylococcus spp    | WILD 79(46.7)  |
| S. epidermidis        | WILD 12(7.1)   |
| S. simulans           | WILD 21(13.5)  |
| S. aureus             | WILD 20(12.4)  |
| S. hominis            | WILD 12(7.1)   |
| S. intermedius        | WILD 24(14.4)  |
| S. xylosus            | WILD 20(12.4)  |
| S. hyicus             | WILD 8(4.8)    |
| S. haemolyticus       | WILD 16(9.9)   |
| S. lentus             | WILD 12(7.1)   |
| Enterococcus spp      | WILD 12(7.1)   |
| E. avium              | WILD 20(12.4)  |
| E. durans             | WILD 12(7.1)   |
| E. faecium            | WILD 25(15.7)  |
| E. faecalis           | WILD 12(7.1)   |
| Kocuria varians       | WILD 12(7.1)   |
| Micrococcus spp       | WILD 12(7.1)   |
| Streptococcus uberis  | WILD 12(7.1)   |
| Gamella morbillorum   | WILD 12(7.1)   |
| Pelicoccus pentosaceus| WILD 12(7.1)   |
| Lactococcus lactis    | WILD 12(7.1)   |

Table 2. Comparison of Gram Positive Rods in Wild and Captive Baboons

| ISOLATE NAME          | No (%) ISOLATE |
|-----------------------|----------------|
| Bifidobacterium spp   | CAPTIVE 14(9.0)|
| Cellulomonas spp      | CAPTIVE 2(4.4) |
| Bacillus cereus       | CAPTIVE 12(7.1)|
| B. magermentierii     | CAPTIVE 8(5.1) |
| B. firmus             | CAPTIVE 2(13.5)|
| B. circulans          | CAPTIVE 1(6.0) |
| Clostridium spp       | CAPTIVE 11(7.1)|
| C. clostridiforme     | WILD 3(3.6)    |
| C. paraputrificum     | WILD 6(4.8)    |
| C. tetani             | WILD 5(6.0)    |
| C. beijerincki        | WILD 8(5.1)    |
| C. botulinum          | WILD 3(3.6)    |
| C. bifermontans       | WILD 14(9.0)   |
| C. baratti            | WILD 1(6.0)    |
| Corynebacterium spp   | WILD 10(6.4)   |
| C. renale             | WILD 13(8.3)   |
| C. propinquum         | WILD 1(1.2)    |
| C. grahamii           | WILD 8(5.1)    |
| C. auris              | WILD 3(1.9)    |
| Lactobacillus spp     | WILD 2(1.3)    |
| L. delbrueckii        | WILD 3(1.9)    |
| L. plantarum          | WILD 12(7.7)   |
| L. paracasei          | WILD 1(1.2)    |
| L. brevis             | WILD 3(1.9)    |
| Actinomyces israelii  | WILD 3(1.9)    |
| A. naeslundii         | WILD 3(1.9)    |
| Listeria monocytenogen| WILD 1(0.6)    |

Table 3. Comparison of Gram Negative Rods in Wild and Captive Baboons

| ISOLATE NAME          | No (%) ISOLATE |
|-----------------------|----------------|
| Escherichia coli      | CAPTIVE 59(69.4)|
| Klebsiella spp        | CAPTIVE 2(4.4)  |
| K. pneumoniae         | CAPTIVE 12(4.8)|
| K. oxytoca            | CAPTIVE 11(5.4)|
| Proteus mirabilis     | CAPTIVE 19(9.4)|
| P. vulgaris group     | CAPTIVE 2(4.4)  |
| Serratia marcescens   | CAPTIVE 8(4.0)  |
| S. liquefaciens       | CAPTIVE 14(6.9)|
| S. ficaria            | CAPTIVE 3(1.5)  |
| S. odorfera           | CAPTIVE 2(1.0)  |
| Citrobacter freundii  | CAPTIVE 6(3.0)  |
| C. koseri             | CAPTIVE 2(1.0)  |
| Enterobacter cloacae  | CAPTIVE 6(3.0)  |
| E. sakazakii          | CAPTIVE 3(1.5)  |
| Aeromonas hydrophilia | CAPTIVE 1(0.5)  |
| Salmonella choleraesuis| CAPTIVE 2(4.4)|
| Raoultella aquatilis  | CAPTIVE 1(0.5)  |
| R. ornithinolytica    | CAPTIVE 5(2.5)  |
| R. planticola         | CAPTIVE 2(1.0)  |
| R. terrigena          | CAPTIVE 1(0.5)  |
| Pantoea spp           | CAPTIVE 2(4.4)  |
| Klyvera Spp           | CAPTIVE 5(5.9)  |
| Helicobacter alvei    | CAPTIVE 3(4.7)  |
| Cellulomonas spp      | CAPTIVE 1(0.5)  |
| Enterobacter aerogenes| CAPTIVE 2(4.4)  |
| E. amnigenus          | CAPTIVE 1(0.5)  |
| Providencia retgeri   | CAPTIVE 1(0.5)  |
| Pseudomonas oryihabitans| CAPTIVE 42(0.0)|
| P. aeruginosa         | CAPTIVE 1(0.5)  |
4. Discussion

The baboon gut is populated by an array of bacterial species and a differences and similarities in gut microbial composition exist between the wild and captive baboons. The present study of fecal swab samples from 67 wild and 50 captive baboons provide valuable evidence of what comprise the baboon gut. Our study was not without limitation as we used the conventional methods of cultivation which have been reported to have inability to readily cultivate some of the microbes [21]. However, its significance cannot be disregarded especially in low and middle-income countries.

With the help of the identification software, we identified diverse array of microbes including Gram-positive cocci, Gram-positive rods and Gram-negative rods (Figure 1a-i and Table 1-Table 3). Of the nine species of Gram-positive cocci that were common to both groups of animals, Staphylococci and Enterococci were predominant (Table 1). Ten Gram-positive and 6 Gram negative rods were also found to be common isolates in both groups of animals. As demonstrated in our study, previous studies have also shown that there is high similarity of gut microbiota in mammals of the same species [40].

Despite similarities observed, four species of both Gram-positive cocci and Gram-positive rods, and fifteen species of Gram-negative rods were unique to the fecal samples collected from wild animals. The unique isolates from the captive animals included six species of Gram-positive cocci (Table 1) and seven of both Gram-negative and Gram-positive rods (Table 2 and Table 3). As previously reported [22], our study found that there was overwhelming diverse composition and appreciable differences in the composition of the baboon gut microbiota between individual animals and between the two groups. Overall, 34.9% of isolated bacterial species were unique to wild baboons with 27.7% being unique to captive. This uniqueness can be attributed to various factors but most important is social interactions. A handful of recent studies in humans and other primates provide circumstantial evidence for social effects on the gut microbiome. Social interactions have been reported to predict gut microbiome composition in wild baboons [23].

The baboon gut harbors some microbes which have been considered pathogenic in addition to those considered beneficial to humans. Similar to previous studies [24,22], our results demonstrated that there was overwhelming dynamic and appreciable differences in the composition of the microbiota from one baboon gut to another in both groups. A recent study [22] has also found that baboon gut microbiota appears to be highly dynamic such that samples collected from the same individual only a few days apart are as different from each other as sample collected over years apart. However, many previous studies have uncovered multiple variables that are likely to influence gut microbial composition:- and they include: diet, host immune system, gut morphology, and phylogenetic. The presence of resilient species such as E. coli in more than half of the swabs from this study suggest that they are common inhabitant of intestinal tract of many animals and humans and can be easily transferred when there is close contact.

Studies of wild populations have the potential to provide unique insights into factors affecting colonization of the intestinal tract by bacteria. The largest number and greatest variety of bacteria comprised Gram-negative rods. Our results also demonstrate that the wild baboon gut is colonized by more Gram-negative rods than their captive counterparts. One possible explanation could be the frequent contact with resistant bacteria in food or environmental fomites [27]. Also, wild baboons often walk through fecal materials of other animals including humans, and drink from common contaminated water bodies shared by other species.

Despite the similarity in environment and diet in captive baboons, there were still differences in bacterial isolates between individual animals. Isolates such as Aerococcus spp, Enterococcus spp, E. faecalis, B. circulans, L. monocytogenes, C. baratii, L. paracasei, P. rettgeri, E. amnigenus, Cellulomonas spp, C. koseri and R. ornithinolytica each comprised less than 1% of the captive baboon gut bacteria population and isolated from different animals. Some degree of these unique microbial populations are inherent to their hosts, irrespective to their dietary effects [28].

Previous research indicate that diet plays an important role in shaping the differences of gut microbial compositions in nonhuman primates and humans. The sampled captive baboons had been continuously fed on a controlled diet of monkey pellets, vegetables and clean water ad libitum. Their environment was also kept clean which limits their exposure to a variety of different bacterial species. There was an observed difference in enteric isolates with wild baboons showing a richer microbial diversity. This was especially high in enteric species isolates with 69.6% being unique to wild compared to 30.4% of captive animals. Wild baboons have a more diversified dietary intake and live in microbially heterogeneous environments. This could lead to higher turnover in gut microbial species [22].

Potential pathogens, including K. pneumoniae, Klebsiella spp, Salmonella spp, P. aeruginosa, P. mirabilis, B. cereus, C. tetani, Corynebacterium spp and E. faecalis were observed from both captive and wild baboon stool samples. From the viewpoint of social evolution, social interactions can mediate exposure and susceptibility to bacteria, and socially mediated transmission is able to affect the evolutionary costs and benefits of social relationships. In light of the increase in uncontrolled interaction and conflict between baboon and humans [10,11], there is risk of transmission and infection of such pathogenic microorganisms. Studies in humans and other primates suggest that direct contact during social interactions may alter the composition of the gut microbiome in an individual. This could explain why there is a strong association between social interactions and health in humans and other social animals in relation with disease transmission. The risk of transmission is also supported by findings from previous studies that humans and NHPs harbor similar enterotypes (59,60). Shigella dysenteriae type 1 and Salmonella typhi, have only rarely been isolated from NHPs; nevertheless, several others including S. flexneri, S. sonnei, and Salmonella typhimurium, which are pathogenic to man, have been isolated [34].

There are many reports of infection in primates and reports of transmission to human beings [35]. This illustrates the
potential danger for infants and children in contact with the species [36]. The above results concur with these findings as a good percentage of the isolated species, both wild and captive, are either known human pathogens or opportunistic pathogens.

Past research on fecal-oral or social network-mediated transmission has focused almost solely on pathogens or parasites. With perspective to health effects and evolution, species with beneficial effects should also play part in such research. As observed above, we identified several potential pathogens including K. pneumoniae, Salmonella spp, P. aeruginosa, Corynebacterium spp. However, we found several other bacterial isolates thought to be beneficial to hosts. For example, Bifidobacterium spp, L. delbrueckii, L. brevis, L. plantarum and other Lactobacillus spp, are commonly thought to have probiotic effects in humans due to their role in complex carbohydrate digestion, pathogen inhibition, and vitamin production [32,33]. Wild baboons are exposed to many different sources of microbes in their environment. These physical interactions may indeed help to share beneficial bacteria that are likely to support their physical, immunological and neurological development. These beneficial bacteria may serve similar purpose in case of zoonotic transfer to other species such as humans. Recent findings suggest that, social partners not only share more similar sets of gut microbes, but also similar loads of individual microbial species [40]. A possible explanation to this observation is that when bacteria from a host colonize a social partner, they arrive pre-adapted to occupy the available gut microbial niches in their new host [41]. Due to the fact that members of a single bacterial species can have markedly different gene contents, a given member of a gut microbial species may perform different functions in different hosts [41,42]. However, if social partners transmit bacteria with similar competences to each other, these bacteria may serve similar functions in both hosts and thus be found in similar abundances. In light of the observed genetic similarity between baboons and man, this hypothesis could be further tested to assess if bacterial species isolated from social partners tend to represent shared strains that perform similar biological functions. An understanding of the ecological function of baboon gut microbiota is essential and needs to be explored so as to recognize and pathologic abnormalities.

Statement of Competing Interest

The authors declare no competing interests.

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