

Original Research

Antimicrobial Resistant Profile of Bacterial Pathogen Isolated from Macaque species Rescued in the Center for Rescue, Conservation and Creature Development, Phong Nha-Ke Bang Nation Park, Vietnam

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***Correspondence**Corresponding author: Nguyen Van Chao
E-mail address: nvchao@hueuni.edu.vn**Abstract**

Macaque species play important roles in the cultures, and religions of many societies. They are an essential component of tropical biodiversity, contributing to forest regeneration and ecosystem health. The close phylogenetic relationship between humans and *Macaque* species also creates a high potential for pathogen exchange. A total of 228 *Macaques* which belong to four species, including *Macaca arctoides*, *Macaca leonine*, *Macaca assamensis*, and *Macaca mulatta*, were rescued in the Center Rescue, Conservation and Creature Development, Phong Nha-Ke Bang National Park (PN-KB NP). Of 228 *Macaques*, 149 (65.4%) individuals successfully reintegrated into the wild. The prevalence and the antimicrobial resistance (AMR) profile of *Escherichia coli* (*E. coli*), *Salmonella*, and *Staphylococcus aureus* (*S. aureus*) isolates from *Macaques* rescuing in the Center were investigated. The fecal and nasal samples from 19 *Macaques* were collected. These samples were positive for *E. coli* (73.7%), *Salmonella* (36.8%), and *S. aureus* (57.9%). All of the tested bacterial strains showed 100% resistance to penicillin and vancomycin. The multi-drug resistant (MDR) profile was observed in *S. aureus* (71.4%), *E. coli* (95.3%), and *Salmonella* (100%). This is the first report on the rescue and natural reintegration of the *Macaque* species status in Vietnam and the prevalence of AMR in zoonotic bacterial pathogens isolated from these *Macaques*. This result indicated that AMR of the zoonotic bacterial pathogens could colonize in *Macaques* and may transmit to humans.

KEYWORDS

Antimicrobial resistant, Bacteria, *Macaque*, Rescue, Reintegration**INTRODUCTION**

Phong Nha-Ke Bang National Park (PN-KB NP) is located in the west of the Bo Trach district, Quang Binh Province, Vietnam. It is close to Hin Namno National Protected Forest in Laos. Many researchers have conducted studies on primate fauna in PN-KB NP (Workman, 2004; Haus-Maciej *et al.*, 2009; Hoàng, 2016). There were nine species including the red-shanked douc monkey (*Pygathrix nemaeus*), Hatinh langur (*Trachypitecus hatinhensis*), Southern white-cheeked gibbon (*Nomascus siki*), *Rhesus macaque* (*M. mulatta*), Stump-tailed *Macaque* (*M. arctoides*), Pig-tailed *Macaque* (*M. nemestrina*), and Long-tailed *Macaque* (*Macaca* (*M.*) *fascicularis*) (Haus-Maciej *et al.*, 2009). The diversity of nonhuman primates plays important roles in livelihoods, cultures, religions and offers unique insights into human evolution, biology, behavior, and the threat of emerging diseases. They are an essential component of tropical biodiversity, contributing to forest regeneration and ecosystem health. However, more than 60% of primate species are now threatened with extinction and more than 75% have declining populations (Federman *et al.*, 2016). The main threats to primate species are loss of habitat due to escalating anthropogenic pressures on primates, the expansion of industrial agriculture (Laurance *et al.*, 2014), large-scale cattle ranching, logging, oil and gas drilling (Vijay *et al.*, 2016), mining, dam building, and the construction of new road networks in pri-

mate range regions (Dobrovolski *et al.*, 2014). Other important drivers have increased the illegal hunting and trade of primates to use as pets, bushmeat, and traditional medicines; along with emerging threats, such as climate change and anthroponotic diseases (Estrada *et al.*, 2017).

Primates living in degraded forests are facing the risk of habitat loss (Gomez *et al.*, 2015), that leading to increased exposure to humans and domestic animals (Bublitz *et al.*, 2015). Primates can carry pathogens that may potentially transmit to human or domestic animals and vice versa (Karesh *et al.*, 2005). The close phylogenetic relationship between humans and other primates also creates an exceptionally high potential for pathogen exchange (Cooper and Nunn, 2013), as evidence that they have transmitted many diseases to humans (for example, outbreaks of Ebola and HIV/AIDS pandemic) (Calvignac-Spencer *et al.*, 2012). Zoonotic bacteria diseases such as *E. coli*, *Salmonella*, *Streptococcus* spp. and *S. aureus* are widely distributed on plants, soil, water, humans, and animals (McFall-Ngai *et al.*, 2013). These bacteria genera can cause serious morbidity and mortality in humans and animals (including nonhuman primates) (Whary *et al.*, 2015). While the studies on these bacteria in primates are limited (Xavier *et al.*, 2010). Additionally, the close interaction may increase the transmission of antibiotic resistant strains between humans and wild animals (Allen *et al.*, 2010). Goldberg *et al.* (2007) found that bacteria isolate from apes were genetically similar to those in

humans, who have interacted regularly with apes (eg. researcher, tour guide). This study indicates that shared habitat and human interaction with wild apes facilitate bacterial transmission and increasing AMR. So, further studies are needed to understand the burden and risks of AMR in primate populations with humans.

This study aimed to investigate the situation of rescue and natural reintegration of primates at the Center for Rescue, Conservation and Creature Development in PN-KB NP. In addition, to understanding the prevalence of *E. coli*, *Salmonella*, and *Staphylococcus aureus* in the primates, and the AMR profiles in these bacterial strains. To the best of our knowledge, this is the first report on the prevalence of the bacteria such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and the AMR profile of these bacterial strains in the rescued and natural reintegrated *Macaques* in Vietnam.

MATERIALS AND METHODS

Data collection

The data on the *Macaque* species receiving, rescuing, and reintegration into the wild between 2015 and June 2021 were collected. The data were obtained by reviewing documents, annual reports and monitoring reports at the Center for Rescue, Conservation and Creature development in PN-KB NP. The process of rescuing, nurturing and restoring wild behaviors was implemented according to the decree No. 06/2019/NĐ-CP of the Government of Vietnam (Government of Vietnam, 2019), and the guidelines in Decision No. 38/2016/QĐ-UBND dated November 29, 2016, of People’s Committee of Quang Binh Province (People’s Committee of Quang Binh Province, 2016).

Sampling

The samples were collected from all *Macaques* that were being raised while waiting for reintegration into the wild at the Center in 2021. These animal were immobilized by ketamine (100 mg/mL) (Flecknell et al., 2015) for rectal and nasal swabs collection. Rectal swabs were collected from the perirectal area and nasal swabs were collected from the nostrils. All samples were stored in Amies Transport Medium (M&G, Products Co. Ltd, JiangSu, China), and transported to the laboratory of Microbiology and Infectious diseases, the Faculty of Animal Science and Veterinary Medicine, Hue University of Agriculture and Forestry for microbiological analyses.

Bacterial isolation

E. coli was isolated from the rectal swab samples. The rectal

swabs were streaked onto plates of Eosin Methylene Blue agar (EMB, Conda Laboratories, S.A., Spain). One to three colonies on EMB agar from each sample were picked up and subcultured onto Tryptic Soy Agar (TSA, Difco, Detroit, MI, USA). The procedure of *E. coli* identification was carried out as previous described by (Hamner et al., 2007) using API 20E strips (Biomerieux, USA). Additionally, the *E. coli* isolates were reconfirmed using PCR primers specific for *E. coli* (Maheux et al., 2009).

Salmonella isolation and identification was performed as described by Yue et al. (2014). After enrichment in Tetrathionate Broth base (TTB, Thermo Scientific TM, UK) at 42°C for 24 h, the TTB enrichment broth was inoculated into Xylose-Lysine Deoxycholate agar (XLD, Merck, Darmstadt, Germany), and incubated at 37°C for 24 h. One to three suspected *Salmonella* colonies from each sample were picked up and subcultured onto TSA (Difco, USA) for further purification and identification. *Salmonella* identification was accomplished by oxidase test and biochemical tests with ATB ID 32E strips (bioMerieux, Marcy l’Étoile, France). Additionally, the *Salmonella* isolates were reconfirmed using PCR primers specific for *Salmonella* (Ziemer and Steadham, 2003).

S. aureus isolation and identification was performed as described by Akobi et al. (2012) with slight modification. Briefly, the enrichment was carried out in 3 mL of sterile Brain Heart Infusion Broth (BHI, Sigma, St. Louis, MO, USA), and incubated at 37°C overnight. And then, a loopful was streaked on Mannitol Salt Agar (MSA, Sigma, Saint Louis, Missouri 63103, USA) and incubated at 37°C for 48 h. Preliminary identification of *S. aureus* was based on positive Gram stain, and positive results for catalase, coagulase. Molecular identification and confirmation of the isolates were based on sequencing analysis of the hsp60 gene as previously reported (Kwok and Chow, 2003).

Antimicrobial susceptibility testing

The bacterial strains were examined for antimicrobial susceptibility with 10 antimicrobial agents (AMs). The agar disk diffusion method was carried out according to the standards of the Clinical Laboratory Standards Institute CLSI (2020). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 strains were used as control strains. Strains resistant to two or more antimicrobial agents were considered multidrug-resistant (MDR).

RESULTS

The situation of rescued *Macaque* species at the Center

From 2015 to June 2021, the Center Rescue, Conservation and Creature Development, PN-KB NP received a total of 228 *Macaques* from local people who voluntarily hand over and from

Table 1. *Macaque* species received by the Center from 2015 to June 2021.

Year	No. of individual	<i>M. arctoides</i>		<i>M. leonina</i>		<i>M. assamensis</i>		<i>M. mulatta</i>	
		No.	Ratio (%)	No.	Ratio (%)	No.	Ratio (%)	No.	Ratio (%)
2015	38	18	47.4	5	13.2	8	21.1	7	18.4
2016	28	8	28.6	6	21.4	7	25	7	25
2017	36	9	25	11	30.6	4	11.1	12	33.3
2018	44	11	25	14	31.8	3	6.8	16	36.4
2019	34	7	20.6	11	32.4	2	5.9	14	41.2
2020	29	10	34.5	5	17.2	2	6.9	12	41.4
2021	19	7	36.8	4	21.1	1	5.3	7	36.8
Total	228	70	30.7	56	24.6	27	11.8	75	32.9

state management agencies (forest rangers, customs office, border guards). Of 228 *Macaque* individuals, *M. mulatta* was the most predominant species (32.9%), followed by *M. arctoides* (30.7%), *M. leonina* (24.6%), and *M. assamensis* (11.8%). These individuals were fed, cared, nurtured and restored to wild behavior. A total 228 *Macaques* individual was rescued, in which 149 (65.4%) were successfully natural reintegrated (Table 2). The rate of natural reintegration changed by year, which were 89.5% in 2021, 75.0% in 2018, and 44.4% in 2017. The *M. mulatta* had the highest rate of reintegration (72.0%), followed by *M. arctoides* (65.7%), *M. leonina* (64.3%), and *M. assamensis* (48.2%).

The results isolation of bacteria and antimicrobial susceptibility

Among 19 fecal samples tested, 14/19 (73,7%) samples were *E. coli* positive, 7/19 (36.8%) were *Salmonella* positive (Table 3). Moreover, 11 out of 19 (57.9%) nasal swab samples were *S. aureus*, positive. A total 43 *E. coli* strains obtained from 14 positive fecal samples, 43/43 (100%) were resistant to at least one AM tested (table 5). Most of the strains were resistant to PEN (97.7%), followed by RFP (86.1%), and AMP (65.1%), whereas resistant to ST, and GEN was observed only in 13.9%, and 7.0% of the strains, respectively (table 4). Fifteen *Salmonella* strains were resistant to at least four to eight AMs tested. Resistant to RFP (100%), PEN (100%), and VAN (86.7%) was commonly observed, whereas most of the strains were susceptible to GEN and ST (93.3%). Out of 14 *S.*

aureus strains, there were 85.7%; 64.3%, and 57.1% strains resistant to PEN, RFP, VAN, and ERY, respectively. Most of the strains were susceptible to GEN (71.4%), CHL and ST (share 64,3%), and CP and TET (share 50.0%). Overview, all of the bacteria isolates were shown high frequency resistant to PEN and VAN. Moreover, all of the bacterial isolates were resistant to at least one to eight AMs. In which, 71.4% of *S. aureus*; 95,3% of *E. coli*; and 100% *Salmonella* isolates are MDR; in fact, 4 out of 14 (28.6%) *Salmonella* strains were resistant to seven AMs.

DISCUSSION

All of the macaque species that were received, rescued, and reintegrated into the wild between 2015 and June 2021 at the Center for Rescue, Conservation and Creature development in PN-KB NP are protected by Vietnamese law (Government of Vietnam, 2006). Additionally, these species are included in Viet Nam’s Red Data Book (Ministry of Science and technology, 2007) as “Vulnerable”. IUCN Red List Category: Vulnerable (VU) Criteria: A4cd. Although all *Macaques* are protected under the wildlife protection law, the main threat for the primates is poaching, mostly for the use in traditional medicine but also for food and for the pet trade (Nadler et al., 2007). Habitat destruction through firewood collection, logging, agriculture encroachment, road building, and forest fragmentation has also an increasing impact on the population decrease (Estrada et al., 2017). Most, if not all populations are already highly fragmented (Nadler et al., 2007). So that, more

Table 2. Results of natural reintegration for *Macaque* species at the Center.

Year	NR/RC ^a	Ratio (%)	<i>M. arctoides</i>		<i>M. leonina</i>		<i>M. assamensis</i>		<i>M. mulatta</i>	
			NR/RC	Ratio (%)	NR/RC	Ratio (%)	NR/RC	Ratio (%)	NR/RC	Ratio (%)
2015	23/38	60.5	13/18	72.2	5-Apr	80	8-Feb	25	7-Apr	57.1
2016	20/28	71.43	8-Jun	75	6-Mar	50	7-Jun	85.7	7-May	71.4
2017	16/36	44.4	9-Apr	44.4	11-May	45.5	4-Jan	25	12-Jun	50
2018	33/44	75	11-Sep	81.8	14-Sep	64.3	3-Feb	66.7	13/16	81.3
2019	20/34	58.8	7-Mar	42.9	11-Aug	72.7	2-Feb	100	14-Jul	50
2020	20/29	68.9	10-Apr	40	5-Apr	80	0/2	0	12-Dec	100
2021	17/19	89.5	7-Jul	100	4-Mar	75	0/1	0	7-Jul	100
Total	149/228	65.4	46/70	65.7	36/56	64.3	13/27	48.2	54/75	72

a NR: the number of individuals was natural reintegration; RC: the number of individuals has been received.

Table 3. Isolation of bacteria from rescued *Macaques* in the Center

Bacteria	No. sample	Positive samples	Ratio (%)
<i>Salmonella</i> sp.	19	7	36.8
<i>Escherichia coli</i>	19	14	73.7
<i>Staphylococcus aureus</i>	19	11	57.9

Table 4. AMR profiles of the bacteria isolates from rescued *Macaques* in the Center (%).

Antimicrobial agents	<i>S. aureus</i> (n=14)	<i>E. coli</i> (n=43)	<i>Salmonella</i> (n=15)
Chloramphenicol (CHL)	28.6	53.3	53.3
Penicillin (PEN)	85.7	100	100
Ampicillin (AMP)	35.7	80	80
Rifampin (RFP)	64.3	100	100
Tetracycline (TET)	28.6	53.3	53.3
Vancomycin (VAN)	64.3	86.7	86.7
Gentamicin (GEN)	14.3	0	0
Erythromycin (ERY)	57.1	20	20
Streptomycin (ST)	28.6	6.7	6.7
Ciprofloxacin (CP)	7.1	13.3	13.3

Table 5. The MDR of the bacteria isolates from rescued *Macaques* in the Center.

No. of antimicrobial agents	<i>S. aureus</i> (n = 14)		<i>E. coli</i> (n = 43)		<i>Salmonella</i> (n = 15)	
	No. strain	Cumulative rate (%)	No. strain	Cumulative rate (%)	No. strain	Cumulative rate (%)
1	4	100	2	100	0	
2	1	71.4	3	95.3	0	
3	0	64.3	8	88.4	0	
4	2	64.3	3	69.8	3	100
5	2	50	10	62.8	9	80
6	1	35.7	11	39.5	2	20
7	4	28.6	5	14	0	6.7
8	0		1	2.3	1	6.7

studies are necessary to improve knowledge the protection and to save the survival of several endangered species.

Generally, the AMR observed in this study is similar to previous studies on wild animals (Furness *et al.*, 2017; Grassotti *et al.*, 2018). The AMR isolates from wild primates are a matter of concern since these animals did not have a history of therapeutic antimicrobial exposure. The presence of resistant bacteria in wild primates emphasizes the role of human activities on the environment. In this study, the AMR strains were isolated from the *Macaques* rescued in the Center, they were received from many different sources. That means, they have been contacted by humans or domestic animals that may be a source of antimicrobial resistant strains. Previous studies investigated wild animals that live near a public hospital and water treatment plant and found that these environments sources of AMR strains in the wild animals (Grassotti *et al.*, 2018; Tang *et al.*, 2021).

Moreover, the AMR strains found in primates were derived from the gut microbial communities. In a study of (Tsukayama *et al.*, 2018) shown that AMR is an ancient feature of the intestinal microbial communities in primates and the sharing habitats with humans may have an important impact on the structure and function of this microbiota. Wildlife is not typically administered antimicrobial drugs but can acquire AMR bacteria through contact or shared resources with humans, domestic animals, and the environment. Proximity to humans has been associated with a higher prevalence of AMR in some wildlife populations, and exposure to antimicrobials from anthropogenic sources affects AMR in the gut bacteria of wild animals (Rwego *et al.*, 2008). In addition, wildlife may serve as sentinels of emerging resistant bacterial pathogens or AMR genes in the environment (Carroll *et al.*, 2015). In our study, most of the isolated strains were resistant at least to one or more drugs. The MDR strains have been isolated from wild and captive animals (Tang *et al.*, 2021). It is important to note that an elevated number of the MDR isolates from wild primates that live near human and domestic animals was detected.

CONCLUSION

This is the first report on the status of rescue and natural reintegration of the *Macaque* species in Vietnam and the prevalence of AMR in zoonotic bacterial pathogens isolated from these *Macaques*. From 2015 to 2021, a total of 228 individuals of four *Macaque* species have been rescued, and 149 out of 228 (65.4%) individuals have successfully reintegrated into the wild. The AMR of the zoonotic bacterial pathogens were found in the *Macaques*. Most *E. coli* strains were resistant to PEN, RFP, AMP, VAN, and ERY; *Salmonella* sp. strains were resistant to RFP, PEN, VAN, CHL, and TET; *S. aureus* strains resistant to PEN, RFP, VAN, and ERY; and *Streptococcus* sp. strains resistant to TET, VAN, ERY, CHL, AMP, and ST. Besides, the MDR was found in most of the bacterial strains isolated. This study therefore raises a warning about the risk of transmitting these pathogens from *Macaques* to

domestic animals or humans and vice versa.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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