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Phenotypic Characterization of Antibiotic-Resistant Pseudomonas Species in Habituated Gorillas (Gorilla Gorilla Gorilla) Of Moukalaba-Doudou National Park (Nyanga, Gabon)

Pierre Philippe Mbehang Nguema[1*], Anicet-Clotaire Dikoumba[2,3], Roméo Wenceslas Lendamba[4], Emelie Arlette Apinda Legnouo[1], Aimé Lionel Loïc Bekale Obame[1], Reine Véronique Ngolo Mbadinga[1], Christophe Roland Nzinga Koumba[1]

[1]*Institut de Recherche en Écologie Tropicale, Centre National de la Recherche Scientifique et Technologique (IRET/CENAREST), Microbiology Laboratory, PB : 13345, Libreville, Gabon.*

[2]*Department of Medical Biology, Hôpital d'Instruction des Armées Omar Bongo Ondimba (HIAOBO), Libreville.*

[3]*Department of Microbiology, Université des Sciences et techniques de la Santé (USS), Libreville.*

[4]*Centre de Recherches Médicales de Lambaréné (CERMEL), Department of Clinical Operations, PB : 242, Lambaréné, Gabon.*

Corresponding Author:** ***Pierre Philippe MBEHANG NGUEMA, *Institut de Recherche en Écologie Tropicale, (IRET/CENAREST), Microbiology Laboratory, PB: 13345, Libreville Gabon.*

Abstract: *This study was conducted following WHO recommendations, which advocate monitoring the emergence of specific resistance phenotypes, such as carbapenem-resistant Pseudomonas. This study aims to assess the prevalence of antibiotic-resistant Pseudomonas spp. in habituated gorillas within Moukalaba Doudou National Park (MDNP). Following bacterial coproculture on Cetrimide agar, 44 samples produced green colonies, all exhibiting the same characteristic aromatic odour and possessing oxidase activity. These colonies were identified as Pseudomonas spp., yielding a prevalence exceeding 50%. Biochemical identification using API galleries for non-enteric bacteria revealed the presence of Pseudomonas aeruginosa, Pseudomonas fluorescens, and other Pseudomonas spp. Antibiotic susceptibility testing demonstrated an overall resistance rate exceeding 50% (36/44). This resistance appears to be high, with prevalence varying between 4% and 75%. Specifically, resistance was observed in 75% (33/44) of isolates for ceftazidime, 63.6% (28/44) for ticarcillin-clavulanic acid, 54.5% (24/44) for tobramycin, 52.3% (23/44) for imipenem, 25% (11/44) for piperacillin-tazobactam, and 4.5% (2/44) for piperacillin alone. Furthermore, antibiotic susceptibility results indicated that two Pseudomonas spp. strains exhibited resistance to three different antibiotic classes. These findings confirm that Pseudomonas is an omnipresent genus in the environment, particularly within wildlife. However, our study revealed a high prevalence of resistance to β-lactam antibiotics (ticarcillin-clavulanic acid, imipenem, ceftazidime, and piperacillin-tazobactam), although the incidence of multidrug resistance was relatively low. Despite the high levels of acquired resistance, the presence of antibiotic-resistant Pseudomonas spp. in more than 50% of habituated gorillas in MDNP represents a significant potential risk to human health, particularly for individuals in direct contact with these primates. The intrinsic and acquired resistance of Pseudomonas spp. to multiple antibiotics further emphasises the urgent need for the development of new strategies to combat this microorganism.*

Keywords: *Moukalaba Doudou, Pseudomonas, gorillas, antibiotic resistance.*

1. Introduction

The genus *Pseudomonas*, within the family *Pseudomonadaceae*, comprises approximately 200 species, characterised by substantial metabolic variability [1]. *Pseudomonas* species are found in both soil and water and may act as colonisers or pathogens in plants, animals, and humans [2]. Due to its evolutionary history, the *Pseudomonas* genus is one of the most ecologically diverse and significant bacterial groups worldwide. Notably, its phototrophic nature, metabolic versatility, and capacity to withstand various stress conditions are defining features of its members [3].

Pseudomonas species are of medical importance, despite their variable frequency of isolation from clinical samples [4]. While most species predominantly inhabit environmental niches, some cause

diseases in humans and animals. *Pseudomonas aeruginosa* is currently the most significant pathogenic species within the genus, particularly in humans [5]. Other species, such as *Pseudomonas stutzeri* [6], *Pseudomonas fluorescens* [7], and *Pseudomonas putida* [8], have also been implicated in human and animal infections. Among Gram-negative bacteria, *Pseudomonas* species are associated with high morbidity and mortality rates due to their intrinsic ability to develop antibiotic resistance [9] and their potential to exhibit diverse multidrug resistance phenotypes [1].

In primates, *Pseudomonas* species have been identified in cynomolgus macaques, where they are linked to lipid digestion [10]. *Pseudomonas simae* has been reported as the causative agent of acute bronchopneumonia and bacteraemia in a captive Geoffroy's marmoset (*Callithrix geoffroyi*) [11]. *Pseudomonas aeruginosa* has been associated with chronic endobronchitis in rhesus macaques [12] and has also been isolated from chimpanzees (*Pan troglodytes*) [13,14] and orangutans (*Pongo pygmaeus*) [15].

From a human health perspective, monitoring animal health is crucial, as humans and animals coexist in shared environments, facilitating the exchange of pathogens [16]. In forest ecosystems, particularly in regions involving logging activities, wildlife tourism, and research, pathogens such as *Pseudomonas* may circulate between wildlife and human populations [17,18]. This underscores the relevance of the "One Health" approach, which recognises the interconnectedness of human, animal, and environmental health.

In Moukalaba Doudou National Park, located in southwest Gabon, several studies on great ape health have been conducted as part of the Biodiversity Conservation Project in Tropical Forests for Sustainable Human-Animal Coexistence (PROCOBHA) from 2009 to 2015. These studies identified antibiotic-resistant Gram-negative bacilli isolated from the faeces of western lowland gorillas [19-21]. The bacteria isolated primarily belonged to the *Enterobacteriaceae* family and exhibited resistance to β-lactams, aminoglycosides, quinolones/fluoroquinolones, tetracyclines, and sulfonamides [21]. However, *Pseudomonas* spp. had not yet been systematically monitored, although resistance phenotypes such as carbapenem-resistant *Pseudomonas* are actively monitored by the World Health Organization (WHO) [22]. Consequently, the present study aims to contribute to the surveillance of these pathogens to enhance human health protection under WHO recommendations.

The primary objective of this study is to assess the level of antibiotic resistance in *Pseudomonas* spp. isolated from habituated gorillas in Moukalaba Doudou National Park.

2. Materials and Methods

2.1. Study Framework

This study forms part of the health monitoring programme for habituated gorillas in Moukalaba Doudou National Park, undertaken by the bacteriology team at IRET. The objective is to understand the circulation of antibiotic resistance between humans, wildlife, domestic animals, livestock, bats, birds, and water sources. These studies commenced in 2007 as part of a collaborative initiative between IRET and the postgraduate programme at Kyoto University (Japan).

2.2. Biological Material, Target Animals, and Sample Collection Site

Faecal samples from gorillas were collected within Moukalaba Doudou National Park, located in Nyanga Province (Figure 1).

2.3. Sampling

Faecal sample collection was conducted in the forest between March and July 2024, during both the rainy and dry seasons, as part of the tracking of habituated gorillas (the Nidaï group). Fresh faeces (defecated no more than two hours prior) were sampled during direct observations of the gorillas in their habitats, specifically in the Boutsiana and Moukalaba areas (Figure 1). To prevent contamination, only uncontaminated portions of faeces were collected, avoiding contact with soil. Samples were stored in small plastic bags, shielded from direct sunlight. Upon arrival at the Doussala Research Station (a village on the park's periphery), the samples were transferred to plastic urine containers containing an alkaline phosphate-buffered saline (PBS) solution (70%) supplemented with glycerol (30%) and stored in a shaded environment within a protective container.

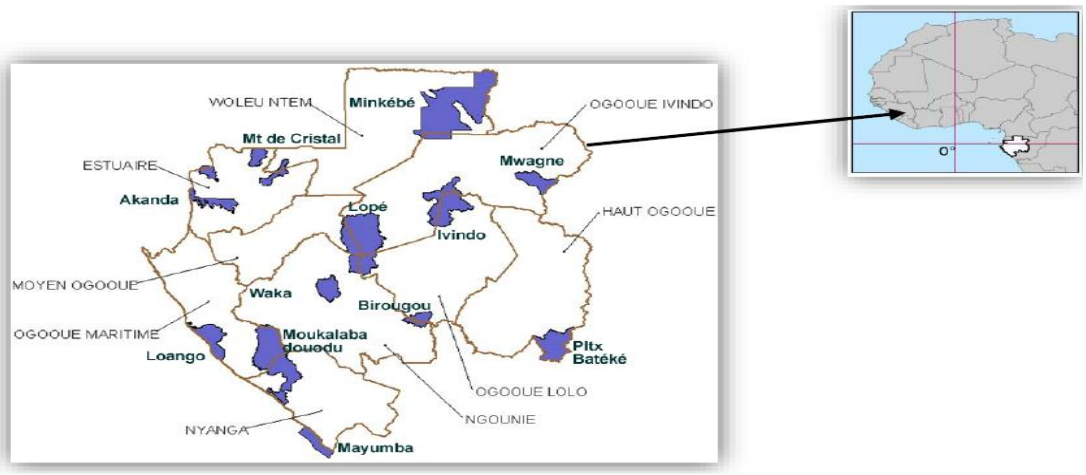

Figure 1. *Study sites of Gorillas (Source: IRET)*

2.4. Laboratory Analyses

2.5. Bacterial Culture

2.6. Bacterial Enrichment

A total of 100 gorilla faecal samples were collected. For each faecal sample, a faecal solution was prepared by mixing approximately 1g of faeces with 1 ml of phosphate-buffered saline solution. To enrich the bacteria, 100µl of gorilla faecal solution was mixed with 1.5ml of Cetrimide broth and incubated at 42°C for 48 hours.

2.7. Bacterial Culture and Identification Of Pseudomonas Colonies

Following 48 hours of incubation of the inoculated broths, 100µl from each tube was streaked onto Cetrimide agar plates using the streaking method and incubated at 37°C for 24 hours. The identification of *Pseudomonas* colonies was initially performed visually, selecting only green colonies (either dark or pale) that exhibited an aromatic odour. All these colonies tested positive for oxidase activity. Identification was further confirmed using biochemical methods with API Non-Enteric strips, followed by result interpretation via the ApiWeb software (BioMerieux, France).

2.8. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method [23]. The following antibiotics, commonly used to treat bacterial infections in local clinics, were tested: ceftazidime (30 µg), imipenem (10 µg), piperacillin-tazobactam (30 µg and 6 µg, respectively), ticarcillin-clavulanic acid (75 µg and 10 µg, respectively), tobramycin (10 µg), and ciprofloxacin (5 µg). The diameters of the inhibition zones were measured using a digital calliper (Carbon Fibre Composite Digital Caliper, France) and interpreted according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) 2023.

Interpretation of the inhibition zone diameters allowed classification into three categories: Susceptible (S), Intermediate (I), and Resistant (R). However, for this study, S and I were both considered Susceptible (S), resulting in only two phenotypic categories: Resistant (R) and Susceptible (S).

3. RESULTS

3.1. Identification of *Pseudomonas*

Following incubation of the inoculated Cetrimide agar plates, 44 out of 100 plates exhibited bacterial colonies. Identification of these colonies yielded the following results: *Pseudomonas* spp. (23; 52.3%), *Pseudomonas fluorescens* (12; 27.3%), and *Pseudomonas aeruginosa* (9; 20.5%).

3.2. General Antibiotic Resistance

As shown in Table 1, the overall antibiotic resistance exceeded 50% for the following antibiotics: ceftazidime (75%; 33/44), followed by ticarcillin-clavulanic acid (63.6%; 28/44), tobramycin (54.5%; 24/44), and imipenem (52.3%; 23/44), indicating high resistance levels. Resistance was lower for

piperacillin-tazobactam (25%; 11/44) and ciprofloxacin (4.5%; 2/44). These results suggest that ciprofloxacin and piperacillin-tazobactam remain effective antibiotics for infections caused by these *Pseudomonas* strains due to their low resistance frequencies (Table 1).

Table 1. *Overall susceptibility to antibiotics*

TTC		TPZ		IMP		CAZ		TOB		CIP	
R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
28	16	11	33	23	21	33	11	24	20	2	42

Figure 2 further confirms that resistance is highest for four antibiotics: ceftazidime, ticarcillin-clavulanic acid, tobramycin, and imipenem, all with resistance prevalence above 50%. In contrast, piperacillin-tazobactam and ciprofloxacin, with respective prevalence rates of 25% and 4.5%, appear to be the most effective against these bacterial strains.

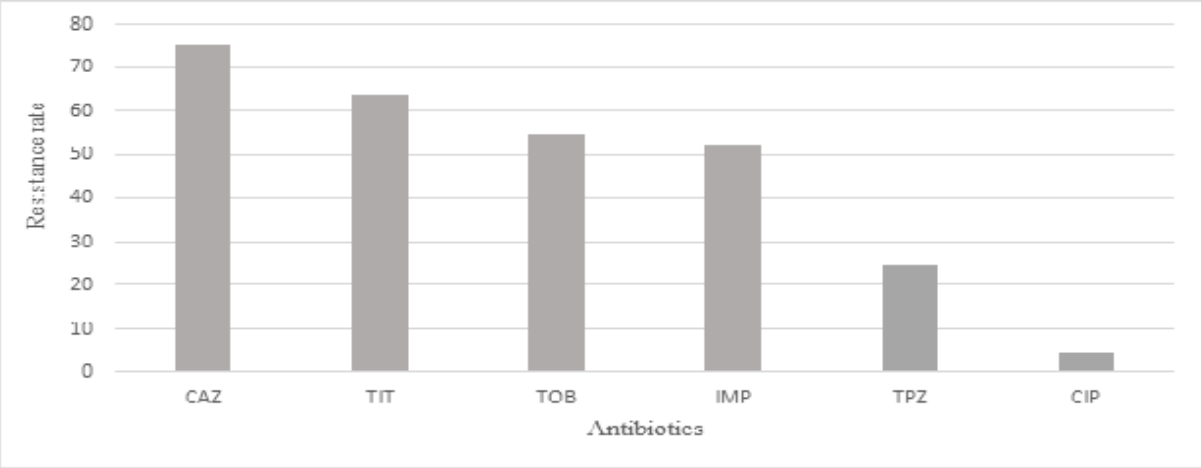

Figure 2. *Assessment of Resistance to Tested Antibiotics*

3.3. Antibiotic Resistance by Identified Species (Table 2)

Table 2 illustrates that antibiotic resistance is higher in *Pseudomonas fluorescens* compared to *Pseudomonas aeruginosa*. Notably, resistance to imipenem in *P. fluorescens* is nearly twice as high as in *P. aeruginosa*.

Table 2. *Antibiotic Resistance by Isolated Pseudomonas Species*

Bacteria	TTC	TPZ	IMP	CAZ	TOB	CIP
Pseudomonas aeruginosa	7 (15,9%)	2 (4,5%)	**4 (9,1%)**	6 (13,6%)	4 (9,1%)	0
Pseudomonas fluorescens	11(25%)	4 (9,1%)	**8 (18,2%)**	10 (22,7)	4 (9,1%)	1 (2,3%)
Pseudomonas spp	9 (20,4%)	5 (11,4%)	**11(25%)**	17 (38,6%)	16 (36,4%)	1 (2,3%)

3.4. Resistance Phenotypes (Table 3)

Table 3 presents the different resistance phenotypes observed following antibiotic susceptibility testing. The results indicate varying resistance profiles, ranging from resistance to a single antibiotic from one or more classes to resistance to multiple antibiotics from at least one class. It is important to highlight that three antibiotic classes were tested: β-lactams (TTC, CAZ, IMP, TPZ), aminoglycosides (TOB), and fluoroquinolones (CIP).

The findings reveal a low prevalence of multidrug resistance (4.5%; 2/44), defined as resistance to at least three antibiotics from three different families.

Table 3. *Resistance phenotypes*

Bacteria	Resistance phenotypes						Multidrug resistance(MDR)
Pseudomonas aeruginosa	TOB						
Pseudomonas aeruginosa	TOB						
Pseudomonas aeruginosa	TTC	CAZ					
Pseudomonas aeruginosa	TTC	CAZ					
Pseudomonas aeruginosa	TTC	CAZ					

Pseudomonas aeruginosa	TTC	IMP	TOB				
Pseudomonas aeruginosa	TTC	IMP	CAZ	TOB			
Pseudomonas aeruginosa	TTC	TPZ	IMP	CAZ			
Pseudomonas aeruginosa	TTC	TPZ	IMP	CAZ			
Pseudomonas fluorescens	TOB						
Pseudomonas fluorescens	TTC	CAZ					
Pseudomonas fluorescens	TTC	CAZ					
Pseudomonas fluorescens	TTC	IMP	TOB				
Pseudomonas fluorescens	TTC	IMP	CAZ				
Pseudomonas fluorescens	TTC	TPZ	IMP	CAZ			
Pseudomonas fluorescens	TTC	CAZ	TOB	CIP			MDR
Pseudomonas fluorescens	TTC	IMP	CAZ				
Pseudomonas fluorescens	TTC	TPZ	IMP	CAZ			
Pseudomonas fluorescens	TTC	TPZ	IMP	CAZ			
Pseudomonas fluorescens	TTC	TPZ	IMP	CAZ			
Pseudomonas fluorescens	TTC	IMP	CAZ	TOB			
Pseudomonas spp	CAZ						
Pseudomonas spp	CAZ						
Pseudomonas spp	CAZ						
Pseudomonas spp	TTC						
Pseudomonas spp	TTC						
Pseudomonas spp	TOB						
Pseudomonas spp	CAZ	TOB					
Pseudomonas spp	IMP	TOB					
Pseudomonas spp	IMP	TOB					
Pseudomonas spp	TTC	IMP	TOB				
Pseudomonas spp	TTC	CAZ	TOB				
Pseudomonas spp	TTC	CAZ	TOB				
Pseudomonas spp	TTC	TPZ	CAZ				
Pseudomonas spp	IMP	CAZ	TOB				
Pseudomonas spp	IMP	CAZ	TOB				
Pseudomonas spp	IMP	CAZ	TOB				
Pseudomonas spp	IMP	CAZ	TOB				
Pseudomonas spp	TTC	IMP	CAZ	TOB			
Pseudomonas spp	TTC	IMP	CAZ	TOB			
Pseudomonas spp	TTC	TPZ	IMP	CAZ			
Pseudomonas spp	TTC	TPZ	CAZ	TOB			
Pseudomonas spp	TTC	TPZ	CAZ	TOB			
Pseudomonas spp	TTC	TPZ	IMP	CAZ	TOB	CIP	MDR

4. DISCUSSION

Pseudomonas spp. are Gram-negative bacilli implicated in various human and animal infections [24]. This bacterium is intrinsically multidrug-resistant to several antibiotics belonging to different families [25, 26]. It may also exhibit both acquired and intrinsic (natural) multidrug resistance [25, 26], which complicates the treatment of diseases and infections it causes [26]. This resistance is often associated with high morbidity and mortality, particularly in hospital settings [27].

4.1. **Prevalence of Pseudomonas spp.**

The results of our study indicate a prevalence of 20.5% for *Pseudomonas aeruginosa* and 27.3% for *Pseudomonas fluorescens*. These prevalence rates are lower than the 50% prevalence of *Pseudomonas aeruginosa* reported by Mugisha et al. in chimpanzees (*Pan troglodytes*) [13]. Additionally, *Pseudomonas* spp. have been isolated in 10% of monkeys studied [28]. These findings confirm that this pyogenic Gram-negative bacillus is frequently found in primates, although its prevalence remains below 50%.

4.2. Antibiotic Resistance

Our results show a 4.5% prevalence of resistance to ciprofloxacin and resistance to β-lactams ranging from 25% to 75%. These findings are similar to those reported by Cristobal-Azkarate et al., who observed a 2% prevalence of ciprofloxacin resistance and aminoglycoside resistance in monkeys [28].

The same author also reported the production of extended-spectrum β-lactamase (ESBL) in *Pseudomonas* spp. isolates from monkeys [28]. This implies that such isolates would be resistant to all antibiotics within this class, except for carbapenems [29]. While our results align with these findings, no ESBL production was detected in our study.

Our study identified a 9.1% prevalence of carbapenem (imipenem) resistance among *Pseudomonas* isolates. This resistance level is lower than those reported in studies conducted on wild animals in 2024, where frequencies ranged from 15.7% to 51.8% [30, 31]. These findings suggest that carbapenem-resistant *Pseudomonas* can be found in animals [32], although their prevalence varies depending on the animals' habitat. Carbapenem resistance appears to be lower in animals residing in less human-influenced environments (as in our study) compared to those living in areas with greater human activity [33].

5. CONCLUSION

In summary, *Pseudomonas* is a bacterium present in humans, animals, and the environment. It exhibits both intrinsic and acquired resistance to multiple antibiotics from different families, which is concerning and highlights the urgent need for new strategies to combat this organism.

Our study identified *Pseudomonas* isolates, particularly *P. aeruginosa* and *P. fluorescens*, in western lowland gorillas, demonstrating a low prevalence (4.5%) of acquired multidrug resistance (MDR) and resistance to a carbapenem (imipenem). These findings suggest that wildlife could serve as a natural reservoir for MDR *Pseudomonas*, potentially posing a significant public health risk to individuals frequently in contact with these wild animals.

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