PSEUDOMONAS AERUGINOSA INFECTION ASSOCIATED WITH PNEUMONIA IN A DRILL MONKEY IN THE ZOOLOGICAL GARDEN, UNIVERSITY OF IBADAN: A CASE REPORT

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ABSTRACT

Pseudomonas aeruginosa, an important bacterium has been implicated in life-threatening conditions in animals. P. aeruginosa was indicated in transient and persistent lung infections in immunocompromised animals. Pseudomonas species are frequently associated with wildlife infections but are often not reported. In this study, a female drill monkey died 14 days after showing clinical signs of anorexia and nasal discharge. Vitamins B-complex and C were administered intramuscularly before her death. Postmortem examination and bacteriological analysis of necropsy samples indicated severe pneumonia caused by P. aeruginosa. The gums showed cyanosis of gums and oral mucous membrane. The cranial and middle lobes of the right lungs were consolidated. Enlargement and yellowish diffusion of the liver were observed. Multifocal petechial haemorrhages on small and large intestinal mucosa were observed. The histopathology revealed diffuse thickening of the interstitium due to hypercellular septae, congested capillaries, proteinaceous fluid and inflammatory cells. Alveolar cells were also hypertrophic with hyperplasia of type II pneumocytes, infiltration of inflammatory cells, and fluid exudation. This study showed the importance of surveillance for the incidence of pathogenic bacteria and virulence traits in captive animals.

Keywords: *Pseudomonas aeruginosa,* Pneumonia, Drill monkey, Zoological garden

INTRODUCTION

Pseudomonas species are common gram-negative bacillus found throughout the environment (Farver, 2008). *Pseudomonas* species possess many pathogenic and metabolic factors responsible for infecting a wide range of animals (Ozer *et al.,* 2014). *Pseudomonas* species infections are frequently associated with wildlife infections but are often not reported (Abd El-Ghany, 2021; Anipedia, 2024). The pathogenic potential and pervasive presence of the bacteria in the environment make its infection life-threatening in immunocompromised animals (Ozer *et al*., 2014).

Pseudomonas putida was responsible for multifocal areas of mild pulmonary oedema that led to per-acute endotoxic shock observed after post-mortem examination of the sudden post-anesthesia death of male Cynomolgous Macaque (*Macaca fascicularis*) (Matchett *et al*., 2003). *Pseudomonas simiae* was responsible for uraemic pneumonitis and acute broncho-interstitial pneumonia observed after a post-mortem examination of the sudden death of a female monkey and her offspring (Vela *et al.,* 2006). *P. aeruginosa* was indicated in severe fibrino-necrotic enteritis in a captive monitor lizard (*Varanus niloticus*) (Seixas *et al*., 2014). In immunocompromised animals, *P. aeruginosa* was indicated in transient and persistent lung infections (Sadikot *et al.,* 2005; Qin *et al*., 2022). The clinical classification of *P. aeruginosa* infections as acute and chronic infections exists, though the difference between the two groups remains unclear (Seixas *et al*., 2014). *P. aeruginosa* is the most common Gram-negative bacterium related to healthcare-associated respiratory infections (Reynolds and Kollef, 2021). *P. aeruginosa* showed higher death occurrence than other bacteria in healthcare-associated pneumonia in animals. This is caused by the ability of the bacteria to infect animals in immunocompromised conditions (Pachori *et al*., 2019; Reynolds and Kollef, 2021). The effects of the bacteria on these infections rest on its virulence, impairment of host defence system, and failure to treat the patients on time (Qin *et al*., 2022).

The high incidence of *P. aeruginosa* in infected animals may be due to the prevalence of the bacteria in the environment, soil, use of broad-spectrum antibiotics without antimicrobial resistance tests, level of degree of lung damage, and immunocompromised status of the animals (Lister *et al.,* 2009; Wood *et al*., 2023). Meanwhile, *P. aeruginosa* survives in many wildlife by adapting to the hosts' internal milieu which includes nutrient limitations, pH irregularities, antimicrobial exposure to soil bacteria, abuse of antibiotics used in zoo animals, and extreme temperatures. *P. aeruginosa* possesses a complement of genes to encode for the destruction of host defence mechanisms, wide nutrient availability pathways, and virulence characteristics (Qin *et al.,* 2023; Verdial *et al*., 2023). This clinical case study describes *P. aeruginosa* infection associated with pneumonia in a drill monkey that occurred in the Zoological Garden, University of Ibadan.

CASE REPORT

History: A female adult drill monkey from the Zoological Garden, University of Ibadan, showed clinical signs of anorexia and nasal discharge was reported by an Animal Technologist in the Zoo to clinicians on duty at the Veterinary Teaching Hospital, University of Obadiah on 7 July 2024. Vitamin B complex and C (IM) were administered on Thursday 10 July, 2024. The animal became hyporexic 2 days later. Intramuscular injections of vitamins B-complex and C were repeated on Monday 13 July 2024. It was totally weak on Saturday 20 July 2024, and 5% dextrose (S/C) and vitamin B-complex (IM) were administered. The animal died on the morning of Sunday 21 July 2024.

Necropsy Findings: The carcass was fresh and had a body condition score of 5/5. The gums and oral mucous membranes were cyanotic. The lungs were non-collapsed with rib imprints and approximately 50% of the cranial and middle lobes of the right lung were consolidated. Additionally, approximately 20% of the cranial lobe of the right lung was consolidated. There was abundant mesenteric fat. The liver was moderately enlarged and diffusely yellow. The spleen was mildly enlarged and meaty. There was a copious amount of mucus in the lumen of the small and large intestines and the mucosae had multifocal petechial haemorrhages. There were multifocal ecchymotic haemorrhages on the fascia and subcutis of the scalp. A haematoma was observed on the meninges.

Histopathologic Investigation: The harvested lung, preserved in 10% buffered formalin was subjected to dehydration under various concentrations of ethanol (70, 80, 90 and 100%), cleared in xylene and embedded in paraffin wax, mounted on blocks before sectioning using a microtome. Sectioning and staining with Hematoxylin and Eosin were done as described by Bancroft and Gamble (2008), and examined with the light microscope (Olympus CX21) at different magnifications. Photomicrographs were taken using a digital microscope camera, the Amscope 319CU CMOS Camera, and Micrometrics imaging software.

Microphotograph of the lungs showed moderately thickened interstitium (blue arrows) with congested capillaries, proteinaceous fluid and inflammatory cells, Hypertrophic alveolar cells, hyperplasia of type II pneumocytes and exudation of fluid (red arrows) in the alveoli (Figure 1).

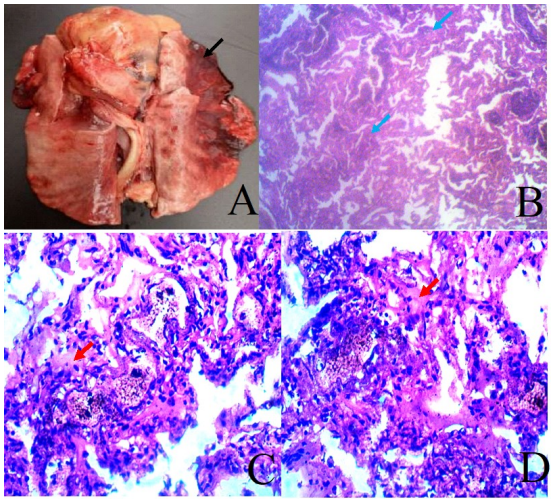

Figure 1: *Pseudomonas aeruginosa* infected lung of a drill monkey. *A) - grossly non-collapsed lungs with rib imprints and approximately 50% of the cranial and middle lobes of the right lung were consolidated (black arrows). B) - Micrograph of the lungs showing moderately thickened interstitium (blue arrows) with congested capillaries, proteinaceous fluid and inflammatory cells (H&E x100). C and D) - Hypertrophic alveolar cells, hyperplasia of type II pneumocytes and exudation of fluid (red arrows) in alveoli (H&E x400)*

Microbial Analysis: Small sections of the harvested lungs were cultured on nutrient agar (Mueller Hinton Agar and MacConkey Agar, HiMEDIA, USA). Agar plates were labelled and incubated for 24 hours at 37^0C. The bacterial isolates were identified morphologically and biochemically (oxidase, catalase, motility, gram staining, citrate, indole, glucose concentration, hydrogen sulfide production, lysine, ornithine, urease and methyl red using Urea Hydrolysis Test and Sugar Fermentation Test (Markey *et al.*, 2013).

The lungs streaked on the Mueller Hinton agar plate showed high growth of white convex colonies with greenish-blue pigment (Figure 2).

Figure 2: Colonies of *Pseudomonas aeruginosa* isolates associated with pneumonia on Mueller Hinton agar

The bacterial isolates were motile and were negative for methyl-red, hydrogen sulphide production, indole production, urea hydrolysis, coagulase, and ornithine decarboxylase. Furthermore, the bacterial isolates showed negative reactions for glucose, maltose, lactose, mannitol, xylose, sucrose, and arabinose (Table 1). The bacterial isolates were confirmed as *Pseudomonas aeruginosa.*

DISCUSSION

The virulence of the bacterium was exacerbated in an immunocompromised host where it is expertly adapted to thrive in a wide variety of environments, and in the process, becomes an opportunistic pathogen (Morin *et al.*, 2021).

Table 1: Morphological and biochemical characteristics of *Pseudomonas aeruginosa* isolates associated with pneumonia in a female drill monkey

Morphological and Biochemical Characteristics	*Pseudomonas aeruginosa* Isolate
Gram Staining Test	
Catalase	Positive
Oxidase	Positive
Motility	Positive
Methyl-red	Negative
Hydrogen sulphide production	Negative
Indole production	Negative
Citrate	Positive
Urea Hydrolysis Test	
Coagulase	Negative
Ornithine decarboxylase	Negative
Sugar Fermentation Test	
Glucose	Negative
Maltose	Negative
Lactose	Negative
Mannitol	Negative
Xylose	Negative
Sucrose	Negative
Arabinose	Negative

Pseudomonas aeruginosa expresses a wide array of virulence traits that are powerful manipulators and destroyers of host cells; however, it is clear that *P. aeruginosa* does not express all of these at all times. Probably the most important aspect of *P. aeruginosa* versatility is its ability to adapt to a new environment quickly and then adapt to prevailing endogenous and exogenous factors that allow it to thrive (Mathee *et al*., 2008). The challenges faced by a microorganism are the lining of the epithelial airway and a sink faucet filled with a waterborne environment. The capacity of an organism to alter its transcriptional profile swiftly to escape destruction by the host defence entails a composite system of environmental sensors. The signals like iron, pH, nutrients and temperature are responsible for the induction of a cascade of actions to prepare *P. aeruginosa* to live in the airway. The presence of *P. aeruginosa* triggers the adaptation of the bacterial infection changes to one of determination (Smith *et al*., 2006).

Lung infections from *P. aeruginosa* are found throughout the world in hospitals with ICU patients, cancer patients, and those with debilitating lung diseases or immunodeficiency. The spectrum of symptoms varies widely and this is almost exclusively a feature of the underlying deficiency either in the patient's host response or the virulence of the infecting *P. aeruginosa.*

The lung infections caused by *P. aeruginosa* led to sepsis and pneumonia, the bacteria achieved this by creating a surface and attaching to pili and fimbriae. After the binding process is established, *P. aeruginosa* releases toxins that damage the host cell lining and modify the integrity of the mucosa lining, thus the bacteria enter the bloodstream by traversing the epithelium (Williams *et al*., 2010). Moreover, *P. aeruginosa* secreted an extra-cellular matrix that created a biofilm encompassing a large colony of the bacterial isolates. Production of biofilm enabled the bacteria to evade antibiotic and phagocytosis effects from neutrophils. The bacteria located in the biofilm continued to establish itself in other organs of the affected patients (Déziel *et al*., 2001). *P. aeruginosa* may also be found in already infected lungs, established in thick, airways mucus, using purulent sputum as a surface for the formation of biofilm (Whitchurch *et al*., 2002; Moreau-Marquis *et al*., 2008; Bjarnsholt *et al.,* 2009). The bacteria can exist in the purulent sputum for years and initiate adaptation including secretion of virulence traits. Moreover, long-term adaptation of *P. aeruginosa* is possible by the mutability of its genome while in the biofilm leading to bacteremia and pneumonia (Boles *et al*., 2005; Smith *et al*., 2006).

Conclusion: *Pseudomonas aeruginosa* infection was responsible for pneumonia in a female adult drill monkey in the Zoological Garden which resulted in the sudden death of the monkey after a brief illness.

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