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Pandrug-Resistant *Pseudomonas Aeruginosa* Isolated from a 4-Year-Old Indigenous Horse with Respiratory Distress: Current AMR Challenges

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ABSTRACT
Respiratory diseases in equids are frequently complicated by opportunistic Gram-negative pathogens capable of biofilm formation, which shields them from both host immunity and therapeutics. This report details the isolation of a pandrug-resistant (PDR) *Pseudomonas aeruginosa* strain from a 4-year-old indigenous Arewa horse with respiratory distress and bilateral nasal discharge in Zaria, Nigeria. Standard microbiological processing of nasal swabs at the AMR Sentinel Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria yielded non-lactose fermenting, gram-negative, motile bacilli that were oxidizers and phenotypically identified as *P. aeruginosa*. *In vitro* antimicrobial susceptibility testing via the Kirby-Bauer disc diffusion method, interpreted using CLSI (2026) guidelines, revealed total resistance across all tested pharmacological classes of antimicrobials. The isolate demonstrated clinical resistance to Ertapenem, Ampicillin, Nalidixic acid, Ceftizoxime, Tetracycline, and Sulphamethoxazole-trimethoprim, and intermediate resistance to Streptomycin, confirming its PDR classification. With resistant *P. aeruginosa* already driving fatal healthcare-associated infections in Nigerian hospitals, this interspecies dissemination underscores a critical breakdown in antimicrobial stewardship, demanding urgent epidemiological surveillance and biosecurity interventions.

Keywords: Horse; Pandrug-resistance; *Pseudomonas aeruginosa*; Respiratory distress

INTRODUCTION

Equine respiratory diseases, particularly those caused by highly contagious pathogens, represent a significant challenge in veterinary medicine worldwide, due to their ability to cause substantial economic losses and reduced performance (Olguin-Perglione & Barrandeguy, 2021). While primary viral and bacterial pathogens are well-documented, opportunistic gram-negative bacteria increasingly complicate underlying respiratory problems. Among these, *Pseudomonas aeruginosa* is a ubiquitous, saprophytic organism capable of causing severe and refractory deep tissue infections, including pneumonia and pleuropneumonia, particularly in immunocompromised individuals or those with compromised mucosal barriers (Wood *et al.*, 2023). The management of *P. aeruginosa* is inherently difficult due to its intrinsic resistance to various antimicrobial classes, its outer membrane permeability barriers, and its exceptional capacity to form biofilms, which shield the bacteria from both host immune responses and conventional therapies (Pottier *et al*., 2022; Wood *et al.*, 2023).

The rapidly escalating global crisis of antimicrobial resistance (AMR) has further compounded the clinical management of pseudomonal infections. In both human and veterinary medicine, While Multidrug-resistant and extensively drug-resistant strains of *P. aeruginosa* are increasingly reported in companion animals and livestock (Pottier *et al.*, 2022), the emergence of PDR strains remains exceedingly rare in veterinary practice. The isolation of a PDR strain signifies an absolute therapeutic dead-end, rendering standard clinical protocols ineffective and posing a profound biosafety risk.

From a One Health perspective, the documentation of PDR pathogens in indigenous livestock and working equids is of critical epidemiological importance. Indigenous horses in developing regions often live in close proximity to human communities and other domestic animals, serving as potential reservoirs for highly resistant genetic lineages. The selection pressure driven by the empirical use of antimicrobials in agriculture and livestock management is a primary driver for the selection of these superbugs, demonstrating that AMR crosses geographical and species boundaries (Velazquez-Meza *et al.*, 2022). Consequently, characterizing the clinical presentation and diagnostic timeline of PDR infections in these populations is essential for mapping the dissemination of extreme resistance traits across the animal-human-environmental interface.

MATERIALS AND METHODS

Case Presentation

A 4-year-old Arewa horse weighing 194 kg was presented to the Large Animal Clinic of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria on the 1st of June 2026 with complaint of off-feed and nasal discharge. History revealed that the horse was presented to the clinic earlier for wound dressing.

Case Management

Sample Collection

Nasal swab samples were collected using sterile swab sticks and transported on ice to the AMR Sentinel Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for

bacterial isolation, identification and antimicrobial susceptibility testing.

Sample Processing

The nasal swab sample was processed according to the standard bacteriological methods (Markey *et al.* 2013). Briefly, the nasal swab was directly smeared over the surface of both the 5% sheep blood and MacConkey agar to make a "well" and using a Pasteur loop, a quadrant streaking pattern were made. All the inoculated plates were incubated aerobically at 37°C for 24-48 hours and further sub-cultured into appropriate media to obtain pure colonies. The colonies observed from the nasal cavity swab on MacConkey agar plate were non-lactose fermenter revealing a typical colorless appearance, while on the blood agar the colonies were small, round, raised, greyish and β-haemolytic.

Microscopy and Biochemical Identification

The isolates were gram stained and microscopically viewed using a 100x objective lens according to the standard method (Markey *et al*., 2013). The colorless non-lactose fermenting and β-haemolytic colonies from MacConkey and blood agar, respectively were found to be microscopically gram-negative bacilli. This was further confirmed using 3% KOH (String test) and the isolates were subjected to Indole, Citrate, Oxidative and fermentative (O-F), Triple sugar iron, Motility, Methyl red, Voges-Proskauer tests.

Antimicrobial Susceptibility Profile of the Pseudomonas Aeruginosa

The Kirby-Bauer disc diffusion method was used in assessing the antimicrobial susceptibility profile of the phenotypically identified *P. aeruginosa* isolate on Muller-Hinton agar (MHA). Briefly, an overnight culture of the isolate was prepared and standardized according to 0.5 MacFarland standard, the inoculum was lawned on MHA, allowed to dry for about 10 minutes. Using a sterile thumb forceps, the following antibiotics from Oxoid (UK) were used; Ertapenem (10µg), Ampicillin (10µg), nalidixic acid (30µg), Streptomycin (10µg), Cefotaxime (30µg) Tetracycline (30µg), Sulphamethoxazole-trimethoprim (25µg; 23.75µg/1.25µg), and the zone of inhibition were measured using a transparent ruler (Fig. 2) and the results were interpreted according to CLSI (2026) guidelines.

RESULTS AND DISCUSSION

The bacterium isolated from the Arewa horse with respiratory distress was identified phenotypically as *Pseudomonas aeruginosa* based on its colony morphology and biochemical characteristics. The organism was a gram-negative bacillus and produced a positive 3% KOH reaction, confirming its gram-negative nature. It exhibited an alkaline/no change (K/NC) reaction on Triple Sugar Iron (TSI) agar, indicating that it did not ferment glucose, lactose, or sucrose. The isolate was motile, citrate-positive, indole-negative, methyl red-negative, and Voges-Proskauer-positive. In the oxidative-fermentative (O/F) test, the organism demonstrated an oxidative pattern of glucose metabolism, which is consistent with the biochemical characteristics of *P. aeruginosa* (Fig. 1; Table 1).

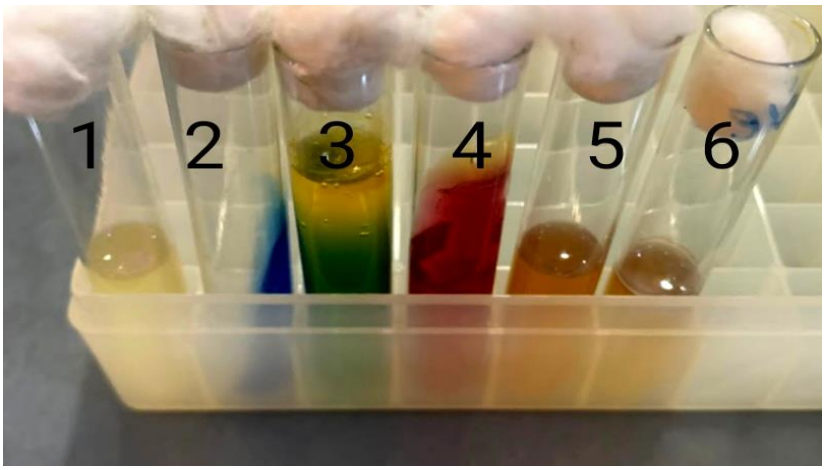

Figure 1: Biochemical test results showing 1= Indole negative, 2= Citrate positive, 3= Oxidizer, 4= Alkaline/no change, 5= Methyl red negative, and 6= Voges-Proskauer negative

Table 1: Phenotypic Identification of *Pseudomonas Aeruginosa* Isolated from the Arewa Horse with Respiratory Distress

Tests	Results
Gram's reaction	Gram negative bacilli
3% KOH	Positive
Triple sugar iron	Alkaline/no change
Motility	Motile
Citrate	Positive
Indole	Negative
Methyl red	Negative
Voges Proskauer	Positive
Oxidative and fermentative(O-F)	Oxidizer

The antimicrobial susceptibility test results revealed that the *P. aeruginosa* isolate was resistant to ertapenem (10 µg), ampicillin (10 µg), nalidixic acid (30 µg), cefotaxime (30 µg), tetracycline (30 µg), and sulphamethoxazole-trimethoprim (25 µg). The isolate exhibited intermediate susceptibility only to streptomycin (10 µg), with an inhibition zone diameter of 15 mm. None of the antimicrobial agent tested demonstrated complete susceptibility against the isolate, indicating a pandrug-resistant phenotype (Fig. 2; Table 2).

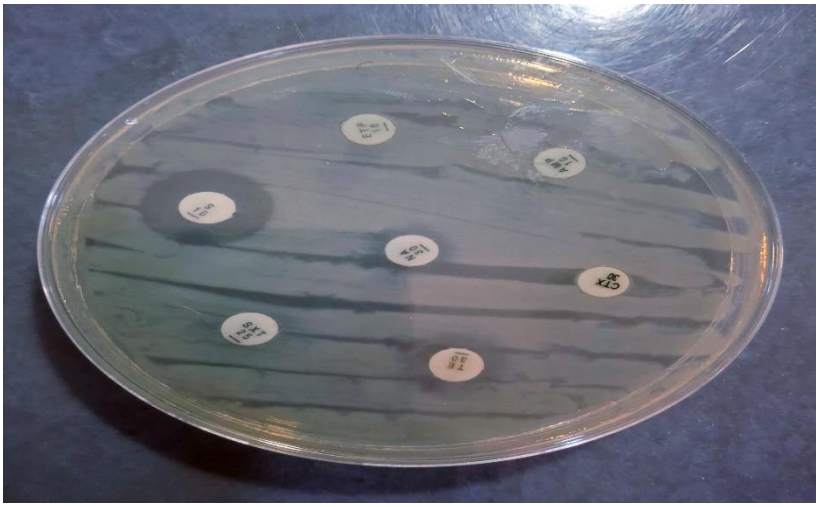

Figure 2: Antimicrobial susceptibility test result showing different susceptibility profile of the *Pseudomonas aeruginosa* isolated from the Arewa horse

Table 2: Antimicrobial Susceptibility Profile of the Isolated *Pseudomonas Aeruginosa*

Isolate	ETP (10µg)	AMP (10µg)	NA (30µg)	S (10µg)	CTX (30µg)	TE (30µg)	SXT (25µg)
Pseudomonas aeruginosa	6 (R)	6 (R)	6 (R)	15 (I)	6 (R)	11 (R)	6 (R)

S = susceptible, R = resistance, I = intermediate, ETP = Ertapenem, AMP = Ampicillin, NA = Nalidixic acid, S = Streptomycin, CTX = Cefotaxime, TE = Tetracycline and SXT = Sulphamethoxazole-trimethoprim.

Discussion

The isolation of a purely PDR *P. aeruginosa* is exceedingly rare in veterinary literature, previous studies predominantly report multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas* species (Pottier *et al.*, 2022). The pandrug-resistant *P. aeruginosa* isolated from this case presents a critical therapeutic challenge in equine infectious diseases. Furthermore, *P. aeruginosa* has been reported from various equine clinical conditions involving the respiratory and other body systems, where it often causes chronic and difficult-to-treat infections (Pottier *et al.*, 2022; Yang *et al.*, 2026). In equine clinical practice, respiratory infections driven by extensively resistant strains are notoriously refractory to standard therapies, frequently leading to chronic respiratory distress, prolonged pathogen shedding, and a poor clinical prognosis (Abdullahi *et al*., 2025).

The profound resistance exhibited by this isolate is driven by a complex interplay of intrinsic and acquired biological mechanisms. *P. aeruginosa* naturally possesses a highly impermeable membrane and constitutively expresses Mex-type efflux pumps and chromosomal AmpC β-lactamases, which collectively expel or degrade a wide array of antibiotics (Elfadadny *et al.*, 2024).

This PDR isolate from an Arewa horse, a breed native to Northern Nigeria indicates a highly refractory upper respiratory infection, likely complicated by poor environmental hygiene or the chronic misuse of broad-spectrum antibiotics. Because Arewa horses are handled closely for sporting and cultural events, the emergence of PDR *P. aeruginosa* in the local equine population represents a severe public health threat that necessitates a One Health response. Horses and veterinary facilities can act as silent amplification reservoirs for high-risk nosocomial clones, facilitating the persistence and dissemination of resistance genes in shared agricultural and clinical environments (Amsalu *et al.*, 2020). With highly resistant *P. aeruginosa* already recognized as a critical pathogen driving fatal, healthcare-associated infections in Nigerian human hospitals, its unchecked circulation in local livestock and environment highlights a dangerous breakdown in antimicrobial stewardship that threatens both animals and human healthcare efficacy (Akinloye *et al.*, 2021; Jibola-Shittu *et al.*, 2025).

CONCLUSION

This case report describes the clinical presentation, diagnostic investigation, and therapeutic challenges associated with a pandrug-resistant *Pseudomonas aeruginosa* isolation from a 4-year-old indigenous horse with respiratory distress. The report represents one of the few documented cases of absolute Pandrug-Resistance in an indigenous equid, highlighting a critical shift in the local epidemiology of antimicrobial resistance since PDR isolates represent an absolute therapeutic dead-end for conventional antibiotics. Improvement in environmental hygiene conditions in the horse stable and prompt reportage of cases to the veterinary hospital is therefore recommended.

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