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Assessment of antibiogram profiles in *Pseudomonas* isolates from canine otitis externa

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Abstract
This study focused on assessing the antibiogram profiles of *Pseudomonas* isolates obtained from canine otitis externa cases. A total of 263 dogs, spanning various ages and breeds, were examined at the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Anjora, Durg, as well as several Government Veterinary hospitals and Private Pet Clinics in the Durg vicinity. Through clinical examination, 52 dogs were identified with otitis externa, and among them, 19 exhibited otitis externa attributed to *Pseudomonas* spp. These specific cases were selected for an in-depth analysis of their antibiogram profiles. The results of the antibiogram assay revealed that imipenem exhibited the highest sensitivity (100%), followed by piperacillin-tazobactam (84.2%), while amikacin and ceftriaxone displayed the lowest sensitivity (5.2%).

Keywords: Antibiogram, *Pseudomonas*, canine, otitis externa, Chhattisgarh

Introduction
Sensorineural hearing disorders in canines, encompassing both biological factors such as otitis externa and physical causes like noise-induced trauma, pose significant challenges as they are often considered incurable. Otitis externa, characterized by inflammation of the external ear canal, stands out as a prevalent clinical ailment, affecting a substantial portion of the canine population, ranging from 5 to 20% (Rougier *et al.*, 2005) [8]. This multifactorial chronic or recurrent illness has been reported with a prevalence as high as 20% (Korbelik *et al.*, 2019) [3], indicating its widespread impact on canine health.

The etiology of otitis externa in dogs can be attributed to various factors, including genetic predisposition or underlying health conditions. Despite advances in antimicrobial treatments, the recurrence of otitis externa remains a challenge, emphasizing the need for a comprehensive understanding of the microbial agents involved. Microbiological cultures have proven instrumental in isolating and identifying numerous bacteria associated with this disorder (Bradley *et al.*, 2020) [1].

Clinical manifestations of otitis externa in dogs commonly include head shaking, erythema, ear canal discharge, scratching, pain upon palpation, ear rubbing, and a noticeable tilt of the head. *Pseudomonas* aeruginosa, a frequently identified pathogen, is notably implicated in both middle and external otitis. Its presence often leads to erosions, ulcers, and the production of substantial quantities of light-yellow secretions, contributing to a chronic and recurrent disease course. Understanding the antibiogram profiles of *Pseudomonas* isolates in canine otitis externa cases becomes crucial in developing effective treatment strategies and mitigating the impact of this debilitating condition on canine auditory health.

Materials and Methods
Sample Collection
This research was conducted on dogs brought to the Teaching Veterinary Clinical Complex at the College of Veterinary Science and Animal Husbandry, Anjora, Durg, as well as various Government Veterinary hospitals and Private Pet Clinics in the Durg region. Sterilized swabs were carefully introduced into the intersection of the vertical and horizontal external ear canal to procure samples of ear exudates. Concurrently, detailed patient information, encompassing owner complaints and clinical symptoms, was meticulously recorded. Observable manifestations included ottorrhoea, with pus discharges ranging from yellowish-brown to white purulent discharges, alongside indicators such as restlessness and frequent ear itching.

Isolation and Identification of *Pseudomonas aeruginosa* from Ear Swab Samples

A comprehensive screening of 263 ear swab samples from dogs was conducted to isolate *Pseudomonas aeruginosa* bacteria. The primary screening involved the inoculation of all samples into *Pseudomonas* selective Cephalothin-Sodium Fusidate-Cetrimide (CFC) broth, followed by plating on *Pseudomonas aeruginosa* agar supplemented with CetriNix. Following a 24-hour incubation period at 37 °C on selective media, the growth was meticulously observed and characterized by distinct pigment production, ranging from green, blue, yellow, brown, to cream colors (Plate III). Nineteen pure cultures exhibiting blue, green, and brown pigmentation on both *Pseudomonas* agar and Nutrient agar were provisionally identified as *Pseudomonas aeruginosa* through morphological assessment and Gram's staining, revealing pink-colored, medium-sized gram-negative bacilli.

The isolation process, phenotypic identification, and biochemical analysis for *Pseudomonas* were executed following the methodology outlined by Penna *et al*., (2011) [7], and Park *et al*., (2020) [6], with slight modifications to suit the laboratory conditions. Consequently, the findings affirm that the recommended method for *Pseudomonas aeruginosa* isolation involves selective enrichment in CFC broth, succeeded by isolation on *Pseudomonas* agar. The primary criteria for identification, post-selective isolation, encompass phenotypic colony characteristics and biochemical testing.

Antibiotic Susceptibility Test

Antibiogram Analysis of *Pseudomonas aeruginosa* Isolates

In accordance with the Clinical and Laboratory Standard Institute guidelines, antimicrobial susceptibility testing (AST) was conducted on *Pseudomonas aeruginosa* isolates. The Kirby-Bauer disc diffusion method was employed, utilizing antibiotic discs outlined in Table 1 and depicted in Figures 1.

Table 1: Antimicrobial Agents for *Pseudomonas aeruginosa* Susceptibility Testing

Test group	Antimicrobial	Concentration (µg)
Penicillin	Piperacillin	100
B-lactam/β-lactamase inhibitor combinations	Piperacillin-tazobactam	100/10
	Ticarcillin-clavulanate	75/10
	Amoxicillin-clavulanate	10
	Ceftriaxone-tazobactam	30/10
Cephems (parenteral)	Ceftazidime	30
	Cefepime	30
	Ceftriaxone	10
Carbapenems	Meropenem	10
	Imipenem	10
Aminoglycosides	Gentamicin	10
	Amikacin	30
Fluoroquinolones	Ciprofloxacin	5
	Ofloxacin	5
	Enrofloxacin	10

Concentrations are given in micrograms (µg)

Result and Discussion

In accordance with the results obtained from the antibiogram, the *Pseudomonas aeruginosa* isolate was classified as sensitive, intermediate-resistant, or resistant, as outlined in Table 2.

Table 2: Drug resistance pattern of *Pseudomonas* isolates

Antimicrobial Agent and Sample Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Piperacillin	S	I	R	I	I	S	R	S	S	S	S	I	R	S	S	I	R	S	S
Ticarcillin-Clavulanate	S	I	I	S	I	I	I	I	I	I	I	I	I	I	S	I	I	I	I
Piperacillin-Tazobactum	S	S	S	S	S	S	I	S	S	S	S	S	I	S	S	S	I	S	S
Amoxicillin-Clavulanate	I	S	I	S	S	R	I	S	I	R	S	I	S	S	I	S	S	S	I
Ceftriaxone-Tazobactum	I	I	I	S	R	S	R	S	S	I	I	S	I	R	I	I	I	I	S
Ceftazidime	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	S	R	R
Cefepime	I	I	I	R	I	S	R	R	R	I	R	I	S	I	I	I	S	R	R
Ceftriaxone	R	I	I	R	R	I	R	S	I	R	I	R	I	I	R	I	I	I	I
Meropenem	I	I	R	R	R	S	R	S	I	R	I	R	I	R	I	I	I	I	I
Imipenem	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Gentamicin	S	S	S	S	S	S	S	R	S	S	S	S	R	I	S	S	R	S	S
Amikacin	I	R	R	R	R	S	R	R	I	I	R	I	I	R	I	R	I	R	I
Ciprofloxacin	I	I	I	I	I	S	I	I	I	I	I	S	I	I	I	I	I	I	I
Ofloxacin	R	R	R	R	R	S	R	S	R	R	R	I	R	I	R	R	R	R	R
Enrofloxacin	I	R	S	I	I	S	I	S	I	I	I	S	I	S	I	R	I	I	I
MAR index	0.2	0.27	0.34	0.4	0.4	0.06	0.53	0.27	0.2	0.33	0.27	0.2	0.2	0.26	0.2	0.26	0.2	0.26	0.2

Comprehensive Antibiotic Sensitivity Analysis of *Pseudomonas* Isolates

The results of antibiotic susceptibility testing for all *Pseudomonas* isolates (n=19) are presented in Table 2 and Fig 2.

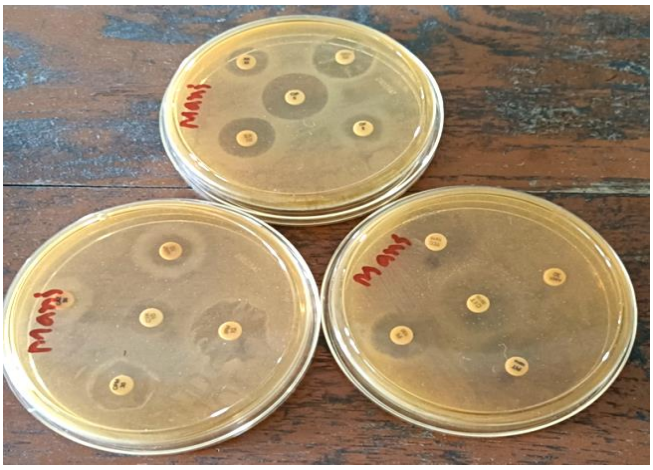

Fig 1: Illustrating the Antibiotic Sensitivity Test of Pseudomonas Isolates

Notably, the analysis revealed that *Pseudomonas* isolates exhibited the highest sensitivity to imipenem (100%), followed by piperacillin-tazobactam (84.2%). Conversely, the lowest sensitivity was observed for amikacin and ceftriaxone, both registering at 5.2%.

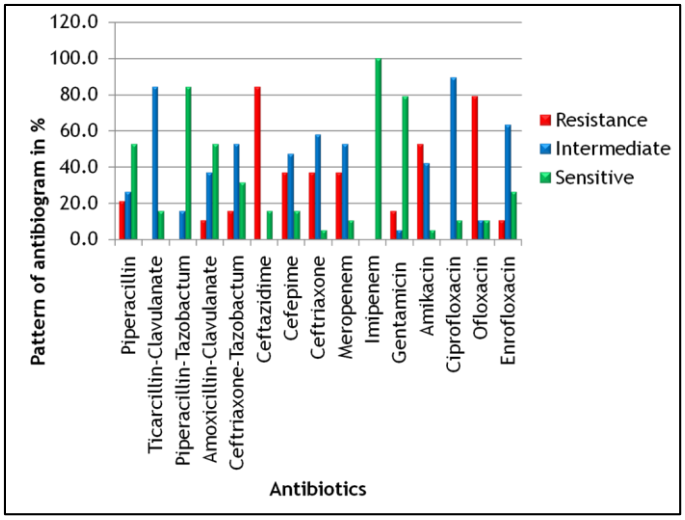

Fig 2: Pattern of antibiogram

The *Pseudomonas* isolates demonstrated notable resistance patterns, with Ceftazidime being the most resisted antibiotic (84.2%), followed by Ofloxacin (78.9%), Amikacin (52.6%), Meropenem (36.8%), Ceftriaxone (36.8%), and Cefepime (36.8%). Conversely, the highest proportion of *Pseudomonas* isolates exhibited intermediate sensitivity to Ciprofloxacin (88.9%), Ticarcillin (84.2%), Enrofloxacin (63.15%), Ceftriaxone (57.8%), and Cefepime (47.3%). In contrast, minimal intermediate sensitivity was observed for Ceftazidime (0%) and Imipenem (0%).

These findings align with the study by Lin *et al.* (2012) [4], which highlighted imipenem's potent anti-*Pseudomonas* activity, emphasizing its efficacy against canine isolates. Imipenem, a derivative of N-formimidoyl thienamycin, demonstrated superior antibacterial properties, inhibiting peptidoglycan cross-linking during cell wall synthesis through the inactivation of penicillin-binding proteins (PBPs), ultimately leading to bacterial cell lysis and death. Notably, the present study also identified resistance among *Pseudomonas* isolates to fluoroquinolones, aminoglycosides (amikacin and gentamicin), and ceftazidime.

Comparisons with studies from the USA (Beir *et al.*, 2014) [2] indicate similar resistance levels against Ciprofloxacin (5%) and Gentamicin (9%) in isolates from various animal species. Consistent with Odumosu *et al.* (2016) [5], our study revealed high-level resistance (100%) to ceftazidime among *Pseudomonas* aeruginosa isolates. Serrano *et al.* (2016) [9] reported varying resistance rates in *Pseudomonas aeruginosa* isolates from animals, with higher resistance to ciprofloxacin (15.1%), piperacillin-tazobactam (12.3%), and ticarcillin-clavulanic acid (17.2%), while lower resistance was observed for cefepime (9.6%), ceftazidime (2.7%), imipenem (1.4%), gentamicin (12.3%), and meropenem (1.4%).

Interestingly, our clinical isolates demonstrated low resistance (4.5%) to cefepime, imipenem, and piperacillin-tazobactam. This sustained susceptibility of *Pseudomonas aeruginosa* to imipenem and other antibiotics suggests a potential positive outcome attributed to the deliberate and prudent use of antibiotics in animals over an extended period.

In our investigation, fourth and third-generation cephalosporins (Ceftazidime and Cefepime) exhibited elevated resistance among *Pseudomonas* aeruginosa strains in dogs, possibly due to frequent and, to some extent, indiscriminate use of these antibiotics in clinical practice. The widespread use of new antibiotics in veterinary practice raises concerns for public health, emphasizing the urgency of monitoring and regulating antibiotic usage, especially as only a limited number of drugs (Imipenem and Piperacillin) have demonstrated sensitivity against extensively or multi-drug resistant *Pseudomonas* isolates.

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