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Isolation, Sensitivity, and Tolerance of *Pseudomonas aeruginosa* from Canine Otitis

Neeraja E [a], Anju JR [a], Shafi Muhammed Kunnuchalil [b], Lija Joy [b], Deepa PM [a], Janus A [a], Bipin KC [a], Archana Chandran [c] and Rathish RL [a*]

[a] Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Pookode, Wayanad, India.
[b] College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India.
[c] College of Dairy Science and Technology, Pookode, Wayanad, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to detect Pseudomonas from cases of canine otitis and profile their antibiotic sensitivity pattern.
Study Design: We collected samples aseptically. Antibiogram was done by disc diffusion test. The broth microdilution method was applied for biocide tolerance against chlorhexidine (CHX) and Cetyl Trimethyl ammonium bromide (CTAB).
Place and Duration of Study: The study period was from March 2023 to March 2024. Samples(n=28) collected were by veterinarians treating the dogs presented at Teaching Veterinary

**Corresponding author: E-mail: rathish@kvasu.ac.in;*

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Clinical Complex Pookode and Peripheral Veterinary Clinics, Kakkavayal, Wayanad District, Kerala, India and submitted to the laboratory.
Methodology: *P. aeruginosa* was identified based on colony characteristics and biochemical tests. Antibiotic sensitivity was estimated by a disc diffusion on Muller Hinton Agar. Broth microdilution assay for the minimum inhibitory concentration (MIC) of biocides was done in 250 ul plate and cation-adjusted Muller Hinton Broth for culturing bacterial isolates. Statistical analysis using Orange Machine Learning Software and Jamovi statistical application.
Results: Nine isolates of *Pseudomonas aeruginosa* were obtained. Ciprofloxacin was most effective against most isolates. The difference was statistically significant. Polymixin B and ceftriaxone-tazobactam had lower median zone sizes and were below the cut-off point for sensitivity. There was variation in the zone diameter for most of the drugs. The distribution of zone diameters was positively skewed for gentamicin, ceftriaxone-tazobactam, levofloxacin, ceftazidime, ciprofloxacin, and ofloxacin. Chlorhexidine had a statistically significant lower MIC than for CTAB.
Conclusion: Ciprofloxacin could be a better therapeutic option for treating canine otitis caused by *P. aeruginosa*. Better environmental sanitation against pathogenic *Pseudomonas aeruginosa* could be attained by chlorhexidine than CTAB.

Keywords: Antibiogram; biocide tolerance; chlorhexidine; ciprofloxacin; Pseudomonas aeruginosa.

1. INTRODUCTION

Canine otitis is a prevalent condition that causes significant irritation, scratching, and distress to affected dogs. Among the various pathogens responsible for this condition*, Pseudomonas aeruginosa* (*P*.aeruginosa) species are often implicated. *P*.aeruginosa are obligate aerobes that thrive in oxygen-rich environments such as soil and are closely associated with increased anthropogenic activities, from where dogs can easily acquire the infection [1]. *P*.aeruginosa is a part of the ESKAPE group of pathogens, known for their ability to rapidly develop resistance to multiple antibiotics [2]. Understanding the sensitivity patterns of *P*.aeruginosa is imperative for better antimicrobial stewardship and informed decision-making in the treatment of canine otitis.

Biocides are chemicals used to control harmful microorganisms through nonspecific mechanisms of action. It was previously believed that resistance to biocides would be unlikely due to their broad-spectrum activity and nonspecific modes of action. However, resistance to biocides has been reported in various bacterial species [3]. Despite the widespread use of biocides like chlorhexidine and quaternary ammonium compounds, there is limited data on the biocide tolerance of *P*.aeruginosa, particularly from veterinary sources.

Given this knowledge gap, we aimed to isolate and identify *P.aeruginosa* from cases of canine otitis, profile their antibiotic sensitivity patterns, and evaluate their tolerance to chlorhexidine gluconate and Cetyl Trimethyl Ammonium Bromide (CTAB), a commonly used quaternary ammonium compound. This research will provide valuable insights into the resistance mechanisms of *P.aeruginosa* guiding more effective treatment strategies and improving outcomes for canine patients.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted during the period from March 2023 to March 2024. Dogs presented at Teaching Veterinary Clinical Complex Pookode and Peripheral Veterinary Clinics, Kakkavayal, Wayanad District, Kerala with clinical signs suggestive of otitis like head shaking, ear scratching, redness of ear and presence of otic discharge studied.

2.2 Isolation and Identification of *Pseudomonas*

Cases presented were clinically examined for the presence of otitis, which included head shaking, ear scratching, redness of the ear, and the presence of otic discharge. Ear swabbing was aseptically collected using a cotton-tipped polypropylene swab (Himedia, Mumbai) and immediately transported to the laboratory for cultural isolation. The swab was streaked on Brain Heart Infusion agar (Cat No. M211; Himedia, Mumbai) and incubated at 37°c for 24 h. Samples form 28 such cases were collected and cultured. *P. aeruginosa* was identified based on Gram staining, oxidase test, catalase test, growth on selective media Cetrimide Agar, and presence of fluorescent pyocyanin [4].

2.3 Antibiotic Sensitivity Profile

The disc diffusion method following CLSI 2021. Bacterial isolates were inoculated in sterile Muller Hinton Broth (MHB, Cat No. M391; Himedia, Mumbai) and incubated overnight at 37°C and turbidity adjusted to 0.5 McFarland Standard. The broth culture was spread over the Muller-Hinton Agar (MHA, Cat No. M173; Himedia, Mumbai) plate using a sterile swab to make a lawn. Plates were allowed to dry, and various antibiotic discs were placed on the surface with enough spaces in between for the antimicrobial to diffuse. Plates were incubated at 37°C for 24 hours and the zone of inhibition of growth of the organism around each disc was measured in millimeters using an antibiotic zone scale (Cat. No. 297; Himedia, Mumbai) and interpreted as sensitive or resistant by comparing the ranges given by the manufacturer. Antibacterial discs used in the study were Piperacillin-Tazobactam - 100/10µg (PIT 100/10), Aztreonam-30µg (AT30), Cefipime- 30µg (CPM30), Imipenem- 10µg (IPM10), Norfloxacin- 10µg (NIX10), Ciprofloxacin- 5µg (CIP5), Ceftazidime- 30µg (CAZ30), Gentamicin- 10µg (GEN10) Ceftriaxone-Tazobactam- 90µg - (CIT90), Tobramycin- 10µg (TOB10), Ofloxacin- 5µg (OF5), Polymixin- B- 300IU (PB300) and Levofloxacin- 5µg (LE5). All the discs were procured form Himedia, Mumbai.

2.4 Broth Microdilution Assay

The biocide tolerance of the isolates was evaluated using chlorhexidine (CHX) and cetyl trimethyl ammonium bromide (CTAB) through a broth microdilution assay on a 96-well U-bottom microtitre plate. The bacterial suspension was prepared at a turbidity of 0.5 McFarland standard. Equal volumes of biocide and bacterial suspension were added to each well.

The procedure began with the preparation of biocide solutions. In the first column of the plate, 100 µL of the required drug solution was added to each well. For wells 2 to 12, 50 µL of sterile cation-adjusted Mueller Hinton Broth (MHB) was added. A serial dilution was then performed by transferring 50 µL from the first well to the second well and continuing this process up to the 11th well, discarding 50 µL from the final well to maintain a consistent volume. To ensure sterility control, the 12th column was left containing only the broth without any biocide or bacterial suspension. Following this, 50 µL of the bacterial suspension, standardized to 0.5 McFarland unit, was added to each well except those in the sterility control column. The plate was then incubated at 37°C for 24 hours to allow bacterial growth. After the incubation period, 20 µL of 1% resazurin dye was added to each well, and the plate was incubated for an additional 30 minutes. Bacterial growth was indicated by a color change in the dye from blue to pink. The initial concentrations of chlorhexidine and CTAB used were 50 µg/mL and 250 µg/mL, respectively.

2.5 Statistical Analysis

The statistical tests and data analyses were performed using Orange [5] and Jamovi [6] software applications. Orange was utilized for its data mining and machine learning capabilities, while Jamovi was employed for its user-friendly interface and robust statistical testing functions.

The antibiotic sensitivity index was calculated. Box plots and stacked density plots of the zone diameter were prepared to determine the variability in the sensitivity of the isolates to the antibiotics tested. The normality of the distribution of zone size was tested by the Shapiro-Wilks test. Drugs that showed normal distribution were analyzed using one-way ANOVA, followed by Tuckey's range test to detect drugs with significant differences. If the distribution was not normal, the Krushak-Wilkis test was applied.

The biocide tolerance of *P.aeruginosa* to CHX and CTAB was tested for normality and then subjected to the Brunner Munzel test with full permutation. Pearson's correlation test was performed to assess the relation between the MIC of the two biocides.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Pseudomonas*

Out of 28 cases, 14 Gram-negative bacteria were isolated. Out of which nine were oxidase-positive. Among these, all the nine isolates produced fluorescence on shining UV light-indicating pyocyanins. Samples were identified as *P. aeruginosa.*

P. aeruginosa is often implicated as a pathogen associated with canine otitis, particularly chronic otitis media and externa [7]. This pathogen is hard to treat because it is often resistant to many antibacterial agents [8]. *P. aeruginosa* forms large, flat, spreading colonies with a greenish-

blue hue and serrated edges. These colonies are known for their characteristic fruity odor [9]. Biochemical tests provide a convenient and cost-effective method to identify Pseudomonas. These bacteria are ubiquitous in oxygen-rich environments and lack the enzymes necessary for carbohydrate fermentation, making them obligate aerobes that generate ATP using carbohydrates via the tricarboxylic acid cycle. *P. aeruginosa* are oxidase-positive and fermentation-negative. They produce pale colonies on MacConkey agar, as they cannot ferment lactose [10]. *P. aeruginosa* typically produces pyoverdin, a fluorescent pigment characteristic of the organism [11]. In our study, all isolates were Gram-negative short rods with bluish-green hued colonies and were catalase and oxidase-positive. All isolates exhibited greenish-blue fluorescence under ultraviolet light and were identified as *P. aeruginosa.*

3.2 Antibiogram

The antimicrobial sensitivity index (ASI) ranged from 0.61 to 0.84, with a mean value of 0.71, indicating that most isolates were sensitive to the majority of the drugs tested. Isolates COPM7 and COPM3 had the highest ASI and were sensitive to eleven of the thirteen antimicrobials tested. COPM3 was resistant to CIT90 and PB300, COPM7 to OF5 and AT30, COPM4 and COPM5 to LE5, PB300, OF5, and CIT90, COPM4 to TOB10, and COPM5 to AT30. (Table 1). Only four of the thirteen antibiotics tested were effective against all isolates: GEN10, PIT100/10, CPM30, and NIX10. Eight isolates were sensitive to IPM10, NIX10, and CAZ30.

P. aeruginosa is a major pathogen and is typically resistant to common antibacterial drugs. It is grouped with ESKAPE pathogens, known for being difficult to treat with antimicrobials. However, the isolates obtained in our study had a higher ASI, indicating that most were sensitive to common antimicrobials. These findings align with existing literature [12], which reported that clinical samples of *P. aeruginosa* are more sensitive to antimicrobials than environmental isolates.

Among the thirteen drugs tested, nine had a median zone diameter greater than the CLSI cutoff limit for sensitivity. PB300, CIT90, and LE5 had median zone diameters below the CLSI cutoffs. The smallest median zone diameters were observed for PB300 and CIT90, with only two and three isolates being sensitive, respectively. The median diameter of the zone of inhibition was moderate for TOB10, GEN10, OF5, CAZ30, and LE5. The interquartile deviation (IQD) was also moderate for these antibiotics, with levofloxacin showing a wider deviation. AT30, IPM10, CPM30, PIT100/10, and CIP5 had higher median zone diameters, and the IQD was least for NIX10, CIP5, and IPM10. Compared to other drugs with higher median diameters, NIX10 exhibited a wider range of zone inhibition. The data revealed a minimal IQD range and low diversity in antimicrobial sensitivity. Results are depicted as a box and whiskers plot (Fig. 1).

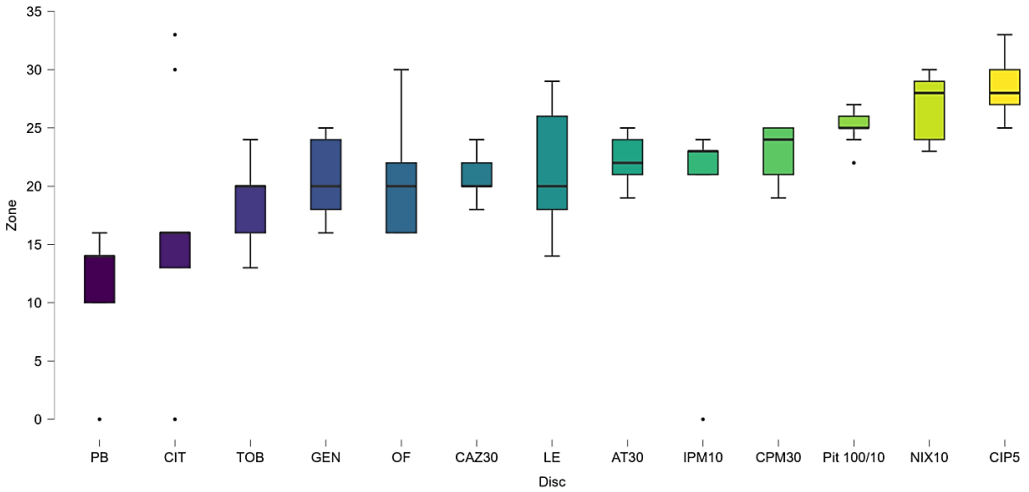

Fig. 1. Box and whiskers plot showing the diameter of the zone of inhibition against different antibiotics by the isolates. The Y axis indicates the zone diameter in mm.

Table 1. Results of the disc diffusion test

Sample	PIT 100/10	AT 30	CPM 30	IPM 10	NIX 10	CIP 5	CAZ 30	GEN 10	CIT 90	TOB 10	OF 5	PB 300	LE 5	Sensitivity index
COPM1	S	S	S	S	S	S	S	S	R	S	S	R	S	0.85
COPM2	S	R	S	S	S	S	S	S	S	S	R	S	S	0.85
COPM3	S	R	S	S	S	S	S	S	R	S	S	S	R	0.77
COPM4	S	R	S	S	S	R	R	S	S	S	S	R	S	0.69
COPM5	S	R	S	S	S	S	S	S	R	S	S	R	R	0.69
COPM6	S	S	S	R	S	S	S	S	R	S	S	R	R	0.69
COPM7	S	S	S	S	S	S	S	S	R	R	S	R	R	0.69
COPM8	S	S	S	S	S	S	S	S	R	R	R	R	R	0.62
COPM9	S	R	S	S	S	S	S	S	R	S	R	R	R	0.62

S= Sensitive; R= resistant

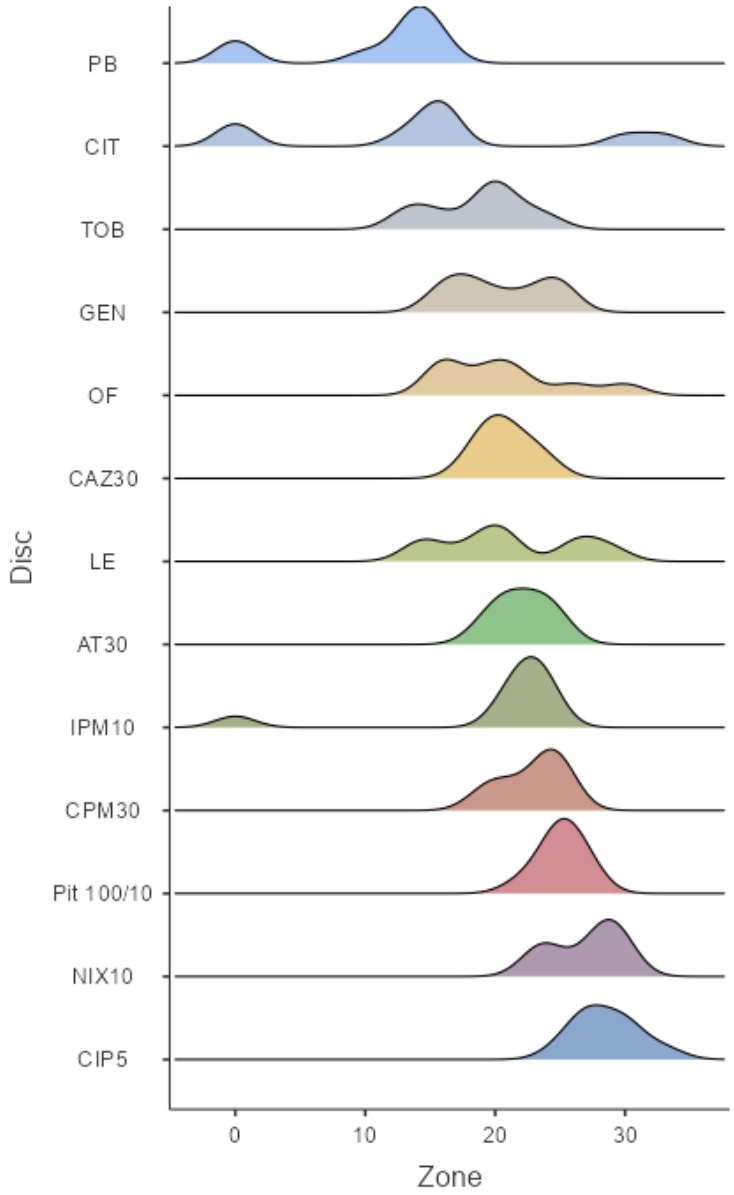

Fig. 2. Stacked density plot showing the diameter of zone of inhibition against different antibiotics by the isolates. The X-axis indicates the zone diameter in mm

The first line of treatment for *P. aeruginosa* infections often involves a combination of an aminoglycoside with a beta-lactam. Our findings support the effectiveness of this combination, as gentamicin, traditionally used against *P. aeruginosa* continued to show high efficacy [13]. Fluoroquinolones are also effective against *P. aeruginosa* by inhibiting bacterial DNA gyrase. Although the high sensitivity of NIX10 against the isolates is favorable, continued monitoring for fluoroquinolone resistance in *P. aeruginosa* is essential due to reports of increased resistance [14].

Density plots (Fig. 2) indicated that most zone sizes were consistent for most drugs. Single peaks, indicating consistency in sensitivity, were observed for CAZ30, AT30, CPM30, PIT100/10, and CIP5. In contrast, relatively flat curves with multiple peaks were observed for PB300, TOB10, GEN10, OF5, and LE5, indicating variation in zone sizes between different isolates. Drugs with lower median zones showed wider variation than those with larger zone diameters (Fig. 2). Among the sensitive drugs, variation in zone diameter occurred for TOB10, GEN10, OF5, LE5, IPM10, and NIX10.

PB300 showed poor zone size and variation in sensitivity, which may be attributed to its large molecular size and poor diffusion in Mueller-Hinton agar (MHA). PB300 is used in conjunction with other antibiotics for treating *P. aeruginosa*. Cases of multidrug-resistant *P. aeruginosa* infections have been treated with polymyxin B in combination with netilmicin [15]. Resistance to PB300 is associated with mutations and modifications in the O antigen of the bacteria [16]. CIT90 was identified as a better choice over ceftriaxone-sulbactam when comparing the zone sizes of both drugs [17]. However, CIT90 was found to be sensitive to only six out of nineteen *P. aeruginosa* isolates in one study [18]. Among the fluoroquinolones tested, LE5 was the least effective. Differences in zone sizes among fluoroquinolones were reported in *Staphylococci* [19], but not observed in *P. aeruginosa* [20].

The moderate interquartile deviation (IQD) of TOB10, GEN10, OF5, CAZ30, and LE5 indicates variation in response to these antimicrobials. Aminoglycosides like GEN10 and TOB10 are widely used in treating Pseudomonas infections. These drugs exert bactericidal action by binding to the A site of the 16S rRNA and inhibiting protein synthesis [21]. Although newer beta-lactams have reduced dependence on aminoglycosides, renewed interest in aminoglycosides has emerged due to resistance development in newer strains of *P. aeruginosa* [22]. The findings of the present study align with the opinions of [23].

The distribution of zone diameters was positively skewed for GEN10, CIT90, LE5, CAZ30, CIP5, and OF5, while the distribution for the remaining drugs skewed negatively. IPM10 had the maximum negative skewness, and OF5 had the maximum positive skewness. Most drugs exhibited a platykurtic distribution, with gentamicin having the lowest kurtosis value. Imipenem exhibited a kurtosis value of more than three. The Shapiro-Wilk test revealed that the data were normally distributed for most antibiotics, except for PB300 and IPM10. One-way ANOVA of the normally distributed drugs revealed an F-value of 10.2 ($p < 0.01$), indicating significant differences in zone sizes among different antibiotics. Tukey's Honest Significant Differences Test showed that the zone sizes of CIP5 differed significantly ($p = 0.05$) from CIT90, GEN10, LE5, OF5, TOB10, and CAZ30. The zone size of CIT90 differed significantly ($p = 0.05$) from CPM30, NIX10, and PIT100/10. The zone size of norfloxacin differed significantly from TOB10 ($p = 0.05$). The Kruskal-Wallis test, applied to compare the zone sizes of PB300 and IPM10 (which did not follow a normal distribution), revealed a significant statistical difference ($p < 0.01$) between the zone sizes of these two drugs.

Positive skewness indicates that more isolates had zone sizes smaller than the mean zone diameter, with fewer isolates having much larger zone sizes. *P. aeruginosa* are well known for their genetic plasticity and ability to acquire resistance from their environment [24]. Positive skewness of fluoroquinolones indicates the presence of resistance mechanisms like efflux pumps working in the bacterial isolates, leading to the narrowing of many zones [25]. Conversely, a negative skew indicates that the majority of isolates had zone sizes larger than the median, and the mean zone size is less than the median due to a few isolates with significantly larger zone sizes. It is interesting to note that CIT90 had a positive skew, while CAZ30 and PIT100/10 had a negative skew, indicating the superiority of CAZ30 and PIT100/10 over CIT90. Since both PIT100/10 and CIT90 had variations in zone size, inhibition of tazobactam [26] by *P. aeruginosa* may be ruled out. Increased sensitivity to newer antibiotics like piperacillin and fourth-generation cephalosporin like CAZ30 suggests that *P. aeruginosa* may be gaining resistance to an older third-generation cephalosporin [27]. Most drugs exhibited a platykurtic distribution, indicating a wider dispersion of zone diameters compared to the median zone, showing increased variability among the isolates in response to the antimicrobials. One-way ANOVA and post-hoc tests showed that CIP5 exhibited superior efficacy against *P. aeruginosa* compared to CIT90, GEN10, LE5, OF5, TOB10, and CAZ30. Cefepime was more effective than CIT90. These findings are in agreement with [27].

3.3 Biocide Tolerance

A comparison of the MIC of CHX and CTAB revealed that the MIC of CTAB was greater than CHX. The mean MIC for chlorhexidine was 0.007 µg/mL, whereas the average MIC for CTAB was 208.3 µg/mL. Isolates Kit and 9 had the lowest MIC against CHX, while isolates Kitten, 10, and 865 had the highest MIC of 0.0125 µg/mL. Isolates COPM1, COPM4 and COPM6 had an MIC of 125 µg/mL against CTAB, while all other isolates had an MIC of 250 µg/mL.

The Shapiro-Wilk test for normality and QQ plot analysis revealed that the MIC data were not normally distributed. Brunner-Munzel test with full permutation revealed that MIC values between groups varied significantly ($p<0.01$). The test indicated that the MIC of chlorhexidine was significantly lower than that of CTAB.

Pearson's correlation test revealed a moderate negative correlation ($r = -0.539$) between the MIC values for CTAB and CHX, but the data were statistically insignificant. These findings suggest a wide variability in biocide tolerance among *P. aeruginosa* isolates from otitis, indicating that tolerance to one biocide is independent of the other. The Brunner-Munzel test was chosen over the Mann-Whitney U test for analyzing the minimum inhibitory concentration (MIC) because the Brunner-Munzel test does not require the assumption of similar distribution shapes and is more robust to differences in variability between groups. This ensures a better and more valid comparison of MIC values between groups. The increased tolerance to CTAB in *P. aeruginosa* has been reported to be caused by the activity of QAC efflux pumps [28].

4. CONCLUSION

The study isolated *P. aeruginosa* bacteria from cases of canine otitis and profiled their antibiotic sensitivity pattern. The tolerance of the isolates against two commonly used biocides- CHX and CTAB was also tested. Most of the isolates were sensitive to the antibacterials. CIP5 exhibited a superior and consistent response compared to other antibiotics. Overall, the antimicrobials had a consistent zone diameter. Polymixin and ceftriaxone-tazobactam had smaller zone sizes. CHX was more efficient than CTAB in eliminating *P. aeruginosa*, as CHX had a significantly lower MIC than CTAB.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during the editing of manuscripts. This include ChatGPT and Grammarly AI for reviewing and editing the text of the manuscript.

ETHICAL APROVAL

IRB approval for the research work : KVASU /DAR/A3/2457/2023(1) Dated 23/12/2023, of the Director of Academics and Research, Kerala Veterinary and Animal Sciences Univeristy, Pookode, Wayanad, Kerala.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Crone S, Vives-Flórez M, Kvich L, Saunders AM, Malone M, Nicolaisen MH et al. The environmental occurrence of *Pseudomonas aeruginosa*. APMIS. 2020; 128(3):220-31.
2. De Oliveira David MP, Forde Brian M, Kidd Timothy J, Harris Patrick NA, Schembri Mark A, Beatson Scott A et al. Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev. 2020; 33(3):10. DOI:1128/cmr.00181-19.
3. Verdial C, Serrano I, Tavares L, Gil S, Oliveira M. Mechanisms of antibiotic and biocide resistance that contribute to *Pseudomonas aeruginosa* Persistence in the hospital environment. Biomedicines. 2023;11(4):1221.
4. Feltham RKA, Barrow GI. Cowan and steel's: Manual for the identification of medical bacteria. 3rd ed, [reprint., 1st paperback ed.] ed. Cambridge: Cambridge University Press; 2003.
5. Demšar J, Curk T, Erjavec A, Gorup Č, Hočevar T, Milutinovič M, et al. Orange: Data mining toolbox in Python. The Journal of Machine Learning Research. 2013; 14(1):2349-53.
6. Karch JD. bmtest: A Jamovi Module for Brunner–Munzel's Test—A Robust Alternative to Wilcoxon–Mann–Whitney's Test. Psych. 2023;5(2):386-95.
7. Secker B, Shaw S, Atterbury RJ. *Pseudomonas spp*. in canine otitis externa. Microorganisms. 2023;11(11):2650.
8. KuKanich KS, Bagladi-Swanson M, KuKanich B. *Pseudomonas aeruginosa* susceptibility, antibiogram and clinical interpretation, and antimicrobial prescribing

behaviors for dogs with otitis in the Midwestern United States. J Vet Pharmacol Ther. 2022;45(5):440-9.
9. Urgancı NN, Yılmaz N, Alaşalvar GK, Yıldırım Z. *Pseudomonas aeruginosa* and its pathogenicity. Turkish Journal of Agriculture-Food Science and Technology. 2022;10(4):726-38.
10. Enemali MU, Yilkahan DI. Antibiotic susceptibility and biofilm formation of clinical isolates of pseudomonas species from wounds specimens. Journal of Scientific Research in Medical and Biological Sciences. 2021;2(3):67-74.
11. Kothari A, Kumar SK, Singh V, Kumar P, Kaushal K, Pandey A, et al. Association of multidrug resistance behavior of clinical *Pseudomonas aeruginosa* to pigment coloration. Eur J Med Res. 2022;27(1):120.
12. Gad GF, El-Domany RA, Zaki S, Ashour HM. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: Prevalence, antibiogram and resistance mechanisms. J Antimicrob Chemother. 2007;60(5):1010-7.
13. Yousefpour Z, Davarzani F, Owlia P. Evaluating of the effects of sub-MIC concentrations of gentamicin on biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. Iranian Journal of Pathology. 2021;16(4):403.
14. Sid Ahmed MA, Abdel Hadi H, Abu Jarir S, Al Khal AL, Al-Maslamani MA, Jass J, et al. Impact of an antimicrobial stewardship programme on antimicrobial utilization and the prevalence of MDR *Pseudomonas aeruginosa* in an acute care hospital in Qatar. JAC-Antimicrobial Resistance. 2020;2(3):dlaa050.
15. Sushmasri K, Joseph J, Chaurasia SR, Ramachandran C, Roy S. An experimental study to evaluate the effect of polymixin E (Colistin) alone or in combination with gentamicin in McCarey-Kaufman corneal preservation medium on various drug resistant bacterial and fungal isolates. Indian J Ophthalmol. 2022;70(8):2950-5.
16. Choi M, Shridhar S, Fox H, Luo K, Amin MN, Tennant SM, et al. The O-glycan is essential for the induction of protective antibodies against lethal infection by flagella A-bearing *Pseudomonas aeruginosa*. Infect Immun. 2024;92(3): e00427-23.
17. Rajpurohit H, Vinay Kumar B, Sharadamma K, Radhakrishna P. *In-vitro* activity of ceftriaxone in combination with sulbactam and tazobactam against Escherichia coli. Int J Pharm Bio Sci. 2011; 1(4):545-50.
18. Kashyap PK, Ali S, Shakya S, Singh J, Gade NE, Rawat N, et al. Assessment of antibiogram profiles in Pseudomonas isolates from canine otitis externa. The Pharma Innovation Journal. 2023;SP-12(12).
19. Kowalski RP, Pandya AN, Karenchak LM, Romanowski EG, Husted RC, Ritterband DC, et al. An *In vitro* resistance study of levofloxacin, ciprofloxacin, and ofloxacin using keratitis isolates of Staphylococcus aureus and *Pseudomonas aeruginosa*1 1The authors have no proprietary interest in any of the products presented in this study. Ophthalmology. 2001;108(10):1826-9.
20. Sihotang TSU, Widodo ADW, Endraswari PD. Effect of ciprofloxacin, levofloxacin, and ofloxacin on *Pseudomonas aeruginosa*: A case control study with time kill curve analysis. Annals of Medicine and Surgery. 2022;82.
21. Schwarz C, Taccetti G, Burgel P-R, Mulrennan S. Tobramycin safety and efficacy review article. Respir Med. 2022; 195:106778.
22. Webster CM, Shepherd M. A mini-review: Environmental and metabolic factors affecting aminoglycoside efficacy. Wrld J Microbiol Biotech. 2023;39(1):7.
23. Böttger EC, Crich D. Aminoglycosides: Time for the Resurrection of a neglected class of antibacterials? ACS Infectious Diseases. 2020;6(2):168-72.
24. Botelho J, Tüffers L, Fuss J, Buchholz F, Utpatel C, Klockgether J, et al. Phylogroup-specific variation shapes the clustering of antimicrobial resistance genes and defence systems across regions of genome plasticity in *Pseudomonas aeruginosa*. EBioMedicine. 2023;90.
25. Al Rashed N, Joji RM, Saeed NK, Bindayna KM. Detection of overexpression of efflux pump expression in fluoroquinolone-resistant *Pseudomonas aeruginosa* isolates. International Journal of Applied and Basic Medical Research. 2020;10(1):37-42.
26. Viola A, Ferrazzano L, Martelli G, Cerisoli L, Ricci A, Tolomelli A, et al. Novel insights into the chemistry of an old medicine: A general degradative pathway for penicillins

from a piperacillin/ tazobactam stability study. Eur J Pharm Sci. 2019;136: 104957.
27. Venturini C, Bowring B, Fajardo-Lubian A, Devine C, Iredell J. Effects of antibiotic treatment with piperacillin/tazobactam versus ceftriaxone on the composition of the murine gut microbiota. Antimicrob Agents Chemother. 2021;65(2):10. DOI:1128/aac.01504-20.
28. Luo Y-H, Lai YS, Zheng C, Ilhan ZE, Ontiveros-Valencia A, Long X, et al. Increased expression of antibiotic-resistance genes in biofilm communities upon exposure to cetyltrimethylammonium bromide (CTAB) and other stress conditions. Sci Total Environ. 2021;765: 144264.

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