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**Research Article**

# Detection of extended spectrum beta lactamases from *pseudomonas aeruginosa* isolates from otitis and pyoderma infections of canines

**Swathi G[1*], Deepika Kumari G[1], Bindu Kiranmayi C.[2] and Anand Kumar P.[1]**

[1]Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram- 521 102, Andhra Pradesh, India; [2]Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavaram- 521 102, Andhra Pradesh, India

## Abstract

***Pseudomonas aeruginosa* is the most frequently isolated opportunistic Gram-negative bacilli from recurrent otitis and chronic suppurative skin infections of canines. Chronic infections are mainly due to the development of various resistance mechanisms, of which production of extended spectrum beta lactamases (ESBLs) is the most important. Therefore, the present study was carried out to detect the ESBLs production from *Pseudomonas aeruginosa* (*P. aeruginosa*) isolates of canines. A total of ninety-seven samples (n=97) were collected from otitis and pyoderma cases of canines. On microscopic, cultural and biochemical examination, 35 isolates were assumed to be *P. aeruginosa*. All 35 isolates were confirmed as *P. aeruginosa* by Polymerase Chain Reaction (PCR) using species-specific 16S rRNA primers that yielded a specific amplicon of 956 bp size. Phenotypic screening of ESBLs production was performed by employing Phenotypic Screening Test (PST), which suggested all isolates as "Suspect ESBLs producers". Twenty one isolates (21/35, 60%) were confirmed as ESBL producers based on the results of Double Disk Synergy Test (DDST). The genetic determinants of ESBLs were detected by using multiplex PCR (mPCR) which revealed the prevalence of $bla_{TEM}$ gene to be 20% (7/35), $bla_{OXA}$ to be 31.42% (11/35) and $bla_{SHV}$ to be 25.71% (9/35).**

**Keywords:** Antibiogram, ESBLs, Otitis, *Pseudomonas aeruginosa*, Pyoderma

## Highlights

- *Pseudomonas aeruginosa* was isolated from 35 samples out of 97 samples collected from otitis and pyoderma cases of dogs, with a prevalence rate of 36.08%.
- Phenotypic detection of ESBLs producers using PST and DDST were found to be 35 (35/35, 100%) and 21 (21/35, 60%), respectively.
- Detection of genetic determinants of ESBLs using multiplex PCR (mPCR) revealed the prevalence of $bla_{TEM}$ gene to be 20% (7/35), $bla_{OXA}$ to be 31.42% (11/35) and $bla_{SHV}$ to be 25.71% (9/35).

## INTRODUCTION

*Pseudomonas aeruginosa* is one of the major causative agents of chronic infections such as otitis and pyoderma in dogs, which signifies its ability to resist many of the antibiotics. Companion animals such as dogs are an important source for the spread of antibiotic resistance to humans, posing an important public health threat. Gram-negative bacteria use several mechanisms to develop resistance against various antibiotics. The production of enzymes such as Extended spectrum beta-lactamases (ESBLs) which include TEM (Temoniera in Greece), SHV (Sulphydral variable) and CTX-M (Hydrolytic activity against cefotaxime, M- Munich) to hydrolyze the beta-lactam ring of the antibiotics is the most important mechanism utilized to confer resistance against beta-lactam antimicrobial classes which includes penicillin, cephalosporins (especially the third and fourth generations), and monobactams. *OXA-1* genes present in plasmids and integrons of the Gram-negative bacteria, are linked to the global spread of CTX-M-15 ESBL (Sfaciotte *et al*., 2021). It is very difficult to treat otitis and pyoderma caused by resistant bacteria in canines. Hence, the current study was conducted to find out the prevalence of resistance-causing ESBLs in otitis and pyoderma cases of canines.

## MATERIALS AND METHODS

### Collection of samples

A total of 97 swab samples were collected from dogs suffering from otitis externa (n=45) and superficial pyoderma (n=52). The samples were collected from different places in Andhra Pradesh in the months of March and April 2023 and also from June to September

*Corresponding Author, E-mail: swathigogana1996@gmail.com

2023. The samples were collected from the external ear canal or the skin of affected canines and were transferred into sterile normal saline, which was stored at 4°C for a minimum period of 24 hrs until cultured/ enriched (Lyskova *et al*., 2007). The details of the collected samples are presented in Table 1.

**Table 1. Details of the samples collected**

| Place | Type of sample | | |
|---|---|---|---|
| | Otitis swab (E) | Pyoderma skin swab (S) | Total |
| VCC, NTR CVSc, Gannavaram | 11 | 06 | 17 |
| VSSH, Vijayawada | 06 | 06 | 12 |
| Prathyusha pet clinic, Vijayawada | 03 | 0 | 03 |
| VPC, Rajahmundry | 12 | 32 | 44 |
| VPC, Kakinada | 11 | 05 | 16 |
| VPC, Guntur | 02 | 03 | 05 |
| **Total** | **45** | **52** | **97** |

**Isolation of *Pseudomonas aeruginosa***

Ear and skin swabs were inoculated directly into Brain Heart Infusion broth (Himedia) and incubated at 37°C for 24 hrs. After observing the turbidity, the BHI broth culture was inoculated on Pseudomonas isolation agar (Himedia). The inoculated plates were incubated at 37°C for 24 to 48 hrs. The isolates were observed for colony morphology and examined for staining characters using Gram's staining technique. Further, the isolates were subjected to biochemical characterization using Indole, Methyl red, Voges- Proskauer and Citrate (IMViC) tests as per Quinn *et al*. (2011).

**Molecular confirmation by PCR**

**Bacterial DNA extraction:** DNA was extracted by boiling and snap chilling method (Ramanarayana *et al*., 2023) from all the suspected *P. aeruginosa* isolates. In a microcentrifuge tube, 2 mL of overnight grown *P. aeruginosa* culture was placed and spun at 10,000 rpm for five minutes. The pellet was suspended in 400 µL of nuclease-free water and kept in a boiling water bath for 10 min. The microcentrifuge tube was immediately chilled. The tube was centrifuged at 8000 rpm for five minutes at 4°C, and the supernatant was used as a template for PCR test.

**Species specific PCR***:* The extracted DNA was subjected to PCR (Table 2) for the detection of the 16S rRNA gene (956 bp) specific for *P. aeruginosa* as described by Spilker *et al*., 2004. Characterized and standard *Pseudomonas* spp. with ATCC no. 27853 was used as a positive control in this study. After performing the PCR, the products were subjected to electrophoresis and examined under the gel documentation system.

**Phenotypic detection of ESBLs production in *P. aeruginosa* isolates**

**Phenotypic Screening Test (PST):** *P. aeruginosa* was tested for susceptibility against four antimicrobial drugs, namely cefotaxime (CTX- 30 ug), ceftazidime (CAZ- 30 µg), ceftriaxone (CTR- 30 µg), and aztreonam (AT- 30 µg) (Table 4). The overnight PIA inoculated isolates were dispersed over Mueller Hinton agar (Himedia) with a sterile swab. After allowing the inoculum to dry, the antibiotic discs were placed on the agar surface with sterile forceps and incubated at 37°C for 18 hrs. The diameter of the zone of inhibition around the discs was measured after incubation using an antibiotic zone measuring scale. Resistance to at least one of the four antibiotics used was considered as a positive in this screening test for the development of ESBLs (CLSI, 2020).

**Double Disk Synergy Test (DDST):** All isolates that were found to be positive in PST were further subjected to Double Disk Synergy Test (DDST) for confirmation as recommended by CLSI (2020) guidelines.

The bacterial suspension was inoculated on MHA plates by uniformly swabbing the entire surface of the agar plates and was incubated at 37°C for 18-24 hrs. Ceftazidime (CAZ, 30µg), cefotaxime (CTX, 30µg), ceftriaxone (CTR, 30µg) and aztreonam (AZ, 30 µg) discs were placed around augmentin (AMC) (amoxycillin/clavulanic acid) 30 µg disc at a distance of 15 mm from it.

A clear visible zone of inhibition of any one of the antibiotic discs towards the zone of amoxyclav disc was regarded as phenotypic confirmation for the production of ESBLs. This enhanced zone of inhibition towards the clavulanate disc is called the keyhole phenomenon/ghost phenomenon/champagne cork phenomenon, which indicates a positive synergy test (Laudy *et al*., 2017).

**Table 2. Species-specific oligonucleotide primers for *P. aeruginosa* (Spilker *et al*., 2004)**

| | Target gene | Nucleotide sequence (5′-3′) | Amplicon size(bp) |
|---|---|---|---|
| ***P. aeruginosa*** | 16S *rRNA* | GGGGGATCTTCGGACCTC<br>ATCCTTAGAGTGCCCACCCG | 956 |

**Table 3. Oligonucleotide primers used for the detection of ESBL genes in *P. aeruginosa* (Spilker *et al*., 2004)**

| Primer | Target | Nucleotide sequence | Amplicon size (bp) |
|---|---|---|---|
| MultiTSO-T | $bla_{TEM}$ | CAT TTC CGT GTC GCC CTT ATT C | 800 |
| | | CGT TCA TCC ATA GTT GCC TGA C | |
| MultiTSO-S | $bla_{SHV}$ | AGC CGC TTG AGC AAA TTA AAC | 713 |
| | | ATC CCG CAG ATA AAA TCA CCA C | |
| MultiTSO-O | $bla_{OXA}$ | GGC ACC AGA TTC AAC TTT CAA G | 564 |
| | | GAC CCC AAG TTT CCT GTA AGT G | |

**Detection of ESBL genes by multiplex PCR**

All DDST-positive *P. aeruginosa* isolates were screened for $bla_{TEM}$ (800 bp), $bla_{SHV}$ (713 bp), and $bla_{OXA}$ (564 bp) genes by using multiplex PCR as per the protocol of Spilker *et al*. (2004). The details regarding the primers and PCR cycling conditions are mentioned in Table 3.

## RESULTS

Among the 97 swab samples collected, 35 samples yielded characteristic translucent green-pigmented smooth mucoid colonies on PIA (Fig. 1) and were Gram-negative small slender rods with IMViC biochemical test results as - - - + (Fig. 2). All 35 isolates showed the presence of 16S *rRNA* gene specific for *P. aeruginosa* in PCR with an amplicon size of 956 bp (Fig. 3). In the present study, the prevalence of *P. aeruginosa* was 40% (18/45) in otitis cases and 32.6% (17/52) in pyoderma cases, with overall prevalence of 36.08% (35/97) after detailed cultural and molecular characterization.

Using commercially available antibiotic discs, all 35 samples were subjected to phenotypic screening test (PST) and Double Disk Synergy Test (DDST).

In PST, all 35 isolates exhibited resistance to either one or more cephalosporin discs tested and were considered as "Suspect ESBL producers" (Fig. 4). The isolates showed 100% resistance to ceftazidime (35/35), 94.28% resistance to ceftriaxone and aztreonam each (33/35) and 82.85% resistance to cefotaxime (29/35). The resistance to the different cephalosporin antibiotics used is summarized in Table 4.

In DDST, 60% (21/35) isolates showed a positive ghost phenomenon (Fig. 5). The PST and DDST results are summarized in Tables 4 and 5.

**Table 4. *P. aeruginosa* isolates exhibiting resistance to cephalosporin antibiotics in PST**

| Name of the antibiotic | No. of *P. aeruginosa* isolates (n=35) exhibiting resistance |
|---|---|
| Ceftazidime (CAZ) | 35 (100%) |
| Ceftriaxone (CTR) | 33 (94.28%) |
| Aztreonam (AT) | 33 (94.28%) |
| Cefotaxime (CTX) | 29 (82.85%) |

**Table 5. PST & DDST results of *P. aeruginosa* for ESBLs production**

| Total no. of positive *P. aeruginosa* isolates | 35 |
|---|---|
| Presumptive ESBLs producers by PST | 35 (100%) |
| DDST | 21 (60%) |

The results of Multiplex PCR of isolates showed bands with amplicon sizes of 564 bp, 713 bp, and 800 bp, confirming the presence of ESBL genes $bla_{SHV}$, $bla_{OXA}$ and $bla_{TEM}$, respectively (Fig. 6). Out of the total 35 isolates, 15 (42.85%) isolates were positive for at least any one of the three ESBL genes $bla_{SHV}$, $bla_{OXA}$ and $bla_{TEM}$. Three isolates were positive only for $bla_{OXA}$ and $bla_{SHV}$ genes, whereas one isolate was positive only for $bla_{TEM}$. Both $bla_{TEM}$ and $bla_{OXA}$ were detected in two isolates (5.71%). Both $bla_{OXA}$ and $bla_{SHV}$ were observed in two isolates (5.71%). All three genes were detected in four isolates (11.42%).

## DISCUSSION

In the present study, the prevalence of *P. aeruginosa* was 36.08%, which was within the limit of the reported results ranging from 23.2% (Colombini *et al*., 2000) to 71.5% (Falodun and Musa 2020).

Biochemical tests were performed to confirm the presence of *P. aeruginosa* by oxidase, catalase, indole, citrate and MR-VP tests. All the 35 presumptively confirmed isolates based on the colony characteristics and Gram's staining were also found to be positive for oxidase, catalase and citrate. All 35 isolates were tested negative for indole and MR-VP tests, which is in correlation with the other research reports (Lakshmi and Rao, 2013; Nwiyi *et al*., 2014; Ramanarayana *et al*., 2022).

The prevalence of *P. aeruginosa* from otitis in the current study was lower than 83.3% reported by Schick *et al*. (2007), whereas it was slightly higher than 27.8% reported by Petersen *et al*. (2002).

These results were found to be much higher than some

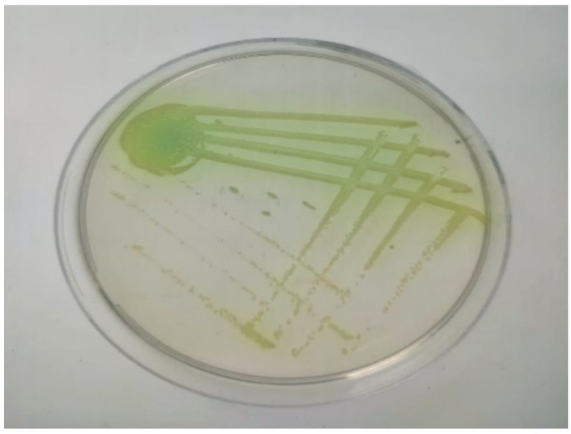


**Fig. 1. *P. aeruginosa* producing characteristic green pigmentation on Pseudomonas isolation agar**

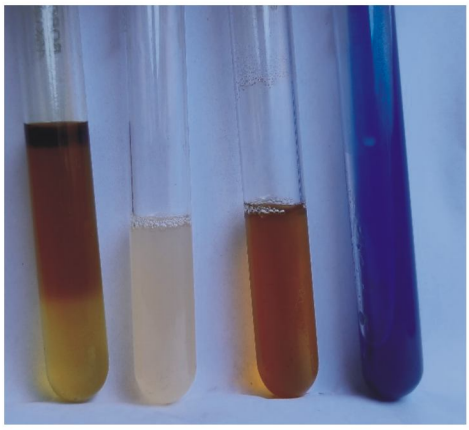
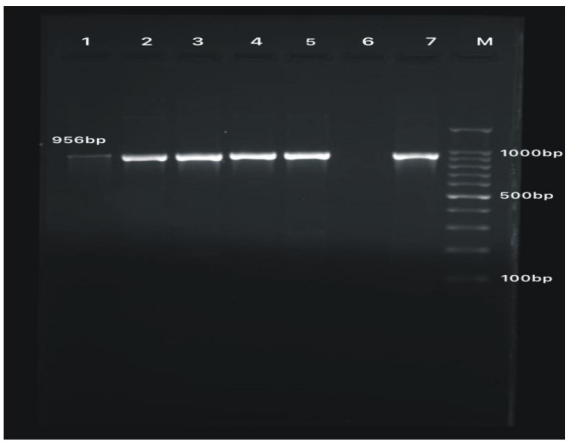


**Fig. 2. IMViC test results of *P. aeruginosa***




[M: Marker of 1100bp; Lane 1: O27; Lane 2: O34; Lane 3: O38; Lane 4: W1; Lane 5: W13; Lane 6: Negative control; Lane 7: Positive control]

**Fig. 3. PCR test with species specific primers (16S rRNA) for detecting the *P. aeruginosa* isolates from dogs**

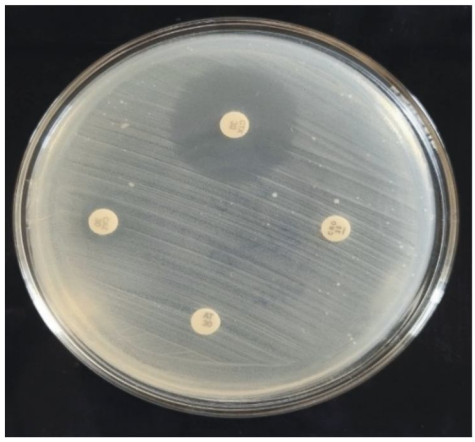


**Fig. 4. Phenotypic Screening Test (PST) of *P. aeruginosa* isolates**

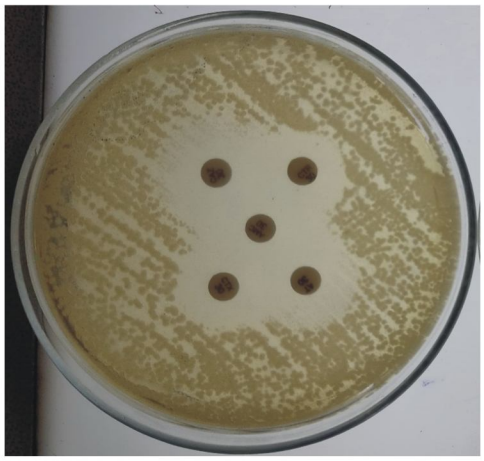
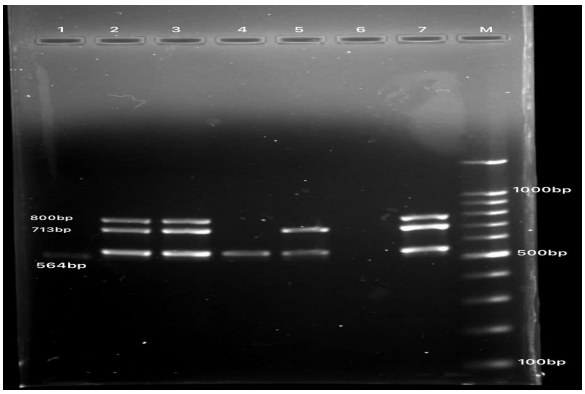


**Fig. 5. Double Disk Synergy Test (DDST) of *P. aeruginosa* isolates**




[M: DNA Marker; Lane 1: O26 isolate showing $bla_{OXA}$ at 564 bp; Lane 2: O13 isolate showing $bla_{OXA}$ (564 bp), $bla_{SHV}$ (713 bp) and $bla_{TEM}$ (800 bp); Lane 3: O14 isolate showing $bla_{OXA}$ (564 bp), $bla_{SHV}$ (713 bp) and $bla_{TEM}$ (800 bp); Lane 4: W15 isolate showing $bla_{OXA}$ (564 bp); Lane 5: W34 positive for $bla_{OXA}$ (564 bp) and $bla_{SHV}$ (713 bp); Lane 6: Negative control; Lane 7: Positive control]

**Fig. 6. Detection of ESBLs genes in *P. aeruginosa* isolates by m-PCR**

of the reports where the isolation rate ranged from 6.7% to 14.6% (Petersen *et al.*, 2002; Lin *et al.*, 2012; Ludwig *et al.*, 2016; Chaudhary *et al.*, 2019; Degi *et al.*, 2021) and slightly higher than Raheem *et al.* (2023) who reported 30% isolation rate. The current study results correlated with the results of Nocera *et al.* (2021), who reported a 36% isolation rate. The variation in the isolation rates of *P. aeruginosa* might be due to the difference in the techniques used for sample processing and culturing.

In the current study, all 35 (100%) positive *P. aeruginosa* isolates were suspected to be ESBLs producers by the PST method, of which 21(60%) were confirmed phenotypically as ESBLs producers by Double Disc Synergy Test (DDST). In another study conducted by Ramanarayana (2022), results obtained by the PST method indicated 90.38% (47/52) to be ESBLs producers, which was lower compared to our current results of 100% (35/35), whereas the results obtained by DDST confirmed 80.76% (42/52) to be ESBL producers which was higher than the current study results of 60% (21/35). Laudy *et al.* (2017) characterized *P. aeruginosa* isolates for ESBL production using DDST and reported 70.15% of isolates to be positive for ESBL production which is slightly higher than the current study.

The ESBL gene $bla_{OXA}$ was noticed in 11 (31.42%) isolates, $bla_{TEM}$ in seven (20%) isolates and $bla_{SHV}$ in nine (25.71%) isolates. In 20 isolates (57.14%) none of the ESBL genes that were used in the present study were detected.

Shahcheraghi *et al.* (2010) and Ramanarayana *et al.* (2022) reported a prevalence of $bla_{SHV}$ to be 22% and 38.46%, respectively, whereas the prevalence of $bla_{TEM}$ to be 9% and 23.07%, respectively. These results were in accordance with the current study reports. Hosu *et al.* (2021) analyzed 82 *P. aeruginosa* isolates and reported $bla_{TEM}$ (79.9%) as the most prevalent genotype, followed by $bla_{SHV}$ (69.5%) and $bla_{CTX\text{-}M}$ (31.5%), which is very high compared to that of the present study.

The presence of variation in the ESBL genes, one gene dominating the prevalence of the other, is usually based on geographical location. The findings of Ehlers *et al.* (2009), Chen *et al.* (2015) and Miranda *et al.* (2015) from South Africa, China and Brazil reported $bla_{TEM}$ to be dominant, whereas Jamali *et al.* (2017) reported $bla_{SHV}$ as the most prevalent genotype, whereas in this study $bla_{OXA}$ showed predominance.

Based on the results of this study, it was concluded that there is a significant presence of *P. aeruginosa* with the selected ESBLs genes in dogs' chronic otitis and pyoderma cases. The results also highlight the variation between the phenotypic and genetic determinants of ESBLs detection, which in turn indicates the presence of other genes that might lead to the current phenotypic findings.

**Conflict of interest:** Authors have no conflict of interest in this study.

**Author's contributions:** SG: Sample collection, carried out the work, analysis and interpretation of the results; DKG, AKP: Designed, supervised and monitored the work regularly; DKG, BKC, AKP: Corrected the manuscript and interpreted the data analysis.

**Data availability statement:** The data used to support the findings of this study are included within the article.

## REFERENCES

Chaudhary AK, Kumar A and Shrivastva M, 2019. Study on prevalence and resistance patterns of bacterial pathogens isolated from canine pyoderma. Int J Cur Microbiol Appl Sci, 8(1): 2305-2311, doi:10.20546/ijcmas.2019.801.241

Chen Z, Niu H, Chen G, Li M, Li M *et al.*, 2015. Prevalence of ESBLs-producing *Pseudomonas aeruginosa* isolates from different wards in a Chinese teaching hospital. Int J Clin Exp Med, 8(10): 19400-19405

Colombini S, Merchant SR and Hosgood G, 2000. Microbial flora and antimicrobial susceptibility patterns from dogs with otitis media. Vet Dermatol, 11(4): 235-239, doi: 10.1046/j.1365-3164.2000.00191.x

Degi J, Moco OA, Degi DM, Suici T, Mare' M *et al.*, 2021. Antibiotic susceptibility profile of *Pseudomonas*

*aeruginosa* canine isolates from a multicentric study in Romania. Antibiotics, 10(7): 846, doi: 10.3390/antibiotics10070846

Ehlers MM, Veldsman C, Makgotlho EP, Dove MG, Hoosen AA *et al*., 2009. Detection of *bla*SHV, *bla*TEM and *bla*CTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. FEMS Immunol Med Microbiol, 56(3): 191-196, doi: 10.1111/j.1574-695X.2009.00564.x

Falodun OI and Musa IB, 2020. *Pseudomonas* species from cattle dung producing extended spectrum and metallo beta-lactamases. Eur J Biol Res, 10(1): 1-10

Hosu MC, Vasaikar SD, Okuthe GE and Apalata T, 2021. Detection of extended spectrum beta-lactamase genes in *Pseudomonas aeruginosa* isolated from patients in rural Eastern Cape Province, South Africa. Sci Rep, 11(1): 7110, doi: 10.1038/s41598-021-86570-y

Jamali S, Shahid M, Sobia F, Singh A and Khan HM, 2017. Phenotypic and molecular characterization of cefotaximases, temoniera, and sulfhydryl variable β-lactamases in Pseudomonas and Acinetobacter isolates in an Indian tertiary health-care center. Indian J Pathol Microbiol, 60(2): 196, doi: 10.4103/0377-4929.208377

Lakshmi K and Rao DT, 2013. Clinico-microbiological and therapeutic studies on canine otitis externa. Int J Pharma Bio Sci, 4(3): B1209-B1214

Laudy AE, Rog P, Smolinska-Krol K, Cmiel M, Sloczynska A *et al.*, 2017. Prevalence of ESBL-producing *Pseudomonas aeruginosa* isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. PLoS One, 12(6): e0180121 doi: 10.1371/journal.pone. e0180121

Lin D, Foley SL, Qi Y, Han J, Ji C *et al.*, 2012. Characterization of antimicrobial resistance of *Pseudomonas aeruginosa* isolated from canine infections. J Appl Microbiol, 113(1): 16-23, doi: 10.1111/j.1365-2672.2012.05304.x

Ludwig C, De Jong A, Moyaert H, El Garch F, Janes R *et al.*, 2016. Antimicrobial susceptibility monitoring of dermatological bacterial pathogens isolated from diseased dogs and cats across Europe (Com Path results). J Appl Microbiol, 121(5): 1254-1267, doi: 10.1111/jam.13287

Lyskova P, Vydrzalova M and Mazurova J, 2007. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. J Vet Med A, 54(10): 559-563, doi: 10.1111/j.1439-0442.2007.00996.x

Miranda CC, de Filippis I, Pinto LH, Coelho Souza T, Bianco K *et al.*, 2015. Genotypic characteristics of multidrug resistant *Pseudomonas aeruginosa* from hospital wastewater treatment plant in Rio de Janeiro, Brazil. J Appl Microbiol, 118(6): 1276-1286, doi: 10.1111/jam.12792

Nocera FP, Ambrosio M, Fiorito F, Cortese L and De Martino L, 2021. On Gram-positive-and Gram-negative-bacteria-associated canine and feline skin infections: A 4-year retrospective study of the University Veterinary Microbiology Diagnostic Laboratory of Naples, Italy. Animals, 11(6): 1603, doi: 10.3390/ani11061603

Nwiyi P, Okonkwo C and Enwere S, 2014. Isolation of pathogenic bacteria and antibiotic susceptibility testing of dogs with otitis externa in Aba, Abia state, Nigeria. Sky J Microbiol Res, 2(8): 59-62

Petersen AD, Walker RD, Bowman MM, Schott HC and Rosser Jr EJ, 2002. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992-1997). J Am Anim Hosp Assoc, 38(5): 407-413, doi: 10.5326/0380407

Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S *et al.*, 2011. Veterinary Microbiology and Microbial Disease. John Wiley and Sons

Raheem SA and Abdalshheed DA, 2023. Evaluating the antibacterial and biofilm activity of *Pseudomonas aeruginosa* isolated from dog's wound infections. Iran J Ichthyol, 10: 96-104

Ramanarayana P, 2022. Assessment of antibiotic resistance pattern and biofilm formation in *Pseudomonas aeruginosa* isolates from buffaloes and dogs. M.V.Sc. Thesis, Sri Venkateswara Veterinary University, Tirupati, India

Ramanarayana P, Kumari GD, Kumar PA and Kiranmayi B, 2022. Isolation and identification of *P. aeruginosa* from clinical samples of dogs. Indian J Anim Health, 61(2): 357- 362, doi: 10.36062/ijah.2022.10022

Ramanarayana P, Kumari GD, Kumar PA and Bindu C, 2023. Cultural characterization and molecular identification of *Pseudomonas aeruginosa* from milk samples. Int J Vet Sci Anim Husb, 8(4): 443-446

Schick AE, Angus JC and Coyner KS, 2007. Variability of laboratory identification and antibiotic susceptibility reporting of *Pseudomonas* spp. isolates from dogs with chronic otitis externa. Vet Dermatol, 18(2): 120-126, doi:10.1111/j.1365-3164.2007.00581.x

Sfaciotte RAP, Parussolo L, Melo FD, Wildemann P, Bordignon G *et al.*, 2021. Identification and characterization of multidrug-resistant extended-spectrum beta-lactamase-producing bacteria from healthy and diseased dogs and cats admitted to a veterinary hospital in Brazil. Microb Drug Resist, 27(6): 855-864, doi: 10.1089/mdr.2020.0043

Shahcheraghi F, Shakibaie MR and Noveiri H, 2010. Molecular identification of ESBL genes $bla_{GES-1}$, $bla_{VEB-1}$, $bla_{CTX-M}$ $bla_{OXA-1}$, $bla_{OXA-4}$, $bla_{OXA-10}$ and $bla_{PER-1}$ in *Pseudomonas aeruginosa* strains isolated from burn patients by PCR, RFLP and sequencing techniques. Int J Biollife Sci, 3(6): 138-142

Spilker T, Coenye T, Vandamme P and LiPuma JJ, 2004. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. J Clin Microbiol, 42(5): 2074-2079, doi: 10.1128/jcm.42.5.2074-2079

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