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CASE REPORT
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Chronic Superficial Infection in a Dog caused by Multidrug-Resistant *Pseudomonas aeruginosa*

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ABSTRACT

***Background*:** *Pseudomonas aeruginosa* is a gram-negative aerobic bacterium and non-glucose fermenting, that usually causes opportunistic infections in animals, including humans. It is rarely involved in primary disease. The antibiotic-resistant bacterial strains are mainly developed due to the inappropriate use of antibiotics, however treating *P. aeruginosa* infections can be difficult owing to their natural resistance to antibiotics. Furthermorer resistant microorganisms such as *P. aeruginosa* grow by developing biofilms. Inaccurate diagnoses and absence of adequate microbiological tests can cause difficulties in resolving cases. This report describes a case of chronic superficial infection in a bitch caused by multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA).

***Case*:** A 6-year-old bitch Shih Tzu, initially presented with an exudative erythematous lesion in the snout region, which progressed to deep lesions, and spread to the back and limbs; furthermore, the animal always experienced a fever before new wounds emerged. Lesion samples, collected using a swab and processed at the Veterinary Microbiology Laboratory of the Federal University of Jatai (UFJ), revealed the presence of *Pseudomonas aeruginosa*. The isolate was multidrug-resistant and a carrier of *TEM* and *ppyR* genes. In the diffusion disk antibiogram, the isolate was found resistant to 14 different antibiotics belonging to 6 classes. Antimicrobial resistance was also tested using the minimum inhibitory concentration (MIC) test against imipenem, ceftazidime, ciprofloxacin, ticarcillin + clavulanic acid and aztreonam present in the MIC test strip. Treatment with amikacin and muporicin proved to be effective; however, owing to lesions extending to the face and palpebral involvement, the animal lost its eyeballs.

***Discussion*:** *Pseudomonas aeruginosa* is frequently associated with nosocomial infections mainly affecting immunosuppressed patients. Among the antibiotics tested, the group with the highest number of ineffective antibiotics was beta-lactams, where sensitivity was only observed for ticarcillin and ceftazidime. Recent studies have demonstrated that ceftazidime can reduce biofilm volume, inhibit motility, and repress the expression of genes associated with bacterial adhesion in *P. aeruginosa*. Therefore, the production of biofilm in *P. aeruginosa* is an important virulence factor as it facilitates a stable environment for the microorganism, which protects the bacteria from contact with antimicrobials. In addition, prolonged exposure to a wide variety of antimicrobials creates an environment of selective pressure between microorganisms, facilitating the emergence of multidrug-resistant strains. Furthermore, it is now well recognized that low doses of antibiotics, administered during continuous and fluctuating treatments, can stimulate biofilm establishment and are partly responsible for biofilm-specific antimicrobial tolerance. The resistance profile of *P. aeruginosa* isolated from dogs varies considerably, and the presence of isolates with a possible biofilm production capacity represents a challenge for the interpretation of the antimicrobial susceptibility profile. Culture and antibiogram is fundamentally important, both clinically and in environmental monitoring, in addition to the use of antibiogram data for decision making in clinical treatment.

Keywords: antimicrobial resistance, susceptibility profile, MDR-PA, biofilm, exudative erythematous lesion.

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative microorganism, a member of the skin microbiota of healthy animals and humans, and its ubiquitous nature largely determines its ecological success as an opportunistic pathogen owing to its high resistance to environmental factors [1]. Although healthy organisms are rarely infected, it represents a serious risk in hospital infections, being the second most frequent cause of skin infections in burn patients and an extremely common one of skin infections in dogs and cats [11].

These difficult-to-treat infections are a challenge in veterinary medicine because of the wide environmental distribution of the microorganism, intrinsic resistance to several antibiotics, and high ability to acquire resistance and virulence mechanisms (mainly the ability to form biofilms). It is a challenging pathogen to culture in cases of acute and chronic canine otitis and occasionally in cases of deep pyoderma. The spread of antimicrobial resistance, particularly carbapenem resistance, is a serious therapeutic challenge worldwide [12]. In this case report, a chronic superficial infection in a bitch is described, as well as the genotypic and phenotypic profile of *P. aeruginosa* isolated from the lesion.

CASE

Samples were collected from a 6-year-old bitch Shih Tzu. The animal initially presented with an exudative erythematous lesion in the snout region, which progressed to deep lesions, spread to the back and limbs, and the dog always experienced fever before emergence of new wounds (Figure 1). The animal was treated at a veterinary clinic in Jataí, GO, Brazil. The sample was collected with a swab moistened with 2 mL of brain heart infusion (BHI)[1]. The samples were processed at the Veterinary Microbiology Laboratory of the Federal University of Jatai (UFJ).

The BHI tube containing the swab was incubated at 37°C for 24 h. The swab was depleted on *Pseudomonas* Agar P (PAP)[2] and F (PAF)[1] plates and incubated at 37°C for 24 - 48 h. From each plate that exhibited bacterial growth, 3 colonies exhibiting characteristics compatible with the *Pseudomonas* genus were randomly selected, stained by the Gram method, inoculated in triple sugar iron (TSI)[3] tubes, and incubated at 37°C for 24 h. The isolates were biochemically identified as belonging to the species *P. aeruginosa* through nitrate to nitrite reduction, motility and oxidase tests, growth at 42°C, pigment production, and alkalinization of acetamide. Antimicrobial resistance was tested using the minimum inhibitory concentration (MIC) test against imipenem (0.002-32 µg/mL), ceftazidime (0.016-256 µg/mL), ciprofloxacin (0.002-32 µg/mL), ticarcillin + clavulanic acid (0.016-256 µg/mL; 2 µg/mL), aztreonam (0.016-256 µg/mL) and gentamicina (0.016-256 µg/mL) present in the MIC test strip[4]. Disk diffusion were developed for the following antibiotics: ampicillin (10 µg), ceftriaxone (30 µg), cefaclor (30 µg), cephalothin (30 µg), cephalexin (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), clindamycin (2 µg), doxycycline (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), penicillin (10 U), oxacillin (1 µg), sulfa + trimethoprim (1.25/23.75 µg) and tetracycline (30 µg).

Bacterial DNA was obtained as follows: the strain was cultured in BHI broth for 12 h at 35°C and then centrifuged at 3.074 *g* for 4 min. The supernatant was discarded and the pellet was washed three times with 200 µL TE buffer. Subsequently, the pellet was resuspended in 100 µL TE buffer, and the microtubes were heated at 95°C in a water bath for 10 min and then centrifuged at 3.074 *g* for 20 s. The supernatant (100 µL) was transferred to a microtube, frozen at -20°C, and stored. The detection of beta-lactamase (β-lactamase) production genes (*TEM*, *SHV*, and *CTX-M*), biofilm formation genes (*pelA*, *pslA*, *ppyR*), primers, and conditions, was performed according the authors [3,20].

Veterinarians have reported unsuccessful antimicrobial treatment prior to microbiological diagnosis. Post culture/antibiogram, the treatment was based on the application of amikacin[5] [Sulfato de Amicacina - 10 mg/kg, BID, for 14 days, IM], muporicin[6] [Bactroban® - 20 mg/g, topical ointment], prednisolone[7] [Prediderm® - 1 mg/kg, for 10 days], dipyrone[8] [Dipirona Sódica Genérico - 25 mg/kg, for 5 days], and laser therapy [5 Joule, as an adjuvant in dermal lesions, for 10 sessions]. Treatment with amikacin and muporicin has proved to be effective; however, owing to the lesions extending to the face and palpebral involvement, the animal lost its eyeballs (Figure 2).

DISCUSSION

Pseudomonas aeruginosa is frequently associated with nosocomial infections, and mainly affects immunosuppressed patients. In veterinary medicine, infections caused by *P. aeruginosa* are increasing and related to intrinsic or acquired resistance mechanisms, which limit the choice of effective agents [26].

Pseudomonas aeruginosa was identified in the collected sample. The isolate was demonstrated to be multidrug-resistant and a carrier of *TEM* (β-lactamase production) and *ppyR* (biofilm production capacity) genes (Table 1). In the diffusion disk antibiogram, the isolate was resistant to 14 different antibiotics belonging to 6 classes (β-lactams, lincosamides, tetracyclines, quinolones, macrolides, and sulfa drugs). Among the antibiotics tested based on minimum inhibitory concentration, the isolate was sensitive to 6 drugs (Table 1). In general,

Table 1. Pathotype and resistance profile by disc-diffusion and minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa* strain isolated from a 6-year-old bitch Shih Tzu.

Antibiotics (disc diffusion)	MIC						Resistance genes			Biofilm formation gene
Resistance: Ampicilin; Cefrtiaxone; Cefachlor; Cephalothin; Cephalexin; Cefotaxime; Cefoxitin; Clindamycin; Doxycycline; Enrofloxacin; Erythromycin; Penicillin; Oxacillin; Sulfa+Trimethoprim; Tetracycline.	Imipenem	Aztreonam	Gentamicin	Ciprofloxacin	Ceftazidime	Ticarcilin + Clavulanic acid	TEM	SHV	CTX-M	ppyR
Susceptibility: Amikacin; Meropenem	4.0	6.0	3.0	1.5	2.0	16.0	●	-	-	●

Figure 1. A 6-year-old bitch Shih Tzu, with exudative erythematous lesion on the muzzle (A) and paws (B).

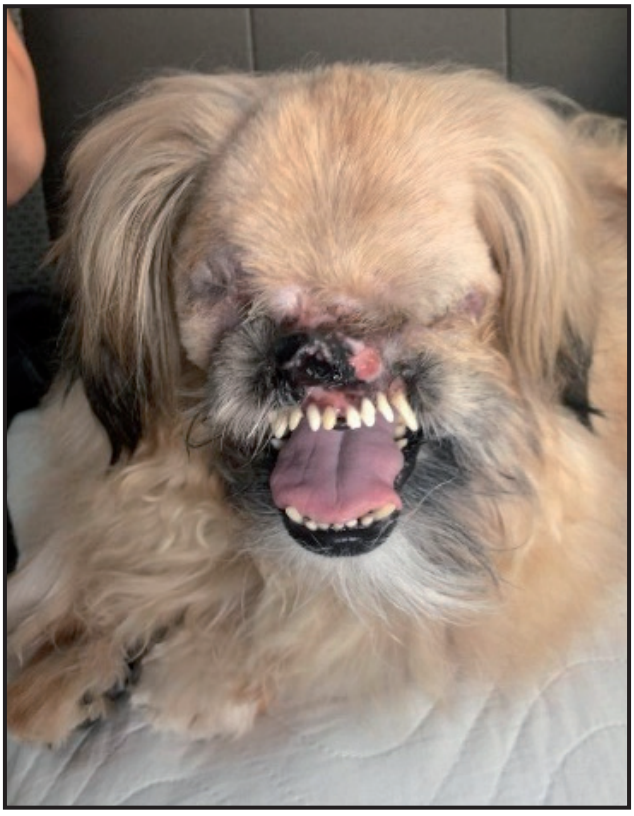

Figure 2. A 6-year-old bitch Shih Tzu, after treatment.

regardless of the technique used (disk diffusion or MIC), sensitivity to carbapenems (imipenem and meropenem) and aminoglycosides amicacin and gentamicin) was observed. The isolate was also sensitive to the antibiotic aztreonam, a monobactam. Among the quinolones, the profile varied, and the isolate proved to be sensitive to ciprofloxacin and resistant to enrofloxacin, depending on the type of quinolone or technique used, which had been previously reported in isolates from dogs [19].

However, this sensitivity is not sustainable as it is known that the MIC of an effective antimicrobial in sessile bacteria has 10 to 1,000 times greater concentration than that active in its planktonic form [24]. This decrease in antimicrobial susceptibility may be caused by several factors. For example, this may be either inherent to the biofilm organization itself (structure and functioning), or acquired through the transmission of resistance factors [16].

The group with the highest number of ineffective drugs was β-lactams (11 antibiotics were tested), with sensitivity observed only for 2 drugs such as ticarcillin and ceftazidime. Recent studies have shown that ceftazidime can reduce biofilm volume, inhibit motility, and repress the expression of genes associated with bacterial adhesion in *P. aeruginosa* [17,23]. The broad resistance of this group can be explained by the presence of the *TEM* gene in the isolate (Table 1), already reported to promote the production of β-lactamase enzymes, which act by inactivating the antibiotics to this group. The results of this case report and other studies suggest that resistance to β-lactams is common among *P. aeruginosa* [2,21,22,25,26].

The resistance profile of *P. aeruginosa* isolated from dogs varied considerably. The resistance of *P. aeruginosa* to fluoroquinolones and cephalosporins in infections of companion animals has been reported in Japan, and the authors speculated that the degree of resistance to cefotaxime, orbifloxacin, and enrofloxacin was greatly affected by efflux pump inhibitors, indicating that overexpression of the efflux pump contributes to this resistance [8]. Other authors have identified MDR isolates with significant resistance to sulfa, tetracycline, and aminoglycosides [4]. Research on isolates from superficial infections reported resistance mainly to ciprofloxacin, piperacycline+tazobactam, tobramycin, imipenem, meropenem, and cefepime [5]. The importance of *P. aeruginosa* isolates from veterinary clinical cases is emphasized with regard to the possibility of the dissemination of carbapenemase-producing clones [10].

Biofilms are complex microbial communities that contain microcolonies surrounded by a self-generated extracellular polysaccharide (EPS) matrix. Biofilm cells exhibit higher resistance to environmental pressures, such as antimicrobial agents, than their planktonic forms [7,27]. Therefore, biofilm production in *P. aeruginosa* is an important virulence factor that facilitates the creation of a stable environment for the microorganism, which protects the bacteria from contact with antimicrobials. Similarly, other authors have reported a significant correlation between biofilm formation and antibiotic resistance in *P. aeruginosa* [13]. The isolate identified in this case report carried the *ppyR* gene, which is strongly related to biofilm production in *P. aeruginosa* [7,13,20]. The involvement of *P. aeruginosa* in a wide range of infections related to biofilm production frequently leads to treatment failure [16,27]. This extraordinary ability of *P. aeruginosa* to form biofilms is promoted by a sophisticated cell communication system called quorum sensing, which functions hierarchically and can be actively induced by increasing cell density or nutrient limitation [27].

The use of amikacin for the treatment of multidrug-resistant *P. aeruginosa* infection has already been

reported [18] in a dog with pneumonia. Isolates from dogs with systemic infections are also sensitive to this antibiotic [26]. In general, aminoglycosides, such as amikacin, prevent the early adherence of clinical strains of *P. aeruginosa* at various concentrations [15]. However, there are reports of isolates from clinical cases that are resistant to this antimicrobial agent in South Africa [25].

Notably, the absence of a microbiological diagnosis with the resistance profile of the isolate led to the spread of the infection in this case, which could have been prevented. In addition, prolonged exposure to a significant variety of antimicrobials creates an environment of selective pressure between microorganisms, facilitating the emergence of multidrug-resistant strains. Furthermore, it is well recognized that low doses of antibiotics administered during continuous and fluctuating treatments can stimulate biofilm establishment and are partly responsible for biofilm-specific antimicrobial tolerance [16].

The importance of culture and antibiograms, both clinically and in environmental monitoring, has been reinforced by multiple authors. There is a need to monitor antimicrobial resistance among bacterial pathogens in animals and the value of antibiograms as a basis for decision making in clinical treatment [14]. Furthermore, the presence of isolates with a possible biofilm production capacity represents a challenge for the interpretation of antimicrobial susceptibility profiles [9]. The importance of antimicrobial susceptibility testing (AST) in *P. aeruginosa* isolates from dogs with superficial infections is highlighted as an adequate treatment plan [5]. Prolonged exposure of the animal to different antimicrobial therapies, owing to inadequate clinical management, can stimulate the production of biofilms by the strains causing the infection. This may lead to genetic exchange and the emergence of increasingly resistant isolates. Furthermore, these isolates pose a risk of environmental contamination and zooanthroponotic transmission [6].

In this case report, multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) was identified, with a marked resistance to antibiotics of the β-lactam group, carrying genes related to the production of β-lactamases and biofilms. The isolate was identified as a persistent infection that initially originated from an exudative erythematous lesion in a dog. Multidrug-resistant bacteria are a challenge in veterinary medical therapy, mainly because of the limited availability of effective drugs, as well as the absence of an antimicrobial susceptibility test that can result in inadequate therapy, causing difficulties in resolving the infection.

MANUFACTURERS

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REFERENCES

1 **Andonova M. & Urumova V. 2013.** Immune surveillance mechanisms of the skin against the stealth infection strategy of *Pseudomonas aeruginosa*. *Comparative Immunology, Microbiology and Infectious Disease*. 36(5): 433-448.

2 **Ansari S., Dhital R., Shrestha S., Thapa S., Puri R., Chaudhary N., Khatiwada S. & Gautam R. 2016.** Growing menace of antibacterial resistance in clinical isolates of *Pseudomonas aeruginosa* in Nepal: an insight of beta-lactamase production. *BioMed Research International*. 2016: 1-8. DOI: 10.1155/2016/6437208

3 **Bajpai T., Pandey M., Varma M. & Bhatambare G.S. 2017.** Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. *Avicenna Journal of Medicine*. 7(1): 12-16. DOI: 10.4103/2231-0770.197508

4 **Biçakcioğlu T., Yörük Ş. & Müştak H.K. 2021.** Antibiotic resistance profiles of Pseudomonas aeruginosa strains isolated from dogs with otitis externa. *Etlik Veteriner Mikrobiyoloji Dergisi*. 32(2): 118-123.

5 **Dégi J., Moţco O.A., Dégi D.M., Suici T., Mareş M., Imre K. & Cristina R.T. 2021.** Antibiotic susceptibility profile of *Pseudomonas aeruginosa* canine isolates from a multicentric study in Romania. *Antibiotics*. 10(7): 846. DOI: 10.3390/antibiotics10070846

6 **Fernandes M.R., Sellera F.P., Moura Q., Carvalho M.P., Rosato P.N., Cerdeira L. & Lincopan N. 2018.** Zooanthroponotic transmission of drug-resistant *Pseudomonas aeruginosa*, Brazil. *Emerging Infectious Diseases*. 24(6): 1160-1162.

7 **Ghazalibina M., Morshedi K., Farahani R.K., Babadi M. & Khaledi A. 2019.** Study of virulence genes and related with biofilm formation in *Pseudomonas aeruginosa* isolated from clinical samples of Iranian patients: A systematic review. *Gene Reports*. 17: 100471. DOI: 10.1016/j.genrep.2019.100471

8 **Harada K., Arima S., Niina A., Kataoka Y. & Takahashi T. 2012.** Characterization of *Pseudomonas aeruginosa* isolates from dogs and cats in Japan: current status of antimicrobial resistance and prevailing resistance mechanisms. *Microbiology and Immunology*. 56(2): 123-127.

9 **Hattab J., Mosca F., Di Francesco C.E., Aste G., Marruchella G., Guardiani P. & Tiscar P.G. 2021.** Occurrence, antimicrobial susceptibility, and pathogenic factors of *Pseudomonas aeruginosa* in canine clinical samples. *Veterinary World*. 14(4): 978-985.

10 **Hayashi W., Izumi K., Yoshida S., Takizawa S., Sakaguchi K., Iyori K., Minoshima K., Takano S., Kitagawa M., Nagano Y. & Nagano N. 2021.** Antimicrobial Resistance and Type III Secretion System Virulotypes of *Pseudomonas aeruginosa* Isolates from Dogs and Cats in Primary Veterinary Hospitals in Japan: Identification of the International High-Risk Clone Sequence Type 235. *Microbiology Spectrum*. 9(2): e00408-21. DOI: 10.1128/Spectrum.00408-21

11 **Hillier A., Alcorn J.R., Cole L.K. & Kowalski J.J. 2006.** Pyoderma caused by *Pseudomonas aeruginosa* infection in dogs: 20 cases. *Veterinary Dermatology*. 17(6): 432-439.

12 **Hyun J.E., Chung T.H. & Hwang C.Y. 2018.** Identification of VIM 2 metallo β lactamase producing *Pseudomonas aeruginosa* isolated from dogs with pyoderma and otitis in Korea. *Veterinary Dermatology*. 29(3): 186-e68. DOI: 10.1111/vde.12534

13 **Karballaei Mirzahosseini H., Hadadi-Fishani M., Morshedi K. & Khaledi A. 2020.** Meta-analysis of biofilm formation, antibiotic resistance pattern, and biofilm-related genes in *Pseudomonas aeruginosa* isolated from clinical samples. *Microbial Drug Resistance*. 26(7): 815-824.

14 **Lin D., Foley S.L., Qi Y., Han J., Ji C., Li R., Wu C., Shen J. & Wang Y. 2012.** Characterization of antimicrobial resistance of *Pseudomonas aeruginosa* isolated from canine infections. *Journal of Applied Microbiology*. 113(1): 16-23.

15 **Olivares E., Badel-Berchoux S., Provot C., Jaulhac B., Prévost G., Bernardi T. & Jehl F. 2017.** Tobramycin and amikacin delay adhesion and microcolony formation in *Pseudomonas aeruginosa* cystic fibrosis isolates. *Frontiers in Microbiology*. 8: 1289. DOI: 10.3389/fmicb.2017.01289

16 **Olivares E., Badel-Berchoux S., Provot C., Prévost G., Bernardi T. & Jehl F. 2020.** Clinical impact of antibiotics for the treatment of *Pseudomonas aeruginosa* biofilm infections. *Frontiers in Microbiology*. 10: 2894. DOI: 10.3389/fmicb.2019.02894

17 **Otani S., Hiramatsu K., Hashinaga K., Komiya K., Umeki K., Kishi K. & Kadota J.I. 2018.** Sub-minimum inhibitory concentrations of ceftazidime inhibit *Pseudomonas aeruginosa biofilm* formation. *Journal of Infection and Chemotherapy*. 24(6): 428-433.

18 **Park K.M., Nam H.S. & Woo H.M. 2013.** Successful management of multidrug-resistant *Pseudomonas aeruginosa* pneumonia after kidney transplantation in a dog. *Journal of Veterinary Medical Science*. 75(11): 1529-1533.

19 **Pintarić S., Matanović K. & Martinec B.Š. 2017.** Fluoroquinolone susceptibility in *Pseudomonas aeruginosa* isolates from dogs-comparing disk diffusion and microdilution methods. *Veterinarski Arhiv*. 87(3): 291-300.

20 **Pournajaf A., Razavi S., Irajian G., Ardebili A., Erfani Y., Solgi S., Yaghoubi S., Rasaeian A., Yahyapour Y., Kafshgari R., Shoja S. & Rajabnia R. 2018.** Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis *Pseudomonas aeruginosa* isolates. *Le Infezioni in Medicina*. 26(3): 226-236.

21 **Rafiee R., Eftekhar F., Tabatabaei S.A. & Tehrani D.M. 2014.** Prevalence of extended-spectrum and metallo β-lactamase production in AmpC β-lactamase producing *Pseudomonas aeruginosa* isolates from burns. *Jundishapur journal of microbiology*. 7(9): 1-6.

22 **Rafiee R., Eftekhar F., Tabatabaei S.A. & Minaee-Tehrani D. 2016.** Detection of AmpC and extended-spectrum beta-lactamases in clinical isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *Medical Laboratory Journal*. 10(3): 28-32.

23 Roudashti S., Zeighami H., Mirshahabi H., Bahari S., Soltani A. & Haghi F. 2017. Synergistic activity of sub-inhibitory concentrations of curcumin with ceftazidime and ciprofloxacin against *Pseudomonas aeruginosa* quorum sensing related genes and virulence traits. *World Journal of Microbiology and Biotechnology*. 33(3): 1-8.

24 Schurek K.N., Breidenstein E. & Hancock R.E. 2012. *Pseudomonas aeruginosa*: a persistent pathogen in cystic fibrosis and hospital-associated infections. In: Dougherty T. & Pucci M. (Eds). *Antibiotic Discovery and Development*. Boston: Springer, pp.679-715.

25 Sebola D., Eliasi U.L., Oguttu J.W. & Qekwana D.N. 2020. Antimicrobial resistance patterns of *Pseudomonas aeruginosa* isolated from canine clinical cases at a veterinary academic hospital in South Africa. *Journal of the South African Veterinary Association*. 91(1): 1-6.

26 Silva L.C.A., Pessoa D.A.N., Maia L.Â., Matos R.A.T. & Silva M.M.M. 2016. Systemic infection by *Pseudomonas aeruginosa* in a dog. *Acta Scientiae Veterinariae*. 44(Suppl 1): 164. 5p.

27 Thi M.T.T., Wibowo D. & Rehm B.H. 2020. *Pseudomonas aeruginosa* biofilms. *International Journal of Molecular Sciences*. 21(22): 8671. DOI: 10.3390/ijms21228671