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Lifestyle changes in wild and veterinary isolates of *Pseudomonas aeruginosa* exposed to three veterinary care antibiotics

Mudanças no estilo de vida de isolados selvagens e veterinários de Pseudomonas aeruginosa expostos a três antibióticos de uso veterinário

Cambios en el estilo de vida en aislados silvestres y veterinarios de Pseudomonas aeruginosa expuestos a tres antibióticos de uso veterinario

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ABSTRACT

This study aimed to evaluate the planktonic and sessile activity of wild and veterinary *Pseudomonas aeruginosa* isolates exposed for 4 h to veterinary formulations composed of florfenicol (FLO) and sulfamethoxazole+trimethoprim (SXT), under different levels of Chemical Oxygen Demand (COD). Additional tests for pyocyanin production, antibiogram and cell wall hydrophobicity were performed. All isolates produced up to 3.26 µg/L of

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pyocyanin and had a strongly hydrophobic cell wall. Both the COD of 20,000 mg/L and the presence of FLO favored inhibition of cell adhesion by up to ≈45%, and no antibiofilm effect was found. FLO/SXT in concentrations 0.30/0.24 mg/mL exhibited greater activities. An increase of up to ≈16% was observed in the presence of FLO and lower percentages in the presence of SXT. There was a significant increase in planktonic cells by up to ≈32%. Two factors may have accounted for this: active detachment in the biofilm and duplication of tolerant cells, as swimming and running velocities were reduced but the cells remained viable (SXT > FLO). The results of the antibiogram indicated that veterinary isolates of *P. aeruginosa* were more sensitive to the antibiotics than wild isolates, however, in the *in vitro* biofilm formation test wild isolates were more susceptible to alteration than veterinary isolates. In the medium with a higher organic matter content, however, the switch from the sessile to the planktonic state seems to have served as a survival strategy for *P. aeruginosa* in subinhibitory concentrations of FLO and SXT.

Keywords: Microbial lifestyle; Biofilm; Florfenicol.

RESUMO

Este estudo teve como objetivo avaliar a atividade planctônica e séssil de isolados silvestres e veterinários de *Pseudomonas aeruginosa* expostos por 4 h à formulações veterinárias compostas de florfenicol (FLO) e sulfametoxazol+trimetoprima (SXT), sob diferentes níveis de Demanda Química de Oxigênio (DQO). Foram realizados testes adicionais para produção de piocianina, antibiograma e hidrofobicidade da parede celular. Todos os isolados produziram até 3,26 µg/L de piocianina e apresentaram parede celular fortemente hidrofóbica. Tanto o DQO de 20.000 mg/L como a presença de FLO favoreceram a inibição da adesão celular em até ≈45%, e nenhum efeito antibiofilme foi encontrado. FLO/SXT nas concentrações 0,30/0,24 mg/mL exibiram as maiores atividades. Foi observado um aumento de até ≈16% na presença de FLO e percentuais menores na presença de SXT. Houve um aumento significativo nas células planctônicas em até ≈32%. Dois fatores podem ter sido responsáveis por isso: descolamento ativo no biofilme e duplicação de células tolerantes, pois as velocidades de natação e corrida foram reduzidas, mas as células permaneceram viáveis (SXT > FLO). Os resultados do antibiograma indicaram que os isolados veterinários de *P. aeruginosa* foram mais sensíveis aos antibióticos do que os isolados selvagens, porém, no teste *in vitro* de formação de biofilme os isolados selvagens foram mais suscetíveis a alterações do que os isolados veterinários. No meio com maior teor de matéria orgânica, entretanto, a passagem do estado séssil para o planctônico parece ter servido como estratégia de sobrevivência para *P. aeruginosa* em concentrações subinibitórias de FLO e SXT.

Palavras-chave: Estilo de vida microbiano; Biofilme; Florfenicol.

RESUMEN

Este estudio tuvo como objetivo evaluar la actividad planctónica y sésil de aislados silvestres y veterinarios de *Pseudomonas aeruginosa* expuestos durante 4 h a formulaciones veterinarias compuestas de florfenicol (FLO) y sulfametoxazol+trimetoprima (SXT), bajo diferentes niveles de Demanda Química de Oxígeno (DQO). Se realizaron pruebas adicionales para la producción de piocianina, antibiograma e hidrofobicidad de la pared celular. Todos los aislados produjeron hasta 3,26 µg/L de piocianina y tenían una pared celular fuertemente hidrófoba. Tanto la DQO

de 20.000 mg/L como la presencia de FLO favorecieron la inhibición de la adhesión celular hasta ≈45%, y no se encontró efecto antibiofilm. FLO/SXT en concentraciones de 0,30/0,24 mg/mL exhibió mayores actividades. Se observó un aumento de hasta ≈16% en presencia de FLO y porcentajes menores en presencia de SXT. Hubo un aumento significativo de células planctónicas de hasta ≈32%. Dos factores pueden haber explicado esto: el desprendimiento activo en lo biofilm y la duplicación de células tolerantes, ya que las velocidades se redujeron, pero las células permanecieron viables (SXT > FLO). Los resultados del antibiograma indicaron que los aislados veterinarios de *P. aeruginosa* eran más sensibles a los antibióticos que los aislados silvestres; sin embargo, en la prueba de formación de biopelículas *in vitro* los aislados silvestres fueron más susceptibles a la alteración que los aislados veterinarios. Sin embargo, en el medio con mayor contenido de materia orgánica, el cambio del estado sésil al planctónico parece haber servido como estrategia de supervivencia para *P. aeruginosa* en concentraciones subinhibitorias de FLO y SXT.

Palabras clave: Estilo de vida microbiano; Biofilm; Florfenicol.

INTRODUCTION

Bacterial resistance is a natural microbial behavior that can be a threat to global public health when it results from exposure to antimicrobials (Mancuso et al., 2021). Antibiotics for veterinary use often belong to the same classes as antimicrobials used with humans. They exceed twice the volume of antibiotics used in humans (Kim & Ahn, 2022), however, the research on the administration of antibiotics in veterinary activities is a very neglected problem (Ardakani et al., 2023). Antibiotics pressure microbes to adapt, effectively contributing to the selection and propagation of resistant bacteria, as has been reported in veterinary care environments (Schimmunech et al., 2022), zoos (Min et al., 2023), aquaculture farms (Gazal et al., 2020) and related to livestock activities (Berman et al., 2023).

More than eleven classes of antibiotics for use in veterinary care environments have been identified, such as beta-lactams, fluoroquinolones, tetracyclines, macrolides, amphenicols and sulfonamides (Cheng et al., 2019; Cui et al., 2018; Holmström et al., 2003). In addition, effluents generated on farms can transport pharmacologically active compounds into aquatic systems (Heberer, 2002), contributing to shaping the microbial communities present in them, in terms of resistance to antimicrobials (Bojarski et al., 2020).

The level of organic matter present in water can determine the abundance of antimicrobial-resistant cells organized in biofilm (Wang et al., 2019). Resistant bacteria have been reported in samples from a river adjacent to a swine waste composting facility

(Awad et al., 2014). In addition, there is an increase in the frequency of bacteria resistant to sulfamethoxazole + trimethoprim and florfenicol on aquaculture farms (Cabello et al., 2013). Florfenicol is used in more than 80% of cases, being administered to small, medium and large mammals, birds, crustaceans and fish (Trif et al., 2023).

In terms of antibiotic resistance, *Pseudomonas aeruginosa* is an opportunistic pathogen that requires attention. It exhibits three main antibiotic resistance mechanisms: reduced outer membrane permeability, multidrug efflux systems, and intrinsic resistance (Pang et al., 2019). Furthermore, *P. aeruginosa* is the most common non-fermenting Gram-negative rod isolated from hospital environments (Oliveira et al., 2021), being recognized by the World Health Organization as a pathogen, for which priority must be given to develop new antibiotics to prevent infections (Tacconelli et al., 2018).

In the field of veterinary medicine, *P. aeruginosa* has been identified as a cause of chronic otitis externa, pyoderma, conjunctivitis, septicemia, lower urinary tract infections, pneumonia, and bacterial endocarditis (Eliasi et al., 2020). *P. aeruginosa* exhibits the ability to express a wide range of virulence factors, such as pyocyanin, enzyme production, motility and biofilm formation (Gonçalves & Vasconcelos, 2021). The present work aimed to isolate *P. aeruginosa* from water samples (from a university veterinary hospital and a reservoir) and to assess the effect of exposure to veterinary care pharmaceuticals on bacterial lifestyle and virulence factors.

MATERIAL AND METHODS

Antibiotics

Two prescribed veterinary pharmaceuticals were purchased: injectable Vetflor® (JA Saúde Animal, Patrocínio Paulista-SP, Brazil), composed of florfenicol (FLO) 30%, in a 30 mL vial; and injectable Trissulfin® (Ourofino, Cravinhos-SP, Brazil), composed of 10 g of sulfamethoxazole + 2 g of trimethoprim (SXT), in a 50 mL vial.

Pseudomonas aeruginosa

In September 2022, six isolates were recovered from water samples (500 mL) by using the method 9213F (APHA et al., 2012). Four isolates from tap water from the University Veterinary Hospital (6.84243° S, 38.29885° W): small animal outpatient clinic (JVD05, SisGen #A8A9D5A); hospital laundry (JVD06, SisGen #A048BA8); food laboratory (JVD03, SisGen #AAA6559); and women's bathroom (JVD04, SisGen #A78C5F3); and two isolates from fresh water samples from water bodies in the municipality of Sousa,

the Alto Sertão zone of the state of Paraíba, Brazil, from the São Gonçalo reservoir (6.85187° S, 38.32468° W) (JVD07, SisGen #AD6CFAA and JVD08, SisGen # AC65073). *P. aeruginosa* was confirmed by the production of pyocyanin in cetrimide agar after incubation for 48h at 36±1 °C.

Extraction and quantification of pyocyanin

The isolates were inoculated on the surface of cetrimide agar (20 mL) and incubated for 48h at 36±1°C. The pigment was extracted using the solid-base extraction method (Devnath et al., 2017). The surface of the agar was washed with sterilized distilled water and the agar was cut into small cubes, transferred to centrifuge tubes with a capacity of 50 mL, to which 20 mL of 99% chloroform were added (Quemis, Joinville, Brazil). The tubes were vortexed for 10 min. The organic phase (blue) was separated and acidified with 0.2 mol/L HCl (red). After a brief rest, the acidified phase was neutralized with drops of 1.5 mol/L Tris-Base buffer solution, returning the blue color. The optical density of this solution (OD) was measured at λ= 520 nm (Kasvi, K37-VIS) and pyocyanin concentration (PYO, in µg/L) was determined by the equation 1:

$$PYO = [(OD_{520} - 6x10^{-4}) \div 14.026) \times 1000] \div 20 \quad (1)$$

Cell wall hydrophobicity test

The MATH assay (Tyfa et al., 2015) was carried out to identify the hydrophobicity of the cell wall. Suspensions of fresh cells of *P. aeruginosa* were prepared in a PBS buffer (pH 7.2), with absorbance varying between 0.4-0.6 at λ= 600 nm (Ai). Then, 0.5 mL of xylene was added to 2.5 mL of the suspension. The sample was vortexed for 1 min and kept for 10 min at 25ºC. After that, the tubes were shaken for 1 min and rested for 50 min. The optical densities of the aqueous phase at λ= 600 nm were measured at intervals of 10 and 60 min (Af) (Kasvi, K37-VIS) and cell wall hydrophobicity was calculated using the equation 2:

$$\%\ hidrophobicity = [(1 - Ai) \div Af] \times 100 \quad (2)$$

Hydrophobicity was classified as strong (≥ 50%), moderate (20-50%) or weak (≤20%).

Synthetic wastewater

The standard solution designed for the *in vitro* biofilm formation test was carefully prepared in order to simulate different levels of water contamination. The composition of synthetic wastewater with Chemical Oxygen Demand (COD) at 20,000 mg/L was (mgL): sucrose (17); yeast extract (3.92); urea (1.24); calcium chloride (0.055); magnesium sulfate (0.045); monopotassium phosphate (0.065); potassium dihydrogen phosphate (0.055) and Iron (III) chloride (0.005) (Reyes-Lara & Reyes-Mazzoco, 2009). The synthetic water was diluted with sterilized distilled water in order to obtain solutions with COD of 400 and 2,000 mg/L.

In vitro tests of biofilm formation

The purpose of the experiment was to determine which isolates were most resistant to FLO and SXT concentrations. The isolates were selected from an antibiogram with 15 antibiotics used in the empirical antipseudomonal therapy (Zakhour et al., 2022). The test was carried out by microdilution technique. The first well was filled with 100 µL of synthetic wastewater (COD = 400; 2,000 and 20,000 mg/L), 100 µL of antibiotic solutions and 10 µL of inoculum prepared in 0.9% sodium chloride solution, with turbidity standardized with tube # 0.5 on the MacFarland scale. Antimicrobials were tested alone and in combination (Table 1). The antibiotic solutions were prepared in DMSO 1%: FLO (0.30 mg/mL) and SXT (0.24 mg/mL), considering, for this formulation, the concentration of the active ingredient, sulfamethoxazole.

The microplates were incubated for 4 h at 36±1ºC. After the incubation, planktonic cells were measured on an automatic microplate reader (BIOTEK ELx800) at λ= 630 nm. Then, each well was carefully emptied to quantify the cells adhered to the wall using the crystal violet test at 590 nm (Batista et al., 2017).

In comparison to the control, the percentage of increase or decrease of the number of planktonic cells (P) and the cell adhesion were calculated using equations 3 and 4:

$$P(\%) = \left[\left(OD_{cp} - OD_{tp}\right) \div OD_{cp}\right] x\ 100 \quad (3)$$

$$A\ (\%) = \left[\left(OD_{cs} - OD_{ts}\right) \div OD_{cs}\right] x\ 100 \quad (4)$$

Where, OD_{cp} = optical density (630 nm) of the control; OD_{tp} = optical density (630 nm) in the test; OD_{cs} = optical density (590 nm) of the control; and OD_{ts} = optical density (590nm) in the test. Cell adhesion was classified according to Charlton (2008) as weak (≤ 40%), moderate (40-80%) or highly adherent (≥80%). Additionally, an antibiofilm effect is considered when inhibition is ≥80%.

Motility test

The assay was carried out with isolates JVD 05 and JVD 07, in a BHI medium to which has been added 0.30 mg/mL FLO and 0.24 mg/mL SXT. Swimming, swarming and twitching motilities were evaluated (Rossi et al., 2018). The plates were incubated for 24 h at 36±1ºC, followed by measuring the diameter of the running zone (mm) from the point of inoculation. The control had no antimicrobials. The cell velocity was calculated by relating the running distance S (µm) to the time t (s), using the following equation 5:

$$V\,{}^{\mu m}/_{s} = S\ (mm) * {}^{1000}/_{t\ (84.6000)} \quad (5)$$

The reduction (%) was calculated using equation 6:

$$\boldsymbol{Red}\ (\%) = [(\boldsymbol{V_c} - \boldsymbol{V_t}) \div \boldsymbol{V_c}]\ \boldsymbol{x}\ \mathbf{100} \quad (6)$$

Where, V_c = cell velocity in the control; and V_t = cell velocity in the test.

Statistical analysis

All tests were carried out in triplicate. The values were expressed as averages. The data were processed using the R software, version 4.2.1, open access, in which the Shapiro-Wilk test was applied to assess the normality of the data. The test result indicated a non-normal distribution. Because the data did not conform to a normal distribution, it was decided to apply the Kruskal-Wallis Test.

Results

Phenotypic characterization of isolates

There was production of pyocyanin (Fig. 1), both wild (JVD 07 and JVD 08) and veterinary isolates (JVD 03, JVD 04, JVD 05 and JVD 06). All isolates exhibited

concentrations lower than 5 µg/L. Pyocyanin production, from highest to lowest concentration, was observed in this order: JVD 05 (3.26 µg/L) > JVD 04 (2.69 µg/L) > JVD 03 (1.46 µg/L) > JVD 07 (1.41 µg/L) > JVD 06 (0.69 µg/L) > JVD 08 (0.56 µg/L). Because JVD 05 exhibited more pyocyanin, it was selected to compare to wild isolates (JVD 07 and JVD 08).

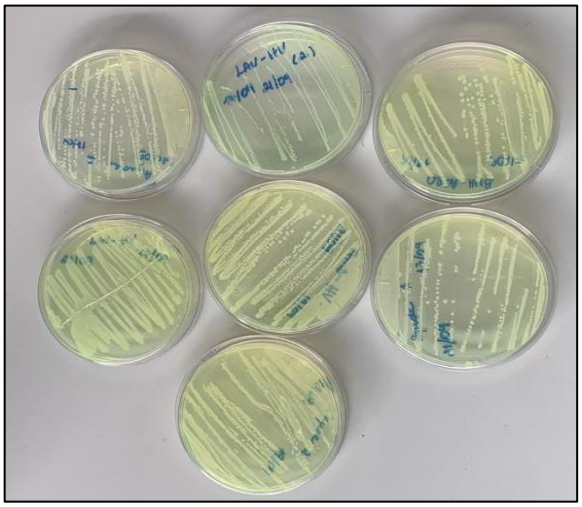

Figure 1. *Pseudomonas aeruginosa* isolates grown on cetrimide agar exhibiting pyocyanin diffusion into the culture medium after 48 h of incubation [source: the authors]

In addition, the homogeneity between the specimens recovered in this study was also observed in the cell wall hydrophobicity test. All isolates were identified as strongly hydrophobic, with percentages ranging between 90 and 110%. The order from highest to lowest percentage: JVD 03 (110%) > JVD 07 (107%) > JVD 05 (106%) > JVD 08 (97%) > JVD 04 (94%) > JVD 06 (90%).

In vitro test of biofilm formation

Table 1 summarizes the results of the test of sessile and planktonic cells, in synthetic wastewater with different levels of organic contamination (20,000 and 2,000 mg/L), compared to raw water (400 mg/L).

The higher COD and the presence of FLO collaborated to inhibit adhesion, which reached approximately 45%, compared to the control, but no antibiofilm effect was observed. On the other hand, adherence increased by up to around 16%. These responses, however, were much more related to the COD content than to the action of antibiotics.

The greatest reductions in COD content were achieved in isolate JVD 08 (FLO/SXT combination of 0.30/0.24 mg/mL). Meanwhile, the greatest increases in adhesion were observed in isolate JVD 07. Although JVD 05 did not improve its adhesion to the surface, the reduction percentages were more than 100% lower than the other isolates (p= 0.05).

Table 1.

Adhesion profile of *Pseudomonas aeruginosa* isolates exposed to antimicrobials at different chemical oxygen demand (COD)

COD (mg/L)	FLO/SXT (mg/mL)	Adhesion (%)					
		JVD 05		JVD 07		JVD 08	
		Bf	Pc	Bf	Pc	Bf	Pc
400	0.00/0.24	-1.2	20.7	1.3	24.4	-34.0	9.9
400	0.30/0.00	-18.4	16.9	**3.1**	25.3	-33.8	9.1
400	0.30/0.24	-22.3	14.1	**4.5**	31.7	-36.7	-1.1
2000	0.00/0.24	**0.8**	29.8	-1.2	9.4	-24.1	7.2
2000	0.30/0.00	-14.1	11.4	-2.1	16.9	-41.5	10.9
2000	0.30/0.24	-10.1	-1.0	**15.8**	11.5	-42.8	2.7
20000	0.00/0.24	**0.9**	17.4	-13.7	14.6	-38.3	14.8
20000	0.30/0.00	**11.8**	17.4	-37.9	19.4	-31.9	19.8
20000	0.30/0.24	-7.7	5.9	-31.1	13.8	-45.6	5.3

Note: FLO: florfenicol, SXT: sulfamethoxazole+trimethoprim; Bf: biofilm cells; PC: planktonic cells. Numbers in bold represent increased adherence compared to control. Negative signs represent percentages of reduction in adherence and/or number of planktonic cells (standard deviation = 0.1).[own elaboration]

In order to verify whether the reduction in adhesion occurred due to cell migration or death, planktonic cell counts were carried out. A significant increase in planktonic cells was observed, between approximately 3 and 32% (p= $5.367x10^{-6}$). The reductions were negligible, reaching 1%, including in those conditions where there was a reduction in adhesion.

There was a significant reduction in motility of the flagella and *pili*, which was reflected in the decrease in the cells' swimming and running velocities, with total interruption of swimming in both isolates (Table 2). The most pronounced effect occurred in JVD 05, compared to the wild isolate JVD 07. On the other hand, when exposed to SXT, the reduction percentages were moderate to low, with significant alterations observed in the swimming velocity of JVD 05. Both antibiotics demonstrated an effect on *P. aeruginosa* with FLO the most effective.

Table 2.

Changes in motility of *Pseudomonas aeruginosa* isolates in 24 h

Motility		JVD 05			JVD 07		
		red. (%)	Velocity (µm/s)		red. (%)	Velocity (µm/s)	
			Control	Test		Control	Test
FLO	Swimming	100.0	0.15	0.00	100.0	0.22	0.00
	Swarming	82.80	0.34	0.06	68.00	0.29	0.09
	Twitching	77.00	0.15	0.03	53.00	0.20	0.09
SXT	Swimming	61.50	0.15	0.06	26.30	0.22	0.16
	Swarming	72.40	0.34	0.09	56.00	0.29	0.13
	Twitching	77.00	0.15	0.03	11.80	0.20	0.17

Note: FLO: florfenicol 0.30 mg/mL, SXT: sulfamethoxazole+trimethoprim 0.24 mg/mL; control (without antibiotics); red: reduction [own elaboration]

DISCUSSION

In this study we evaluated the lifestyle changes and virulence factors of *P. aeruginosa* isolates in response to the exposure to veterinary antimicrobials. Antibiotics are administered to animals for therapeutic, metaphylactic and prophylactic means. Few countries still allow the use of antibiotics dispensed in subtherapeutic doses in feed or water as a growth factor in meat animals (Canton et al, 2021; Muaz et al., 2018).

FLO is a broad-spectrum bacteriostatic compound most used as a specific antibiotic for veterinary care, especially in aquaculture (Qiao et al., 2018; Zhang et al., 2015). The SXT combination (co-trimoxazole) is prescribed for the treatment of various infections in small to large animals. Both drugs carry a high risk of environmental spread because they have a low rate of metabolism in the body (Boxall et al, 2003) and have been reported to contaminate water bodies (He et al., 2018; Straub, 2016).

P. aeruginosa was chosen as the target of the study because it represents a significant concern in aquatic ecosystems (Hajjartabar, 2004). The persistence of the bacteria is guaranteed by its notable viability in environments with a high stress level (Jurado-Martín et al., 2021), being able to develop tolerance to different compounds. For instance, pyocyanin is one of the intrinsic factors involved in tolerance to antibiotics, through mechanisms that are not yet fully understood (Zhu et al., 2019).

According to Nowroozi et al. (2012), the pigment concentration in aquatic isolates of *P. aeruginosa* is lower than in soil isolates. This was consistent with the findings of the present work, where the concentration of pyocyanin was at levels considered very low, i.e., < 5 µg/mL (Abdelaziz et al., 2022), however, consistent with that expected for certain specimens (El-Fouly et al., 2015).

In terms of the cell wall, the property of being strongly hydrophobic implies low affinity for water-soluble molecules and allows the adhesion of *P. aeruginosa* to many surfaces (Colling et al., 2020). This cell wall characteristic also implies a low permeability of the outer membrane (Strateva & Yordanov, 2009). It is estimated that this permeability is 10 to 100 times lower than in *Escherichia coli*, which gives *P. aeruginos*a advantages in terms of resistance to challenging environments (Breidenstein et al., 2011).

The strong hydrophobicity of the cell walls of the isolates may explain what we observed in wild *P. aeruginosa*, in terms of sensibility, compared to the veterinary isolate JVD 05, naturally exposed to antimicrobial stress. Trimethoprim is a bacteriostatic antibiotic, and combined with sulfamethoxazole, a bactericidal effect can be observed (Masters et al., 2003). While sulfamethoxazole is a hydrophilic molecule, both trimethoprim and FLO antibiotics are lipophilic molecules (Mathenge et al., 2017; Zou & Zheng, 2013). This means that they exhibit affinity to the *P. aeruginosa* membrane as well as the micropores on the biofilm surface that are more lipophilic to prevent evaporation or increase repellency to water (Guo et al., 2018), however, resistance proteins will act through intrinsic mechanisms to extrude the antibiotic from the cytoplasm to the periplasm and consequent export to the extracellular environment through efflux pumps (Oliveira-Tintino et al., 2021), as well as the loss of porins in the outer membrane, altering the passage of hydrophilic compounds (Scheffer et al., 2010).

Although the mechanisms are not yet understood, within the same species, cells with both planktonic and sessile lifestyles can respond differently to antibiotics (Penesyan et al., 2020). Planktonic cells can change morphology without demonstrating significant differences in cell viability; sessile cells can actively promote destabilization of the biofilm structure and lead to detachment (Van Laar et al., 2015). Both strategies, however, involve double-edged sword processes because, on the one hand, they expose the cells, but, on the other, parts of the biofilm can be detached as cellular aggregates that can reconstitute a new colony composed of tolerant cells (Petrova & Sauer, 2016). Additionally, although the planktonic cells were viable, their reduction in swimming and consequently in velocity may suggest a change in phenotype as a survival strategy.

Based on the increase in planktonic cells observed, we hypothesized that the reason was the increase in cell migration associated with the growth and maintenance of tolerant cells in the synthetic water due to the nutrient content. Cells organized in

monolayers may detach from the structure as a result of contact with subinhibitory concentrations of antibiotics inside the biofilm (Luo et al., 2017). This can produce coordinated cell signaling events that alter cell phenotypic characteristics, modulated by the intracellular concentration of cyclic di-GMP (Boyd & O'Toole, 2012). Thus, with this altered morphology, cells can detach and restructure themselves into complex three-dimensional biofilms (Dale et al., 2017).

On the other hand, the intrinsic resistance of *P. aeruginosa* to antibiotics may explain the results observed in planktonic cells, which, among some strategies, can extend the doubling time (Balaban et al., 2004), resulting in a gradual increase in the number of cells tolerant to antibiotics (Mangalappalli-Illathu et al., 2008). We have observed that in an aqueous environment with normal levels of COD (400 mg/L), as well as high levels of COD (2,000 and 20,000 mg/L), there was a significant increase in planktonic cells with reduced running velocity up to 50% lower than the reported in the literature for *P. aeruginosa* (Matz & Jürgens, 2005). In the control, velocity was moderate, and very weak after exposure to antibiotics. As the cells remained viable, there was evidence of a reduction in the number of flagellated cells. This can be interpreted as an indicator of the risks associated with the velocity of resistant pathogens in waters with different levels of organic matter. Therefore, it is of great importance to evaluate the impact of the velocity of *P. aeruginosa* in the environment, as well as to develop strategies for treating liquid effluents generated in veterinary facilities.

CONCLUSION

Under the experimental conditions tested, we observed that both veterinary and wild isolates exhibited different responses in terms of exposure to FLO and SXT. The cell wall hydrophobicity profiles and pyocyanin production, however, were similar. There was phenotypic variation with respect to motility and lifestyle that appears to be governed by nutrient concentration and specificities of the isolates.

The reduction of motility and maintenance of viability also demonstrate advantages over the planktonic lifestyle compared to the sessile lifestyle. Although the observations described in this study are not a specific behavior of *P. aeruginosa*, alteration of the phenotypes is a protective advantage of the cells. By considering the entire virulence profile of *P. aeruginosa*, this survival strategy makes this rod successful in terms growth in the presence of FLO and SXT in waters with low to high nutrient concentrations.

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