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Pseudomonas aeruginosa Isolated from the Environment of a Veterinary Academic Hospital in Brazil - Resistance Profile

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ABSTRACT

***Background*:** The presence of resistant and potentially virulent bacterial strains in a veterinary hospital environment is a neglected problem. *Pseudomonas aeruginosa* is an opportunistic microorganism present and circulating in the veterinary hospital environment, of clinical importance and zooanthroponotic transmission of *P. aeruginosa* has also been reported. The aim of this study was to characterize the population of *P. aeruginosa* present in a veterinary hospital environment by evaluating their resistance profile and biofilm production.

***Materials, Methods & Results*:** A total of 306 samples were collected from the veterinary hospital environment (swabs from consultation tables, surgical tables, door handles, hospitalization cages, stethoscopes, thermometers, and muzzles). The isolates were biochemically identified as belonging to the species *Pseudomonas aeruginosa* through nitrate to nitrite reduction, motility and oxidase test, growth at 42°C, pigment production, and alkalinization of acetamide. Antimicrobial resistance was tested using the minimum inhibitory concentration (MIC) test. Twenty seven isolates of *P. aeruginosa* were obtained, with a frequency of 8.8%. The detection of beta-lactamase production and biofilm formation genes by polymerase chain reaction (PCR). Two multidrug resistant (MDR) and 3 single-drug resistant (SDR) strains of *P. aeruginosa* were identified. Furthermore, it was observed that the strains carried genes related to beta-lactamase production (*TEM* and *CTX-M* group 25) and biofilm production (*pelA*, *pslA*, *ppyR*).

***Discussion*:** *Pseudomonas aeruginosa* is considered a major cause of opportunistic hospital infections, as it causes significant morbidity and mortality in immunosuppressed individuals, both in animals and in humans. Veterinary hospitals can harbor microorganisms that cause infections, as well as multiresistant agents. Normally, these environments have a large circulation of people and animals, which particularly enables a facilitated dissemination of these resistant microorganisms. Recently, the World Health Organization (WHO) listed carbapenem-resistant *P. aeruginosa* as one of 3 bacterial species in critical need for the development of new antibiotics to treat their infections. The data found in this work strengthen the knowledge on the antimicrobial resistance capacity that *P. aeruginosa* exhibits. The presence of 3 multiresistant strains further highlights the advanced stage of resistance of this bacterial species. The characterization of strains of this species in a veterinary hospital environment is crucial for the control of this population circulating in this environment, and the consequent adoption of more effective measures aimed at controlling its proliferation. The study of this bacterial species in a veterinary hospital environment has a direct impact on human health, due to the mechanisms of resistance and genetic variability that can occur between infections in different animal species and in humans. In view of that, professionals working in veterinary hospitals should be aware of the importance of controlling these microorganisms. Correct measures must be taken to sanitize the environment and utensils between animal care sessions, besides frequent hand washing by all employees and the use of protective equipment such as masks and gloves. The presence of potentially biofilm-producing MDR and SDR strains indicates the free circulation of these bacteria in the veterinary hospital environment. Thus, as a potentially pathogenic microorganism to humans and animals, containment measures must be taken to prevent this possible transmission.

Keywords: bacteria, antimicrobial resistance, multidrug resistant, beta-lactamase, biofilm, veterinary care.

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INTRODUCTION

Pseudomonas is a ubiquitous genus that also causes human and animal diseases. Most studies have focused on clinical *P. aeruginosa* strains from humans, but research is scarce on animal strains [19]. The bacterium is also an opportunistic microorganism present and circulating in the veterinary hospital environment, of clinical importance (hospital infection and otitis in animals), and zooanthroponotic transmission of *P. aeruginosa* has also been reported [6]. Moreover, One Health links the health of humans with that of animals and the environment [9]. Antibiotic overuse is contributing to an emerging crisis of antimicrobial resistance [4]. Therefore, currently, infections caused by this bacterium are difficult to treat due to various resistance mechanisms and different virulence factors [8]. It exhibits resistance to a wide variety of antimicrobial agents [15]. Animals represent a reservoir of *P. aeruginosa* multiresistant to carbapenems, with strains potentially capable of contaminating the environment being able to infect inpatients [11]. Moreover, the fact different strains of *P. aeruginosa* have the inherent capacity for biofilm formation further boosts their resistance under various environmental factors [15]. Identifying and characterizing these bacterial populations circulating in the veterinary hospital environment is of fundamental importance for us to better understand their ecological and environmental relationships. In this sense, the objective of this work was to identify and characterize resistant and potentially biofilm-producing isolates of *Pseudomonas* in a veterinary hospital environment.

MATERIALS AND METHODS

Sampling

A total of 306 samples of the veterinary hospital environment (swabs from consultation tables, surgical tables, door handles, hospitalization cages, stethoscopes, thermometers, and muzzles) were collected at the Veterinary Hospital of the Federal University of Jataí (UFJ), city of Jataí, state of Goiás, Brazil. Samples were taken using swabs moistened with 2 mL of Brain Heart Infusion (BHI)[1] and swiped over the sampled surfaces to be analyzed.

Bacterial isolation, identification and antimicrobial susceptibility test

The BHI tubes containing the swabs were incubated at 37°C for 24 h. The swabs were depleted on *Pseudomonas* Agar P (PAP)[2] and *Pseudomonas* Agar F (PAF)[3] plates and incubated at 37°C for 24 - 48 h. From each plate showing 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)[4] 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 test, 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), and aztreonam (0.016-256 µg/mL) present in the MIC test strip[5].

Polymerase Chain Reaction (PCR)

Bacterial DNA was obtained as follows: The strains were 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 of TE buffer. Subsequently, the pellet was resuspended in 100 µL of 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 production genes (*TEM*, *SHV*, and *CTX-M*), primers and conditions, was performed according to Bajpai *et al*. [2]; the detection of CTX-M groups was performed according to Woodford *et al*. [25] and biofilm formation genes (*pelA*, *pslA*, *ppyR*) was performed according to Pournajaf *et al*. [18].

RESULTS

A total of 306 samples were collected in the veterinary hospital environment. Of those, 27 samples were positive for *P. aeruginosa* isolates, with a frequency of 8.8%. The isolates and collection sites are identified in Table 1. Through the minimum inhibitory concentration (MIC) of the 27 *P. aeruginosa* isolates, the profiles of antimicrobial resistance were defined, which are also detailed in Table 1. Among the isolates analyzed, a resistant strain from a door handle (isolate 4), resistant to imipenem and to very high concentrations of aztreonam, stands out. Another important isolate was strain 17, from

a cage, resistant to 3 different classes of antibiotics. However, strain 21, also isolated from a cage in the isolation area, was the microorganism with the most pronounced multiresistance profile identified in this work. This isolate was resistant to no less than 4 different classes of antibiotics. It was observed that 11 of the 27 isolates carried the *TEM* gene and 3 isolates carried the *CTX-M* gene. In addition, 2 isolates (4 and 24) carried both genes, related to the production of beta-lactamases. Researching the genes related to biofilm production (*pelA*, *pslA*, *ppyR*), most isolates (20/27) carried at least 1 of them. Isolate 17 stands out for its multiresistance profile as well as for carrying the 3 genes for biofilm production.

Table 1. Pathotypes and minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa* strains.

Isolates	Antibiotic (µg/mL)*					Extended-spectrum β-lactamases genes		Biofilm formation genes	Source	Resistance Profile*
	IMI	AZ	CIP	CEF	TC	*TEM*	*CTX-M*			
1	1.0	0.19	0.023	0.125	3.0	●		● *pslA*	Door handle	NDR
2	6.0	3.0	0.64	2.0	12.0			● pelA, *pslA, ppyR*	Table (consultation)	NDR
3	3.0	2.0	0.25	0.25	12.0			● pelA, *pslA, ppyR*	Dog cage (isolation area)	NDR
4	32.0^{R}	1024.0^{R}	0.75	12.0	16.0	●	● (Group 25)	● *pslA, ppyR*	Door handle	SDR
5	1.0	0.094	0.023	0.125	3.0	●		● pelA, *pslA, ppyR*	Door handle	NDR
6	1.0	0.19	0.023	0.125	6.0				Table (ultrasound)	NDR
7	1.0	4.0	0.016	0.5	16.0			● pelA, *pslA, ppyR*	Dog cage (isolation area)	NDR
8	1.5	0.125	0.023	0.125	2.0	●			Table (postoperative)	NDR
9	1.0	0.125	0.023	0.19	2.0	●			Door handle	NDR
10	0.75	0.125	0.016	0.19	4.0			● *pslA, ppyR*	Table (postoperative)	NDR
11	0.75	0.125	0.016	0.125	2.0	●		● pelA, *pslA, ppyR*	Table (postoperative)	NDR
12	6.0	4.0	0.5	2.0	16.0			● *ppyR*	Table (consultation)	NDR
13	0.38	5.0	0.012	0.064	8.0	●		● pelA, *pslA, ppyR*	Table (consultation)	NDR
14	3.0	128.0^{R}	1.0	4.0	2.0				Table (postoperative)	SDR
15	0.75	0.094	0.023	0.125	3.0	●			Table (ultrasound)	NDR
16	1.5	12.0	0.125	3.0	96.0			● pelA, *pslA, ppyR*	Table (consultation)	NDR
17	32.0^{R}	1024.0^{R}	0.75	256.0^{R}	24.0			● pelA, *pslA, ppyR*	Dog cage (isolation area)	MDR
18	0.75	0.064	0.047	0.19	8.0			● pelA, *pslA, ppyR*	Dog cage (isolation area)	NDR
19	0.75	1.5	0.012	0.38	12.0			● *ppyR*	Dog cage (isolation area)	NDR
20	1.5	128.0^{R}	0.75	1.5	1.5			● pelA, *pslA, ppyR*	Table (postoperative)	SDR
21	32.0^{R}	256.0^{R}	2.0^{R}	256.0^{R}	16.0				Dog cage (isolation area)	MDR
22	2.0	0.125	0.023	0.19	6.0				Table (ultrasound)	NDR
23	1.5	0.094	0.012	0.125	4.0			● pelA, *pslA, ppyR*	Table (postoperative)	NDR
24	0.75	0.25	0.016	0.19	8.0	●	● (Group 25)	● *pslA*	Table (consultation)	NDR
25	1.0	0.19	0.016	4.0	8.0		● (Group 25)	● *pslA*	Table (consultation)	NDR
26	1.0	1.5	0.012	0.38	16.0	●		● *pslA*	Dog cage (isolation area)	NDR
27	1.5	0.19	0.023	0.125	6.0	●		● *ppyR*	Table (consultation)	NDR

*Minimum inhibitory concentration (MIC); IMI: Imipenem; AZ: Aztreonam; CIP: Ciprofloxacin; CEF: Ceftazidime; TC: Ticarcilin+Clavulanic acid. Resistance Profile: NDR (non-drug resistance); SDR (single-drug resistance) & MDR (multidrug resistance).

DISCUSSION

Pseudomonas aeruginosa is essentially a free-living bacterium, which can occasionally be found in the intestinal tract of animals, and is considered a major cause of opportunistic hospital infections, as it causes significant morbidity and mortality in immunosuppressed individuals, both in animals and in humans. In this sense, veterinary facilities can harbor microorganisms that cause hospital infections, as well as multiresistant agents. Normally, these environments have a large circulation of people and animals, which

particularly enables a facilitated dissemination of these resistant microorganisms among this population.

Another important aspect is that veterinary staff and professionals are often exposed to zoonotic microorganisms. Recently, the World Health Organization (WHO) listed carbapenem-resistant *P. aeruginosa* as one of 3 bacterial species in critical need for the development of new antibiotics to treat their infections [22]. Chronic infections caused by this bacterium can last for years or even decades, during which time the population undergoes thousands of generations of growth while facing challenges including antibiotic therapy and species competition [16], further aggravating the possibility of genetic recombination and the emergence of new multiresistant strains. The data found in this work strengthen the knowledge on the antimicrobial resistance capacity that *P. aeruginosa* exhibits. The presence of 3 multiresistant strains further highlights the advanced stage of resistance of this bacterial species. The characterization of strains of this species in a veterinary hospital environment is crucial for the control of this population circulating in this environment, and the consequent adoption of more effective measures aimed at controlling its proliferation.

Resistance to carbapenems can manifest in *P. aeruginosa* strains as an effect of efflux pump dysregulation, and, since sensitivity to β-lactams is poorly evaluated in veterinary laboratories, the growing resistance is commonly neglected or underestimated [11]. In this research, ticarcillin (penicillin) + clavulanic acid (inhibitor of beta-lactamase) proved to be efficient against all isolates. As an association, they are generally more efficient antibiotics, however, other studies have shown that the sensitivity of the bacteria to this combination is variable [10,13,21].

Of the 27 isolates tested, only 1 (isolate 21; = 3.7%), was resistant to ciprofloxacin. The resistance profile of *P. aeruginosa* in veterinary settings to this antibiotic is variable [11,14]. The emergence of resistance in dogs seems to be associated with previous prolonged systemic fluoroquinolone administration [24].

Two isolates (=7.4%) were resistant to ceftazidime (3rd generation cephalosporin with a broader spectrum of action); the resistance to this antibiotic is of concern as demonstrated by another authors [1,6]. Three isolates (=11.1%) were resistant to imipenem (a broad-spectrum carbapenem antibiotic that is highly resistant to beta-lactamases). Despite its general efficiency, animal-derived imipenem-resistant isolates have also been found [6,20]. The greatest resistance was observed to aztreonam (18.5%), a β-lactam antibiotic frequently used to treat *P. aeruginosa* infections. Resistance to this antibiotic is usually linked to the production of extended-spectrum beta-lactamases (ESBL) as well as carbapenemases. The association of this antibiotic with a beta-lactamase inhibitor may be a viable alternative [3], as resistance to this important antibiotic has been highlighted worldwide [5,11,12,19,21].

Many isolates in this work carried genes related to the production of beta-lactamases, such as TEM and CTX-M. It is known that *P. aeruginosa* has already acquired resistance to several classes of antimicrobials, which may have occurred through the mutation of intrinsic genes or through horizontal acquisition, through plasmids, for example [16]. There are studies, which warned about the possible risk of transmission of the infection from animals to humans, especially when the immune system of the animal owner is compromised, so antibiotic resistance in animals must be monitored [10]. An effective alternative was the proposal of guidelines aimed at reducing the use of antibiotics in these animals, so that they would consequently reduce the emergence of multiresistant bacteria in Japan [23].

Bacterial communities aggregate themselves to a substratum and encapsulate in a protein polysaccharide matrix evolved during adverse environmental condition which is known as biofilm [15]. Most of the isolates in this research carried genes related to biofilm production, including those resistant to antimicrobials. This link to biofilm-mediated antibiotic tolerance and its importance for the spread of resistance among clinical isolates of *Pseudomonas* in animals has already been postulated [4]. Also, due to their complex biofilm forming ability, *Pseudomonas* species show great resistance to various classes of antibiotics which are used to overcome microbial infection [15]. Furthermore, the identification of biofilm-forming variants during clinical diagnostics is important because of the established link that exists between increased antibiotic tolerance, biofilm formation, and the corresponding treatment failure from biofilm-associated chronic infections [7].

The study of this bacterial species in a veterinary hospital environment has a direct impact on human health, due to the mechanisms of resistance and genetic variability that can occur between infections

in different animal species and in humans. In view of that, professionals working in veterinary hospitals should be aware of the importance of controlling these microorganisms, because the occurrence of *Pseudomonas* species in hospitals helps to form the biofilms on medical instruments and other similar devices along with implants in patients [17]. Correct measures must be taken to sanitize the environment and utensils between animal care sessions, besides frequent hand washing by all employees and the use of protective equipment such as masks and gloves.

Veterinarians should opt for the choice of antimicrobial therapy according to the results of antibiograms, and emphasize the proper administration of the drug by the guardians of animals. When selecting antimicrobial drugs, veterinarians should consider the specific prevalence of resistant *P. aeruginosa* in the region, in addition, improving the monitoring of antibiotic resistant *P. aeruginosa* in companion animals is necessary [26]. The concern with surgical site infections must always be present in the routine of a veterinary hospital, for this, asepsis procedures must always be followed and, therefore, the use of antibiotic therapy reduced. This also emphasizes the importance of veterinary medicine professionals as unique health care providers, as they are able to promote measures to control the growth and transmission of these microorganisms that can also produce infections in humans.

CONCLUSIONS

The occurence of *Pseudomonas aeruginosa* in the veterinary hospital environment samples was 8.8%. Two multidrug resistant (MDR) strains were identified in dog cages (isolation area) and 3 single-drug resistance (SDR) strains, on a door handle and on tables (postoperative). The presence of MDR and SDR strains of *P. aeruginosa*, carriers of genes related to the production of beta-lactamases as well as biofilm, indicates a potential risk to animal and human health. Therefore, containment measures must be taken to prevent this possible transmission.

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