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Article

Antibiotic susceptibility profile of *Pseudomonas aeruginosa* canine isolates from a multicentric study in Romania

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Abstract: Treating infections caused by *Pseudomonas aeruginosa* is increasingly difficult due to high antimicrobial resistance, materialized through the presence of multiple resistance strains, as well as due to rapid development of resistance throughout treatment. The present survey was conducted to investigate the antibiotic susceptibility profile of *Pseudomonas aeruginosa* pathogens, in two University Veterinary hospitals from different geographical regions of Romania (i.e., south-west - Timisoara county and north-east – Iasi county) involved in canine superficial infections. A total of 142 swab specimens were collected from dogs with superficial infections (superficial skin infections, otitis externa, perianal abscess), with the aim of assessing the presence of *Pseudomonas aeruginosa*, based on phenotypic and molecular characterization. Fifty-eight samples (40.84%; 58/142) were positive for *Pseudomonas aeruginosa* (according to their confirmed morphological and molecular features). Susceptibility to usual antibiotics used in the treatment of canine skin conditions was tested for all *Pseudomonas* strains that were isolated from canine superficial infections, using the Kirby Bauer disc diffusion method. Drug resistance was observed in the case of all tested antibiotics. The susceptibility rate of *P. aeruginosa* strains that were tested in this study was in the following order: ampicillin sulbactam (55.17%; 32/58), followed by ceftazidime (53.44%; 31/58), aztreonam (51.72%; 30/58), amikacin (44.82%; 26/58), azithromycin (41.37%; 24/58), gentamycin (37.93%; 22/58), cefepime (36.20%; 21/58) meropenem (25.86%; 13/58), piperacillin-tazobactam (25.86%; 13/58) imipenem (22.41%; 13/158), ciprofloxacin (17.24%; 10/58) tobramycin (8.62; 5/58), and polymyxin B (1.72; 1/58) respectively. The results highlight the importance of antibiotic susceptibility testing in *Pseudomonas aeruginosa* isolates from dogs with superficial infections, in order to use an adequate treatment plan for the management of the skin condition, and other pathology (otitis externa and perianal abscesses).

Keywords: *Pseudomonas;* antibiotic resistance; dog; infection; skin, otitis externa, perianal abscess.

1. Introduction

Pseudomonas aeruginosa is an important pathogen to both humans and animals, but it is rarely involved in primary diseases. In humans, *P. aeruginosa* is an important opportunistic, nosocomial pathogen, particularly present in hospital-acquired pneumonia cases, in immunocompromised patients. In animals, and especially in dogs, it has been assigned as the distinct cause of infections such as otitis externa, superficial skin infections, chronic deep pyoderma, perianal abscesses and wound/urinary tract infections [1, 2].

The prevalence of *P. aeruginosa* infections is 11.5% in Europe and 17% in developing countries [1]. Among the resistant bacteria, *P. aeruginosa* expresses multi-resistance to antibiotics that can be either acquired (plasmids, transposons) or natural. This resistance generally favours the involvement of *P. aeruginosa* in nosocomial infections, food poisoning and biofilm formation, the latter giving *P. aeruginosa* high colonization potential, the capacity to spoil foodstuffs, and resistance to antiseptics, disinfectants and antibiotics [2,3].

Pyoderma is defined as an inflammatory skin condition of bacterial origin, most commonly characterized through a purulent aspect. The skin is a complex ecosystem hosting a variety of microorganisms such as bacteria, yeasts and parasites. Animals with an increased skin humidity index are the perfect environment where such microorganisms thrive [1–3].

Pseudomonas aeruginosa plays an important role in the pathogenesis of canine pyoderma. Isolation and identification of microbial agents involved in skin disorders of dogs are a fundamental starting point for diagnosis and respectively, for initiating a suitable treatment [2,4]. Treatment of *P. aeruginosa* infections is complex because of its high intrinsic resistance to many commonly used antibiotics. Therefore, the choice of antibiotics that can be used to treat *P. aeruginosa* infections is scarce, especially in veterinary medicine [1-4].

Bacteria are found on skin surface, in the superficial part called *stratum corneum* but they are absent in the external area of the hair follicle and up to the sebaceous gland. Several bacterial species can coexist in harmony without causing any damage to the skin; however instability can occur at any point [4–7]. The skin microbiota consists of resident bacteria and occasionally transient bacteria. The resident bacteria are capable of multiplying on the skin surface as well as in the hair follicle but their presence are non-pathogenic. Thus, pyoderma is most often a secondary rather than a primary disease. Canine pyoderma only occurs if there is an association between pathogenic bacteria and the factors that allow their proliferation and penetration through the skin [8,9].

The incidence of *Pseudomonas* infections in dogs with skin disorders has been reported to be around 11-13% [4,10–12]. In chronic, suppurative diseases, *Pseudomonas aeruginosa* is the dominant bacterial species, isolated alone or in association with other microorganisms (especially *Proteus mirabilis* and *Staphylococcus spp.* [1,8,13,14]. Due to its high resistance to antibiotics, treating infections caused by *Pseudomonas aeruginosa* is increasingly difficult. The known risk factors for selection of resistant and multi-resistant strains are excessive drug use and inappropriate dosage regimens without previous antimicrobial susceptibility testing [15].

Therefore, considering all the above mentioned reasons, we value the determination of antimicrobial susceptibility of *Pseudomonas aeruginosa* strains involved in pet infections, to be of the utmost importance [3,16,17].

All these considered, the purpose of the study was to determine the antibiotic susceptibility of *P. aeruginosa* strains isolated from canine skin superficial infections cases in two clinical setting from Romania, University Veterinary Hospital - Timisoara County and University Veterinary Hospital – Iasi County.

2. Results and Discussion

A total of 58 (40.86%) bacterial isolates showing typical characteristics of the *Pseudomonas aeruginosa* species were isolated from superficial infections (including superficial pyoderma, otitis externa and perianal abscess) lesions. The distribution of positive isolates among 142 canine patients is presented in Table 1.

Table 1. Distribution of *Pseudomonas aeruginosa* isolates from dogs with superficial infections.

Type of disease	*Pseudomonas aeruginosa* distribution	
	No. of collected samples (n)	*No. positive samples: n (%)*
Superficial skin infections	56	19 (32.75)
Otitis externa	48	15 (25.86)
Perianal abscess	38	24 (41.37)
Total	142	58 (100)

All 58 isolates were confirmed positive for *Pseudomonas aeruginosa* by molecular methods.

In the present study, the percentage of samples collected from dogs with superficial infections that were positive for *P. aeruginosa* was 40.86% (58/142; *n* = 58). The susceptibility rate of *P. aeruginosa* strains that were tested in the study was in the following order: ampicillin sulbactam (55.17%; *n* = 32), followed by ceftazidime (53.44%; *n* = 31), aztreonam (51.72%; *n* = 30), amikacin (44.82%; *n* = 26), azithromycin (41.37%; *n* = 24), gentamycin (37.93%; *n* = 22), and cefepime (36.20%; *n* = 21) respectively. Also it has been reported that gentamycin could have an increased efficacy against *Pseudomonas* strains of animal origin, regardless of the animal species or isolation site [4,8].

Good susceptibility rates were noticed for polymyxin B as well, a common component of topical preparations [3,9,18]. Susceptibility to ciprofloxacin remains uncertain, and we strongly recommend the antimicrobial susceptibility tests to be performed prior to the use of this antibiotic. Therapy consisting of meropenem, piperacillin-tazobactam and imipenem leads to resolution of canine superficial infections due to *Pseudomonas aeruginosa*. A very small number of strains isolated from dogs with superficial infections proved to be sensitive to tobramycin and polymyxin B. Both antibiotics are included in commercially available topical therapeutic products [16,19,20].

The results of the antimicrobial susceptibility tests performed on 58 *Pseudomonas aeruginosa* strains isolated from canine superficial infections are presented in Table 2, and the antimicrobial resistance / susceptibility pattern of the multi-resistant *Pseudomonas aeruginosa* strains (***n=18***) is presented in Table 3.

Table 2. Antibiotic susceptibility profile of *Pseudomonas aeruginosa* strains isolated from canine superficial infections.

Antibiotics	Antibiotic susceptibility test results	*Pseudomonas aeruginosa* strains isolated from canine superficial skin infections *(n=58)*	
		No.	*%*
Ampicillin sulbactam (SAM/10+10µg)	S	32	55.17
	R	26	44.82
Gentamycin (GM/10µg)	S	22	37.93
	R	36	62.06
Ciprofloxacin (CIP/5µg)	S	10	17.24
	R	48	82.75
Imipenem (IPM/10µg)	S	13	22.41
	R	45	77.58
Meropenem (MEM/10µg)	S	15	25.86
	R	43	74.13
Piperacillin-tazobactam (TZP 110/100+10µg)	S	15	25.86
	R	43	74.13

Ceftazidime (CAZ/30µg)	S	31	53.44
	R	27	46.55
Cefepime (FEP/30µg)	S	21	36.20
	R	37	63.79
Aztreonam (ATM/30µg)	S	30	51.72
	R	28	48.27
Azithromycin (AZM/15µg)	S	24	41.37
	R	34	58.62
Amikacin (AN 30µg)	S	26	44.82
	R	32	55.17
Tobramycin (TM/10µg)	S	5	8.62
	R	53	91.37
Polymyxin B (PB/50µg/300UI)	S	1	1.72
	R	57	98.27

S-susceptible; R-resistant

Table 3. The behaviour of multi-resistant *Pseudomonas aeruginosa* strains isolated from dogs with superficial infections: resistance (full circles) and susceptibility (empty circles)

Antimicrobial agent	*No. of multi-resistant Pseudomonas aeruginosa tested strains*																	
	12	17	21	28	33	37	39	41	43	47	49	50	53	54	55	56	57	58
SAM/10+10µg	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●
GM/10µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
CIP/5µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
IPM/10µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
MEM/10µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
TZP 110/100+10µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
CAZ/30µg	●	○	●	●	●	●	●	●	○	●	○	●	●	●	●	○	●	○
FEP/30µg	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	○
ATM/30µg	●	●	●	●	●	●	●	●	●	●	●	●	●	○	●	●	●	○
AZM/15µg	●	●	●	●	○	●	●	●	○	●	●	●	●	●	●	●	●	●
AN 30µg	○	○	●	○	●	●	●	●	●	●	●	●	○	●	●	●	●	●
TM/10µg	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
PB/50µg/300UI	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Legend: *SAM – Ampicillin sulbactam; GM – Gentamicin; CIP – Ciprofloxacin; IPM – Imipenem; MEM – Meropenem; TZP – Piperacillin-tazobactam; CAZ – Ceftazidime; FEP – Cefepime; ATM – Aztreonam; AZM – Azithromycin; AN – Amikacin; Norfloxacin – NOR; TM – Tobramycin; PB – Polymyxin B.*

Eighteen out of fifty-eight *Pseudomonas aeruginosa* isolates showed resistance to at least ten of the 13 antibiotics tested (Table 3). All fifty-eight isolates from canine superficial infections were resistant to multiple antimicrobial classes, including synthetic antimicrobial agents that are not commonly used in canine infections management, but mainly used in human medicine. An important variability of susceptibility profiles was observed among these isolates – the situation for

each isolate belonging to different type of disease is detailed in Tables 4, 5, and 6. The higher resistance rates were encountered for polymyxin B (98.27%), tobramycin (91.37%), and ciprofloxacin (82.75%), as it is shown in Table 2.

Table 4. Results of antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolates from the skin (***n=19***).

ID of *Pseudomonas* isolate	SAM	GM	CIP	IPM	MEM	TZP	CAZ	FEP	ATM	AZM	AN	TM	PB	No. of resistant antibiotics
3	S*	S	R*	R	R	R	S	S	S	R	S	R	R	7
7	R	R	R	R	R	R	S	S	S	S	S	R	R	8
8	R	S	R	S	S	R	R	R	S	S	S	R	R	7
9	R	R	S	R	S	R	S	R	S	R	S	R	R	8
14	S	R	R	S	R	R	S	S	S	R	S	R	R	7
17	R	R	R	R	R	R	S	R	R	R	S	R	R	11
20	S	R	R	R	S	R	S	R	S	S	R	R	R	8
21	R	R	R	R	R	R	R	R	R	R	R	R	R	13
32	S	R	R	R	R	R	S	S	S	S	R	R	R	8
36	S	S	S	S	S	S	S	S	S	S	S	R	R	2
39	S	R	R	R	R	R	R	S	R	R	R	R	R	11
40	R	R	R	S	R	S	R	S	R	S	S	R	R	8
43	R	R	R	R	R	R	S	R	R	S	R	R	R	11
49	R	R	R	R	R	R	S	R	R	R	R	R	R	12
50	R	R	R	R	R	R	R	R	R	R	R	R	R	13
51	R	R	S	S	R	S	R	R	S	S	R	S	R	7
52	R	S	S	R	S	R	S	R	R	R	S	R	R	8
53	R	R	R	R	R	R	R	R	R	R	S	R	R	12
58	R	R	R	R	R	R	S	S	S	R	R	R	R	10

S-susceptible; R-resistant

Table 5. Results of antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolates from otitis externa (***n=15***).

ID of *Pseudomonas* isolate	SAM	GM	CIP	IPM	MEM	TZP	CAZ	FEP	ATM	AZM	AN	TM	PB	No. of resistant antibiotics
1	S*	S	R**	R	R	R	S	S	S	R	S	R	R	7
2	R	R	S	R	S	R	S	S	S	S	R	R	R	7
6	R	S	R	R	R	R	R	R	S	S	S	R	R	9
12	R	R	R	R	R	R	R	R	R	R	S	R	R	12
13	S	R	R	R	R	R	S	S	S	R	S	R	R	8
15	S	R	R	S	S	R	S	R	R	R	S	R	R	8
16	S	R	S	S	R	S	S	R	S	R	R	R	R	7
23	S	S	R	R	R	R	S	S	S	R	R	R	R	8
25	S	R	R	S	S	S	S	S	S	S	R	R	R	5
28	R	R	R	R	R	R	R	R	R	R	S	R	R	12
29	S	S	R	R	R	R	S	S	S	S	R	R	R	7
30	S	R	S	S	R	S	R	S	R	R	R	R	R	8
35	R	R	S	S	S	R	S	R	R	S	R	R	R	8
56	R	R	R	R	R	R	S	R	R	R	R	R	R	12

57	R	R	R	R	R	R	R	R	R	R	R	R	R	13

S-susceptible; R-resistant

Table 6. Results of antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolates from perianal abscesses (***n*=24**).

ID of *Pseudomonas* isolate	SAM	GM	CIP	IPM	MEM	TZP	CAZ	FEP	ATM	AZM	AN	TM	PB	No. of resistant antibiotics
4	S*	S	R**	R	R	S	S	S	S	S	S	R	R	5
5	R	R	S	S	R	R	R	S	S	S	R	R	R	8
10	S	R	R	R	R	R	S	R	S	S	S	R	R	8
11	S	S	R	R	S	S	S	R	S	R	R	R	R	7
18	S	S	R	R	R	R	R	R	S	S	R	R	S	8
19	S	S	R	R	S	S	S	S	S	R	R	R	R	6
22	S	S	R	R	R	S	S	S	S	S	R	R	R	6
24	S	S	R	R	R	R	S	S	S	R	R	R	R	8
26	S	S	R	R	R	S	R	R	R	S	S	R	R	7
27	S	R	S	S	R	S	R	R	R	R	S	R	R	8
31	S	R	R	S	S	R	S	R	R	S	S	R	R	7
33	S	R	R	R	R	R	R	R	R	S	R	R	R	12
34	S	S	R	R	S	R	R	R	S	S	R	R	R	8
37	R	R	R	R	R	R	R	R	R	R	R	R	R	13
38	S	S	R	R	R	R	S	R	S	R	S	S	R	7
41	R	R	R	R	R	R	R	R	R	R	R	R	R	13
42	S	S	R	R	R	S	S	R	R	R	S	S	R	7
44	S	S	R	R	R	R	S	S	R	R	S	S	R	7
45	S	S	R	R	R	R	R	R	S	S	S	R	R	8
46	S	S	R	R	S	S	R	R	S	S	R	R	R	7
47	R	R	R	R	R	R	R	R	R	R	R	R	R	13
48	S	S	R	R	S	S	R	R	R	R	S	S	R	7
54	R	R	R	R	R	R	R	R	S	R	R	R	R	12
55	R	R	R	R	R	R	R	R	R	R	R	R	R	13

S-susceptible; R-resistant

Bacterial skin conditions are common in dogs and their empirical treatment is a widespread therapeutic approach to reduce the clinical evolution, but in severe forms, antibiotic susceptibility testing should be considered imperative.

The involvement of *Pseudomonas* isolates in canine pyodermatitis has been confirmed in numerous other studies carried out in several geographic regions [21, 22].

In agreement with our findings regarding the presence of multidrug resistant *Pseudomonas* in canine pyodermatitis lesions, multiple data are reported in previous surveys conducted by Wildemuth et al. [20], based on comparing the susceptibility of *Pseudomonas spp*. isolates from skin and ear disorders, towards enrofloxacin, marbofloxacin and ciprofloxacin. Pathological exudates were obtained from dogs examined within the Dermatology ward of veterinary hospitals. The susceptibility rate of the isolates from ears infections was 46.90% to enrofloxacin, 66.70% to marbofloxacin and 75.0% to ciprofloxacin. The isolates from the skin showed the following susceptibility pattern: 76.20% to enrofloxacin, 81.0% to marbofloxacin and 80.0% to ciprofloxacin [20].

In a study conducted by Hillier et al. [8], based on the examinations of 20 dogs with different skin conditions, the authors reported that 33.0% of the cases were cases of pyoderma caused by *Pseudomonas*, susceptible to florfenicol, which was also the treatment option in their study [8].

In another study, Morris [3] reported various results regarding the *in vitro* susceptibility of *P. aeruginosa* strains isolated from the ear, to fluoroquinolones. The differences found in the diffusion susceptibility tests were as follows: 58.0% were enrofloxacin-susceptible strains and 96.0% were marbofloxacin-susceptible strains, out of a total of 26 strains of *Pseudomonas spp*. (of which *25* were *P. aeruginosa* strains), isolated from the ear [3].

3. Materials and Methods

3.1. Animals

One hundred forty-two dogs with characteristic clinical signs of skin superficial infections, otitis externa, and perianal abscesses were presented at the University Veterinary Hospitals of the Faculty of Veterinary Medicine of Timisoara (FVMT), Western Romania, and Faculty of Veterinary Medicine of Iasi, Eastern Romania, respectively, from 1 January to 30 June 2019.

The dogs in this study were examined during routine veterinary visits, as part of a diagnostic work up. No treatment decisions were made based on the results of clinical examination. All methods have been performed in accordance with the relevant guidelines and regulations. Because the samples were intended for diagnosis, the collection protocol was made with the consent of animal owners, according to the code of the Romanian Veterinary College (protocol numbers 34/1.12.2012) and the valid procedures of the University Veterinary Clinics of the Faculty of Veterinary Medicine Timisoara and Iasi.

The animals included in the study were dogs, aged 5 months to 14 years, of both sexes (69 males and 73 females), belonging to 21 different breeds.

The main criteria for inclusion in the study were:

a. the absence of any antibiotic treatment prior to clinical presentation and

b. presence of main clinical signs of superficial infections: excessive scaling, presence of follicular papules, epidermal collarettes, serous crusts, erythema, serohaemorrhagic or purulent exudates, ear discharge or desquamation, local pain according to criteria from Table 7.

Table 7. Main criteria for the inclusion of dogs in the study, in accordance with the clinical score

Clinical score	*Clinical symptoms*	*No. of P. aeruginosa strain isolated*
1	Excessive scaling, erythema,	0
2	Presence of follicular papules, epidermal collarettes	9
3	Presence of follicular papules, epidermal collarettes, serous crusts, erythema, serohemorrhagic exsudates, pruritus, ear discharge or desquamation, swelled perianal glands, local pain	18
4	Presence of follicular papules, epidermal collarettes, serous crusts, erythema, serohemorrhagic or purulent exudates, ear discharge, inflamed perianal glands, intense pain	31

The first assessment of the patients included the following steps: clinical examination, scoring the extent of superficial infections, collecting of the exudates. Exudates from the skin surface, external ear canal, and perianal glands were collected using sterile cotton swabs dipped in sterile saline solution prior to sampling and maintained in Amies transport medium until the laboratory processing. All superficial skin purulent lesions, and perianal zone, were sanitized using sterile saline solution prior to sample collection. This procedure led to a decrease of microbial contamination. Fresh exudates were easily obtained by applying light pressure on the lesion areas (skin and perianal glands).

3.2. Bacteriological examination

The microbiological examinations were performed in the Bacteriology Laboratory of the Infectious Diseases and Preventive Medicine Department, FVMT and Laboratory of Microbiology, FMVI. Examinations were performed within three hours of sampling or, in some cases, after 24-48 hours. During the waiting period, the clinical samples were refrigerated in special storage environments. In order to isolate and identify *Pseudomonas* bacteria, the collected samples were inoculated onto CHROMagar™ Pseudomonas (CHROMagar, France) and Columbia Agar with 5.0% sheep blood (Becton Dickinson, GmbH, Germany). The plates were incubated for 18-24 h at 37°C, in aerobic conditions. The presumptive identification of isolates was based on the cultural, morphological and biochemical characters.

3.3. Molecular tests

The final species identification was performed by PCR using a previously described method [23]. All *Pseudomonas aeruginosa* isolates give an amplified fragment of 956 bp.

3.3.1. Extraction of template DNA

One milliliter of bacteria grown in 10 milliliters of Cetrimide broth, at 37°C in an aerobic atmosphere for 24 hours, was dispensed aseptically in an Eppendorf tube. Bacterial genomic DNA was extracted using the Pure Link™ Genomic Lysis/Binding Buffer (Thermo Fisher Scientific) boiling method, with a freshly prepared proteinase K solution (10 mg/ml). The DNA quantity and quality were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop® Technologies, Thermo Fisher Scientific), by measuring the absorbance at 260 nm.

PCR was done using specific primer for identifying *Pseudomonas aeruginosa* species, PA-SS-F GGGGGATCTTCGGACCTCA and PA-SS-R TCCTTAGAGTGCCCACCCG 1124–1144 as previously described [23]. The enhanced PCR conditions consisted of an initial denaturation at 95°C for 5 min followed by 32 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min, using the thermocycler My Cycler (BioRad®). The amplified products were analyzed for their size by electrophoresis on 2.5% agarose gel, stained with ethidium bromide, and visualized under UV light using a gel documentation system (UV transilluminator – 2035-2, Bio Olympics USA).

The type strain *Pseudomonas aeruginosa* ATCC 9027™ was used as positive control.

3.4. Antimicrobial susceptibility tests

A common method for determining antimicrobial susceptibility, especially in small laboratories and veterinary practices, is the agar diffusion test (Kirby Bauer disc diffusion method). This test was performed using Müller-Hinton Agar (Becton Dickinson, GmbH, Germany).

The following antibiotic discs (Bio-Rad, France) were tested: ampicillin-sulbactam (SAM/10+10µg); gentamycin (GM/10µg); ciprofloxacin (CIP/5µg); imipenem (IPM/10µg); meropenem (MEM/10µg); piperacillin-tazobactam (TZP/110/100+10µg); ceftazidime (CAZ/30µg); cefepime (FEP/30µg), aztreonam (ATM/30µg), azithromycin (AZM/15µg), amikacin (AN/30 µg), tobramycin (TM/10µg) and polymyxin B (PB/50µg-300UI).

Antibiotic susceptibility was evaluated taking into account the inhibition zone diameter based on recommendations of Clinical and Laboratory Standards Institute (CLSI) [24]. According to this criteria, *Pseudomonas aeruginosa* strains were classified as sensitive, intermediate or resistant [25]. The control strain, *Pseudomonas aeruginosa* ATCC 9027™, was also used in this study [8]. The selection of antibiotics was made according to the suggested guidelines for the use of systemic antimicrobials in bacterial skin infections, as proposed by Beco et al. [26].

4. Conclusions

The use of antimicrobial susceptibility tests before choosing the therapeutic protocol is of particularly importance. Ampicillin-sulbactam and ceftazidime are good therapeutic choices in case of canine superficial infections caused by *Pseudomonas aeruginosa* isolates.

Also, the emergence of multidrug resistant *Pseudomonas aeruginosa* strains, especially those that are resistant to not commonly and effectively used antibiotics in dogs (but used in human medicine), is an occurring event that might indicate the over usage of these antimicrobial agents or suggestive of a human-to-dog transfer. As a result, when choosing antimicrobial drugs, veterinary surgeons must consider the site-specific prevalence of antibiotic resistant *Pseudomonas aeruginosa* in dogs.

Generally, in order to perfect more suitable control strategies for canine superficial infections, performing microbiological exams and continuously remaining updated with respect to the susceptibility profile of isolated strains, are practices that are strongly recommended.

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