



## Resistance to Antibiotics of Bacteria Isolated from Pets

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### Abstract

13 strains from previously isolated and identified domestic pets, *Chryseobacterium*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Pantoea*, *Photobacterium*, *Pleisomonas*, *Pseudomonas*, *Salmonella*, *Serratia* and *Vibrio* were analyzed. Tricanged anti-biograms of each strain were performed, with respect to the conventional method called Kirby Bauer, to observe the susceptibility by disc diffusion of each of them with 16 antibiotics of which as a result the following was obtained sensitivity to the total strains analysed; Cefotaxima with 46%, Metronidazole 53%, Dicloxacillin 38%, Cephaxine 30%, Ciprofloxacin 100%, Nitrofurantoin 92%, Erythromycin, Amoxicillin 100%, Trimethoprim-Sulfalmetoxazole 100%, Cephalotin 53%, Gentamicin 100%, Amikacin 100%, Ceftriaxone 61%, Benzipenicillin 31%, Ampicillin 100%, Erythromycin 69%, Chloramphenicol 100%. Also resulting in *Photobacterium* being the genus of bacteria that exhibited the most resistance.

**Keywords:** Bacteria; Pet; Antibiotic; Sensitivity; Resistance

### Abbreviations

RPM: Revolutions Per Minute;  $\mu$ L: Microliters; CFU: Colony Forming Units

### Introduction

Medicines have always been an important component of man's life and within the broad spectrum of medicines are antibiotics [1]. The discovery of penicillin in 1927 by Alexander Fleming marked the beginning of the antibiotic era. The emergence of this drug encouraged the scientific community to develop endless antibiotics. This great discovery suggested that it was the beginning of the end of infectious diseases, which for their effectiveness, have been used for almost a century to control many diseases [2,3]. However, antimicrobial resistance to these drugs has been increasing due to intensive and excessive use of their consumption [4]. In contrast,

bacteria are able to quickly develop mechanisms, which allow them to evade the effects of antibiotics, as it is ultimately an inevitable consequence of the evolutionary process of life [5].

Diseases that are transmitted from animals to humans can be caused by parasites, fungi, viruses and bacteria, reaching for locality either on your skin, gonads, limbs or almost any part of your body [6]. These pathogens can become resistant to various types of antibiotics. It is estimated that more than 4% of the problems of antimicrobial resistance in humans are closely related to animal sources, regardless of the antimicrobial resistance that develops in agriculture and livestock [7]. The impact of irrational use of antibiotics increases expenditure spending within health services and poses a risk to society, as it could lead to higher mortality from infectious diseases [8].

In our country there is little detailed information on the analysis of patients taking infections caused by pet-isolated pathogenic microorganisms, as well as their antibiotic susceptibility, whose knowledge helps to avoid a possible failure cause one of the most pressing public health problems worldwide, bacterial resistance. Therefore, our study is based on determining the resistance or sensitivity to different types of antibiotics of pathogenic bacteria isolated from pets.

### Materials and Methods

51 strains listed in table 1 previously isolated from domestic pet feces (dogs, cats and turtles) were used, fentypically identified by Biochemical testing of the Biomérieux API20E system. A total of 13 genera are included, each of which underwent resistance tests with different types of commonly used antibiotics in the public health sector. Each of the strains was made three replicates, using the methodology of diffusion in agar with sensidisco (Kirby Bauer). The antibiotics used are listed in table 2.

Tests were included for the 7 different groups of antibiotics (beta-lactams, aminoglycosides, macrolides, amphenicoles, quinolones, sulfamides, nitroimidazoles and nitrofurans, with a total of 16 different antibiotics. Each was diluted from its initial commercial concentration, in 1.0ml of sterile distilled water, placed in a 1.5ml eppendorff tube.

The strains were sown in the culture medium liquid LB incubation for a period of 24 hours, at a temperature of 27°C, in constant agitation of 100 revolutions per minute. Subsequently, serial dilutions were made up to 10<sup>-8</sup>, one of these were placed 2µl each in a Petri box with solidified LB medium or incubating at 27°C for

Bacterial Genus	Strains
1. <i>Chryseobacterium indologenes</i>	35
2. <i>Citrobacter bactrim</i>	36
3. <i>Enterobacter aerogenes</i>	31b
4. <i>Hafnia alvei</i>	5,32a,41a,41b
5. <i>Klebsiella oxytoca</i>	40b, 42, 50 <sup>a</sup>
6. <i>Klebsiella pneumoniae</i>	24,33
7. <i>Morganella morganii</i>	28,31a,44,46 <sup>a</sup>
8. <i>Pantoea sp.</i>	49, 50b,12
9. <i>Photobacterium</i>	8b, 9, 32b, 47b
10. <i>Photobacterium damsela</i>	38 <sup>a</sup>
11. <i>Plesiomonas shigelloides</i>	46b
12. <i>Pseudomonas aeruginosa</i>	38b,38c, 43b
13. <i>Pseudomonas sp.</i>	48b
14. <i>Salmonella colera suis</i>	26, 29
15. <i>Serratia marcescens</i>	8a,25,30a, 34a,47a
16. <i>Vibrio vulnificus</i>	1c,1a,1b, 2, 30b, 34b, 37a,37b, 39, 40 <sup>a</sup> ,48 <sup>a</sup> ,51a

**Table 1:** Bacterial genera analyzed.

Antibiotics	Disc Concentration
1. Cefotaxim	1g/1ml
2. Dicloxacillin	500mg/1ml
3. Cefalexim	500mg/1ml
4. Ciprofloxacin	250mg/1ml
5. Nitrofurantoin	100mg/1ml
6. Erythromycin	500mg/1ml
7. Amoxicillin	500mg/1ml
8. Trimethoprim-sulfamethoxazole	800mg/1ml /4000mg/1ml
9. Cephalotin	1g/1ml
10. Gentamicin	80ml/2ml
11. Amikacin	50mg/2ml
12. Ceftriaxone	250mg/2ml
13. Benzipenicillin	1,20000 U/1ml
14. Metronidazole	500mg/1ml
15. Chloramphenicol	5ml/1ml
16. Ampicillin	500mg

**Table 2:** Antibiotics used for Sensitivity Test.

24 hours. The visibly separated CFUs were counted by locating the corresponding dilution, allowing to calculate the number of CFUs per ml. A spectrophotometer marked Themoscintific Genesys 20 was used with an absorbance of 625 nm and UFM sms per ml were calculated for each strain in order to refer to the concentration of cells present in the inoculum. It was subsequently adjusted to the 10<sup>-7</sup> CFU/ml amount of each strain that underwent an antibiogram.

The Muller-Hinton médium was prepared. It is recognized that until now it has been a universally recommended culture medium for antimicrobial sensitivity testing. It was sterilized for 20 minutes in the autoclave at 15 lbs of pressure, then placed in Petri boxes, filling them to 4 mm wide, corresponding to 25 ml of agar for each.

Filter paper discs, each 6mm in diameter, were used for the Kirby-Bauer test, which were sterilized at 12°C for 20 minutes, then sterilized infertility-proof for 24 hours. The plates were massively sown with sterile cotton swab. The filter paper discs were then supplied with the impregnated antibiotic in the paper discs. 4 or 5 discs were used per Petri dish leaving a space of 5 cm between each. The plates inoculated and with the discs that were applied, were inverted incubated at an ambient temperature of 27°C for a period of 24 hours.

The plate was read after incubation, with a Vernier brand Mitutoyo, taking as reference the diameter of the inhibition halo. Two categories of interpretation were considered:

- **Sensitive:** Presents halo inhibiting the growth of the bacteria around the sensidisco.
- **Resistant:** It is called resistant when the strains are not inhibited and therefore do not have inhibition halo. They are not inhibited by the antibiotic. The results were reported in tables and graphs of inhibition.

**Results and Discussion**

After 24 hours of incubation, the measurement of the inhibition halos produced by each antibiotic and strain was performed. The following tables analyze the averages of the diameters of inhibitions produced by each antibiotic for each bacterial genus.

Table 3 shows that all bacterial genera were sensitive to aminoglycosides: amikacin and gentamicin, the genus Citrobacter shows greater sensitivity to these antibiotics 29.42mm and 33.31 mm respectively, in accordance with that of these antibiotics 29.42mm and 33.31 mm respectively, in accordance with that of Citrobacter shows up to 100% sensitivity to Gentamicin and Amikacin [9]. In contrast, the lowest resistance level was presented in Photobacterium with a value of 14.53mm for Gentamicin.

All bacterial genera were sensitive to Amoxicillin, while for bencipenil-lin the sensitivity was 30%, equal for cefalexin and 53% for cephalotin. The genus Photobacterium was resistant to these three antibiotics, while Plesi-omonas and Morganella were resistant to cefalexin. It is also observed that all bacterial genera were sensitive to ampicillin (100%), and for Ceftriaxone has a sensitivity of 61%, Serratia and Citrobacter were resistant. While for Cefotaxima they were sensitive 46% being resistant Vibrio and Citrobacter,

Genus	Aminoglycosides			
	Inhibition Halos Size			
	Amikacin	*	Gentamicin	*
<i>Hafnia</i>	28.05	s	30.37	s
<i>Serratia</i>	28.58	s	27.69	s
<i>Plesiomonas</i>	25.48	s	26.2	s
<i>Chryseobacterium</i>	24.89	s	31.37	s
<i>Klebsiella</i>	28.66	s	28.9	s
<i>Enterobacter</i>	27.69	s	29.3	s
<i>Salmonella</i>	28.04	s	28.1	s
<i>Pantoea</i>	25.08	s	26.59	s
<i>Vibrio</i>	25.48	s	30.23	s
<i>Citrobacter</i>	29.42	s	33.31	s
<i>Pseudomonas</i>	28.66	s	31.93	s
<i>Photobacterium</i>	26.53	s	14.53	s
<i>Morganella</i>	25.73	s	27.32	s
S	17mm	100%	15mm	100%
I	15-16mm		13-14mm	
R	14mm		12mm	

**Table 3:** Average aminoglycoside inhibition halos for all bacterial genera \*S means sensitive; I stands for intermediate sensitivity and R means resistant.

for Dicloxacillin they were sensitive 38%, among which were resistant were Hafnia, Serratia, hryseobacterium, Enterobacter, Vibrio, Citrobacter and Photobacterium.

	Beta-Lactam															
	Inhibition Halos Size															
	Benzipen-icillin		Amoxi-cillin		Cefalex-im		Cepha-lotin		Diclox-acillin		Cefotax-ima		Ampi-cillin		Ceftri-axone	
<i>Hafnia</i>	12.83	R	19.32	S	27.7	S	25.42	S	0	R	21.38	R	27.5	S	17.63	R
<i>Serratia</i>	14.78	R	22.03	S	27.13	S	20.45	S	0	R	17.57	R	25	S	12.59	R
<i>Plesiomonas</i>	14.93	R	27.52	S	0	R	14.52	R	19.62	S	37.01	S	27.5	S	33.34	S
<i>Chryseobacte-rium</i>	11.14	R	22.64	S	19.22	S	13.52	R	0	R	36.82	S	40.3	S	26.29	S
<i>Klebsiella</i>	15.1	R	29.35	S	29.34	S	25.66	S	15.03	R	20.35	R	37.5	S	16.43	R
<i>Enterobacter</i>	22.46	S	29.6	S	30.32	S	20.14	S	0	R	19.98	R	39	S	31.09	S
<i>Salmonella</i>	19.23	R	26.23	S	24.97	S	16.83	R	19.9	S	37.79	S	35.5	S	35.1	S
<i>Pantoea</i>	27.1	S	33.01	S	29.58	S	24.53	S	21.7	S	39.42	S	37.5	S	36.76	S
<i>Vibrio</i>	15.45	R	26.92	S	27.41	S	17.5	R	0	R	14.86	R	36.12	S	15.1	R
<i>Citrobacter</i>	14.2	R	28.35	S	23.62	S	15.8	R	0	R	14	R	41.3	S	12.63	R
<i>Pseudomonas</i>	22.12	S	21.4	S	32.83	S	21.36	S	18.9	S	31.39	S	23	S	35.01	S
<i>Photobacterium</i>	0	R	24.37	S	0	R	0	R	0	R	22.17	R	27.2	S	33.43	S
<i>Morganella</i>	21.25	S	28.13	S	0	R	18.69	S	17.5	S	34.72	S	32	S	35.18	S
S	21mm	30%	18mm	100%	18mm	30%	18mm	53%	13mm	38%	23mm	46%	17mm	100%	21mm	61%
I	18-20mm		14-17mm		15-17mm		15-17mm		11-12mm		15-22mm		14-16mm		14-20mm	
R	17mm		13mm		14mm		14mm		10mm		14mm		13mm		13mm	

**Table 4:** Average of Beta-lactam inhibition halos for all bacterial genera.

Table 5 shows the bacterial genera that were resistant to macrolides among these are: Hafnia, Serratia, Vibrio and the genus that showed the most resistance is Photobacterium, with the measurement of 10.94mm a halo less than all. While the genus showing the greatest sensitivity was Chyseo bacterium, since the measure of the halo is 30.82mm.

	Macrolide	
	Inhibition Halos Size	
	Erythromycin	
Hafnia	17.8	R
Serratia	16.5	R
Plesiomonas	18.55	S
Chryseobacterium	30.82	S
Klebsiella	22.05	S
Enterobacter	25.19	S
Salmonella	25.95	S
Pantoea	25.69	S
Vibrio	15.95	R
Citrobacter	19.5	S
Pseudomonas	28.58	S
Photobacterium	10.94	R
Morganella	23.75	S
S	18mm	69%
I	14-17mm	
R	13mm	

**Table 5:** Average macrolide inhibition halos for all bacterial genera.

The genus Photobacterium shows resistance to Erythromycin, as in studies of isolated bacteria in different environments of different soils in orchards treated with manure fertilizer, the bacterial susceptibility of these soils is highly resistant to Erythromycin [10]. Table 5 shows a very similar result of resistance in Photobacterium to Erythromycin with a diameter of 10.94mm.

In Table 6 the bacterial genera presented are 100% sensitive to chloramphenicol. In the case of Klebsiella the largest inhibition halo of 35.5mm was found, while the smaller was Pseudomonas with 22.5mm.

Studies in Brazil isolated bacteria from oral cavities to the family Enterobacteriaceae and Pseudomonadaceae which were per-

	Anfenicol	
	Inhibition Halos Size	
	Chloramphenicol	
Hafnia	26.5	S
Serratia	29	S
Plesiomonas	28.5	S
Chryseobacterium	31.6	S
Klebsiella	35.5	S
Enterobacter	22.5	S
Salmonella	25.5	S
Pantoea	29	S
Vibrio	34	S
Citrobacter	29.6	S
Pseudomonas	22.5	S
Photobacterium	27.5	S
Morganella	34	S
S	18mm	100%
I	13-17mm	
R	12mm	

**Table 6:** Average of Anfenicoles inhibition halos for all bacterial genera.

formed as antimicrobial susceptibility, resulting in all Enterobacteria including two species of Klebsiella as they are *K. pneumoniae* and *K. oxytoca* obtained in this study were sensitive to chloramphenicol, while with Pseudomonas showed some resistance to this antibiotic but sensibly to Ciprofloxacin and Chloramphenicol [11,12].

Table 7 shows 100% sensitivity of bacterial genera to Ciprofloxacin. All strains comply with the standard measures according to Kirby Bauer because for bacteria to be sensitive to this antibiotic, they must have an inhibition halo of 21mm. In our results, the lowest halo size was for Photobacterium with 29.79mm, while for Pseudomonas the halo was the largest of all with 46.63mm.

The bacterial genera in table 8 show 100% sensitivity to Trimethoprim -sulfamethoxazole although they all have sensitivity, you can check in Vibrio has greater sensitivity 39.5mm and that it is the best antibiotic for this bacterium (global health organization), it is worth while note that the least sensitive was Serratia with a halo of 27.81mm. The antimicrobial agent that remains suitable for Vibrio par excellence is Trimethoprim - sulfamethoxazole, according to records maintained since 2002 by global health organization.

	Quinolone	
	Inhibition Halos Size	
	Ciprofloxacin	
<i>Hafnia</i>	39.44	S
<i>Serratia</i>	34.03	S
<i>Plesiomonas</i>	37.34	S
<i>Chryseobacterium</i>	42.62	S
<i>Klebsiella</i>	39.67	S
<i>Enterobacter</i>	40.66	S
<i>Salmonella</i>	41.04	S
<i>Pantoea</i>	36.57	S
<i>Vibrio</i>	31.18	S
<i>Citrobacter</i>	35.42	S
<i>Pseudomonas</i>	46.63	S
<i>Photobacterium</i>	29.79	S
<i>Morganella</i>	32.82	S
S	21mm	100%
I	16-20mm	
R	15mm	

**Table 7:** Average Quinolone inhibition halos for all bacterial genera.

	Sulfamide	
	Inhibition Halos Size	
	Trimethoprim -sulfamethoxazole	
<i>Hafnia</i>	28.42	S
<i>Serratia</i>	27.81	S
<i>Plesiomonas</i>	30.91	S
<i>Chryseobacterium</i>	35.12	S
<i>Klebsiella</i>	32.14	S
<i>Enterobacter</i>	35.04	S
<i>Salmonella</i>	32.95	S
<i>Pantoea</i>	35.11	S
<i>Vibrio</i>	39.5	S
<i>Citrobacter</i>	34.76	S
<i>Pseudomonas</i>	23.9	S
<i>Photobacterium</i>	32.1	S
<i>Morganella</i>	29.92	S
S	16mm	100%
I	11-15mm	
R	10mm	

**Table 8:** Average Sulfamide inhibition halos for all bacterial genera.

Table 9 results in a total percentage of 53% sensitivity of bacterial genera to the antibiotic Metronidazole, among the resistant bacterial genera we can find *Plesiomonas*, *Chryseobacterium*, *Hafnia*, *Pantoea*, *Pseudomonas*, *Citrobacter* being the latter that did not have a halo of inhibition unlike those already indicated. the

genera that had the highest sensitivity were *Serratia*, *Enterobacter*, *Salmonella*, *Vibrio*, *Photobacterium*, *Morganella*, *Klebsiella* was the one that had the greatest antibiotic sensitivity unlike the others, presenting a halo of 34.5 mm.

	Nitroimidazole	
	Inhibition Halos Size	
	Metronidazole	
<i>Hafnia</i>	16	R
<i>Serratia</i>	29.9	S
<i>Plesiomonas</i>	14	R
<i>Chryseobacterium</i>	15	R
<i>Klebsiella</i>	34.5	S
<i>Enterobacter</i>	18	S
<i>Salmonella</i>	26	S
<i>Pantoea</i>	13	R
<i>Vibrio</i>	33.5	S
<i>Citrobacter</i>	0	R
<i>Pseudomonas</i>	16	R
<i>Photobacterium</i>	28	S
<i>Morganella</i>	25	S
S	18mm	53%
I		
R	17mm	

**Table 9:** Average inhibition halos of Nitroimidazoles for all bacterial genera.

	Nitrofurantoin	
	Inhibition Halos Size	
	Nitrofurantoin	
<i>Hafnia</i>	16.93	S
<i>Serratia</i>	18.24	S
<i>Plesiomonas</i>	15.95	R
<i>Chryseobacterium</i>	17.89	S
<i>Klebsiella</i>	19.53	S
<i>Enterobacter</i>	23.59	S
<i>Salmonella</i>	23.76	S
<i>Pantoea</i>	23.15	S
<i>Vibrio</i>	20.17	S
<i>Citrobacter</i>	21.62	S
<i>Pseudomonas</i>	30.43	S
<i>Photobacterium</i>	20.46	S
<i>Morganella</i>	35.91	S
S	17mm	92%
I	15-16mm	
R	14mm	

**Table 10:** Average Nitrofurantoin inhibition halos shown for all bacterial genera.

One bacterium was resistant to Nitrofurantoin so as a final result 92% sensitivity was obtained against this antibiotic. The resistant bacterium was *Pseudomonas* with an inhibition halo of 15.95mm.

### Conclusion

The bacterial genera analyzed in this study show increased sensitivity to Quinolones, Aminoglycosidics and Anfenicoles, while the genera show greater resistance are Betalactamics so it is noticeable that bacteria currently follow generating resistance mechanisms. It was possible to confirm along with the literature that the antibiotic Dicloxacillin has no favorable antimicrobial spectrum for Gram-negative bacteria. The genus of bacteria that showed the most resistance to the different groups of antibiotics was Photo-bacterium in addition to being a piscicid bacteria, we can find different types of sewage or also sea, rivers, and industrial waters.

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### Conflicts of Interest

None.

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