Isolation and identification of *Pseudomonas aeruginosa* obtained from dogs and cats in Great Cairo regarding status of phenotypic antimicrobial resistance pattern

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Background
Companion animals; dog and cat are closely associated with the daily life of humans and may be implemented in the transmission of many microorganisms to their adopters. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a suited opportunistic and harsh to treat pathogen due to its rife environmental distribution, unique intrinsic and acquired resistance to numerous antimicrobials' categories.
Objective
The current study targeted to survey the existence of *P. aeruginosa* in laboratory samples obtained from diseased dogs and cats. The study also investigated the susceptibility and resistance of recovered isolates against antimicrobials.
Materials and methods
A total of 315 samples gathered from veterinary laboratories in Great Cairo governorates; fecal, ear, eye, respiratory, wounds and urine samples were previously collected from diseased dogs and cats. The samples were examined bacteriologically and biochemically to isolate *P. aeruginosa*. The isolates were assayed for their sensitivity and resistance versus 25 antimicrobials belonging to various categories.
Results and conclusion
Fifty- eight *P. aeruginosa* isolates (18.41%) were obtained from 315 dogs (44/233, 18.88%) and cats' (14/82, 17.07%) clinical swabs. The isolates were confirmed biochemically and via VITEK 2 compact system. All isolates showed alpha-type of hemolysis and pigment production. The obtained *P. aeruginosa* isolates revealed a multidrug resistance pattern by 70.45% in dog isolates while cat isolates demonstrated a higher ratio 78.57%. *P. aeruginosa* isolates were highly resistant to cephalosporins, trimethoprim/sulfamethoxazole, and intermediate resistant to erythromycin fosfomycin. On the other hand imipenem, amikacin, azithromycin then gentamycin and ciprofloxacin were the most efficient on *P. aeruginosa* isolates. The study included that *P. aeruginosa* isolates obtained from canine and feline clinical samples collected from Great Cairo laboratories were characterized by high and intermediate levels of antimicrobial resistance. However, this pattern was directed to some classes of antibiotics, which are not authorized for veterinary use, which could expose an early warning mark and give the need for ongoing monitoring.

Keywords:
antimicrobial susceptibility, cat, dog, Egypt, pets, *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa (*P. aeruginosa*)is an aerobic Gram-negative, non-glucose-fermenting rod-shaped that is broadly distributed over various environments. The bacterium represents an opportunistic pathogen that causes serious illnesses in humans and animals [1]. The pathogen developed its importance due to its ability to constitute a public health concern; transmission from contact animals to humans [2].

Furthermore, *P. aeruginosa* often comprises a challenge in treatment which contributes to the elevating resistance against a broad spectrum of antibiotics because of their unique intrinsic and acquired resistance mechanisms. Therefore, therapeutic choices for *P. aeruginosa* are shortened and only scarce antibiotics are effective [3].

Nowadays, the one Health approach comprises worldwide emerging of resistant strains which are implemented in serious threats for the health of

people and animals, with the human-pet interaction being a major factor in the spread of clinically important multidrug-resistant pathogens [4].

Recently, in Egypt companion animals rearing will be greatly increased and found to serve as a reservoir of different anti-microbial resistant bacteria resulting in increased prospect for zoonotic transmission due to their close contact with humans. Thus, keeping the eye on the zoonotic AMR pathogens as in companion animals is important for grasping the risk to the human population and the environment [5].

P. aeruginosa is considered one of the most prevalent bacterial agents associated with companion animals' infections (36%). In dogs and cats, *P. aeruginosa* has been exposed to be the obvious reason of wound and skin infections as well as chronic eye and ear inflammations, gastrointestinal, respiratory and to less extent in the urinary tract infections [6,7]. Usually, most researches have concentrated on *P. aeruginosa* isolates obtained from humans, but the tracking of different animal species is scanty.

Based on the previous concerns; the present study will aim to recognize the occurrence of *P. aeruginosa* among samples obtained from companion pets; dogs and cats with morphological assessment of their antimicrobial resistance picture.

Patients and methods

Ethics statement

As per CPCSEA guidelines; a study involving free laboratory samples does not require the approval of the Institute Animal Ethics Committee.

Sampling

A total of 315 samples were collected from different pets' veterinarian labs located in Cairo and Giza governorates with knowledge of case history as shown in (Table 1). All samples were promptly put in ice and transported to the lab for additional processing.

Isolation and identification

Swab samples except for fecal were cultured on trypticase soy broth for 18 h at 37 °C, and then a loopful of each enriched broth as well as fecal swabs were streaked on MacConkey agar and incubated for 18 h at 37 °C. After that, the suspected nonlactose fermenter colonies were streaked onto specific cetrimide agar plates and incubated at 37°C for 24 h aerobically [8]. The suspected colonies were morphologically and biochemically identified; Gram staining, nonlactose and glucose fermenters, positive to citrate utilization and urease production. Further advanced biochemical confirmation was performed by microbial identification system; VITEK 2 compact system, version: 9.02, (BioMerieux, France). All asserted *P. aeruginosa* isolates were preserved in Tryptic Soy Broth supplemented with 15% (vol/vol) glycerol and kept at – 80 °C for possibly future use.

Assessment of virulence

Hemolysis was assessed on 5% sheep blood agar, while Pseudosel agar was used for production of the fluorescent pyoverdine pigment.

Antibiotic susceptibility testing of *P. aeruginosa*

Isolates were purified and checked for antibiotic susceptibility via standard disk diffusion procedure. The antimicrobials used were of different classes. The zones of inhibition were noticed, and the output of antimicrobial sensitivity test were interpreted as resistant (R), intermediate (I) and susceptible (S) following to Clinical and Laboratory Standards Institute [9]. The multi-drug resistant (MDR) isolates were defined as those unsusceptible to more than two different classes of antibiotics. A total of 25 antimicrobials were utilized, comprising, amikacin (AK, 30 μg), amoxycillin/clavulanic acid (AMC, 20/10 μg), ampicillin/sulbactam (A/S or SAM 10/10 μg), azithromycin (AZM, 15 μg), cefaclor (CEC, 30 μg), cefixime (CFM, 5 μg), cefoperazone (CEP, 30 μg), cefotaxime (CTX, 30 μg), cefoxitin (FOX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), cefuroxime (CXM, 30 μg), ciprofloxacin (CIP, 5 μg), clindamycin (DA, 30 μg), erythromycin (E, 15 μg), fosfomycin (FF, 30 μg), gentamicin (GEN, 30 μg), imipenem (IPM, 10 μg), levofloxacin (LEV, 5 μg), moxifloxacin (MXF, 5 μg), nitrofurantoin (F, 300 μg), norfloxacin (NOR, 10 μg), Ofloxacin (OFX, 5 μg), rifampin (RIF, 30 μg) and trimethoprim/sulfamethoxazole (SXT, 25 μg).

Table 1 Different types of collected samples

Type of swab Species	Fecal	Respiratory (nasal, tracheal or lung aspiration)	Wounds	Urine	Ear	Eye	Total
Dog	24	30	57	75	33	14	233
Cat	9	4	22	40	7		82
Total	33	34	79	115	40	14	315

Results and discussions

The human-animal bond has evolved over time. Pets, for example, evolved from work animals (protecting homes, catching mice) to animals with a social job, providing companionship. Companion animals can be beneficial to their owners' mental and physical health, but they can also pose a public health hazard to probable adopters by disseminating zoonotic pathogens [10]. One of the most significant zoonotic bacteria is *P. aeruginosa* which can be considered key pathogen of chronic wounds and burns, eye infection, otitis as well as serious respiratory and urinary infections [11].

Isolation and identification

Our results revealed a number of 58 *P. aeruginosa* isolates as a total incidence (18.41%) were obtained; in detail, 44/233 among ill dog laboratory samples (18.88%) while 14 isolated from 82 ill cat ones (17.07%) distributed as shown in (Table 2). *P. aeruginosa* isolates were confirmed biochemically and via VITEK 2 compact system, version: 9.02 as presented in Fig. 1. All *P. aeruginosa* isolates showed alpha type of hemolysis on 5% sheep blood agar and produced pigment on specific media as shown (Fig. 2a, b).

Near results were also obtained in several reports; Bernal Rosas *et al.* [12] reported an incidence (11.2%) in pets clinical centers in Bogotá, D.C. Also, *P. aeruginosa* was isolated by incidence 14.6% and 8% from canine and feline samples gathered from 10 European countries [6].

In detail, the most isolation rate in our study was obtained from wound and pyogenic skin samples (35.44%) followed by otitis (30%) and keratitis (28.57%) then other infections; diarrhea, respiratory and urinary illnesses by (12.12%), (8.82%), and (6.08%), respectively.

Our data were greatly coincided with that obtained by Harada *et al.* [13] who reported the highest isolation from ear, skin then urine by 39.72%, 30.13%, and 17.80%, respectively, followed by nasal (4.11%) and oral samples (2.74%) but the eye infection demonstrated the lowest isolation among canine and feline samples (1.37%). Also, Yukawa *et al.* [14] demonstrated isolation rates of *P. aeruginosa* as otitis (39%), skin and wounds (28%), urine (13%), respiratory (12.5%), oral cavity (2.5%), genitals (1%), and eye (0.5%). Another study revealed that the otitis externa was the most isolation site of *P. aeruginosa* strains (55.7%), persuaded by the respiratory system (17%), and skin (14.6%) in dogs. While in cats, most of isolates were obtained from the nasal cavity (16.23%) [15]. Otitis externa is a common complaint presenting ranges from 7.5% to 16.5% in canine cases [16] at which *P. aeruginosa* constituted 20% isolation rate [17].

Antibiotic susceptibility testing of *P. aeruginosa*

The concern of the antimicrobial-resistant bacteria is the responsibility for transmission of the illnesses that comprise a threat to dog and cat health and consequently may transmit to their adopters. Among those bacteria, European Food Safety Authority (EFSA) identified *P. aeruginosa* with greater than 90% certainty as one of the most three relevant antimicrobial resistant bacteria in the EU relied on the available data [18].

Concerning the antimicrobial susceptibility, our data demonstrated that thirty one *P. aeruginosa* canine isolates exposed multidrug resistance manner as 70.45% while cat isolates showed a higher ratio 78.57%. Meanwhile, *P. aeruginosa* isolates exhibited strict resistance to many antibiotic classes; β-lactames, clindamycin, nitrofurantoin rifampicin, trimethoprim /sulfamethoxazole, most of cephalosporin, and some quinolones. Also, the isolates showed intermediate resistance to erythromycin (79.85%) and fosfomycin (79.85%). On the other hand amikacin, imipenem and azithromycin were the most efficient on *P. aeruginosa* isolates by 90.7%, 80.2%, and azithromycin, respectively, persuaded by ciprofloxacin (77.95%) and gentamycin (69.8%). Some of phenotypic antibiotic susceptibility and resistance of *P. aeruginosa* isolates were shown (Figs. 3–5).

Table 2 Number of *Pseudomonas aeruginosa* isolates in between given samples

Type of illness Species	Diarrhea	Nasal discharge, cough or pneumonia)	Wounds and pyogenic skin infections	Cystitis and lower urinary system infections	Otitis	Keratitis	Total
Dog	3/24 (12.5%)	2/30 (6.67%)	21/57 (36.84%)	4/75 (5.33%)	10/33 (30.3%)	4/14 (28.57%)	44/233 (18.88%)
Cat	1/9 (11.11%)	1/4 (25%)	7/22 (31.81%)	3/40 (7.5%)	2/7 (28.57%)	–	14/82 (17.07%)
Total	4/33 (12.12%)	3/34 (8.82%)	28/79 (35.44%)	7/115 (6.08%)	12/40 (30%)	4/14 (28.57%)	58/315 (18.41%)

Figure 1

bioMerieux customer **Laboratory Report** Printed by: Labadmin
System#:
Isolate: 2022122506-1 (Approved)
Card Type: GN Bar Code: 241069240303232393 Testing Instrument: 000015F23B41(12170)
Setup Technologist: Laboratory Administrator (Labadmin)

Bionumber: 036735757764210
Organism Quantity: **Selected Organism: *Pseudomonas aeruginosa***

Comments:	

Identification Information	**Card:** GN	**Lot Number:** 2410692403	**Expires:** Aug 05, 2024 13:00 CDT
	Status: Final	**Analysis Time:** 7.00 hours	**Completed:** Mar 7, 2023 12:18 CST
Organism Origin	VITEK 2		
Selected Organism	99% Probability **Pseudomonas aeruginosa** **Bionumber:** 036735757764210 **Confidence:** Excellent identification		
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s) Pseudomonas aeruginosa			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	-	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	LATK	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

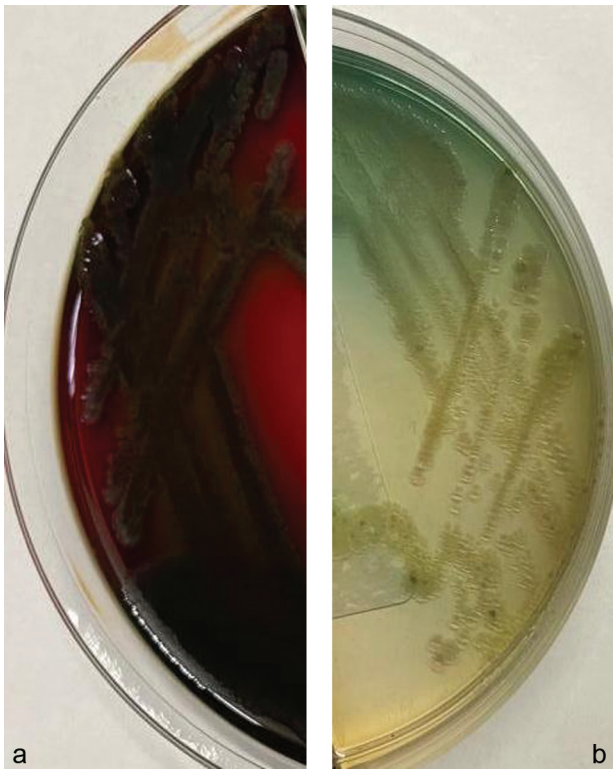
Biochemical profile of *Pseudomonas aeruginosa* isolates using VITEK 2 compact system.

Several reports stated parallel results; in Africa, a study conducted in Pretoria, South Africa, a total 155 *P. aeruginosa* isolates were identified between January 2007 and December 2013. The isolation sites included ear canal (34%), urine (22%), skin (10%) then abscesses (4%) with a considerable ratio (30%) were categorized as 'others'. Notably, 100% of the isolates were resistant to at least one antimicrobial, while 92% were MDR. High susceptibility values were observed to imipenem (94%), amikacin (84%) and gentamicin (82%), while the isolates showed high resistance pattern to penicillin-G (96%), amoxycillin-clavulanic acid (93%), trimethoprim-sulfamethoxazole (90%) cephalothin (90%), ceftazidime (77%) and fluoroquinolones (80%) [19].

Daodu *et al.* [20] identified six *P. aeruginosa* out of 62 oral swabs (5.9%) collected from hunting dogs in rural areas of Ogun State, South Western Nigeria. All isolates showed a strict multidrug resistance pattern. Commonly, more than 50% in *P. aeruginosa* strains identified in African studies are resistant to amoxicillin and clavulanic acid, penicillin G, ceftazidime, tetracycline, doxycycline, and chloramphenicol tylosin. While, there is a low resistance detected to imipenem, tobramycin and ciprofloxacin. However, 92% of these strains showed a multidrug resistant pattern [21].

Regarding, Asian continent, Japan; Harada *et al.* [13] and Yukawa *et al.* [14] reported high susceptibility to amikacin (97.5%), ciprofloxacin (79.5% and 91%) and to gentamicin (95.9% and 87.5%) while showed moderate resistance to fosfomycin (35.5%). Also, collected clinical isolates of *P. aeruginosa* from dogs and cats in Japanese animal hospitals demonstrated high susceptibility to amikacin (99.58%), gentamicin (97.92%) and imipenem (93.33%) with less susceptibility rate (82.08%) was recorded to ciprofloxacin [22]. Another study by Shahini *et al.* [23] examined the resistance manners of *P. aeruginosa* isolates obtained from various

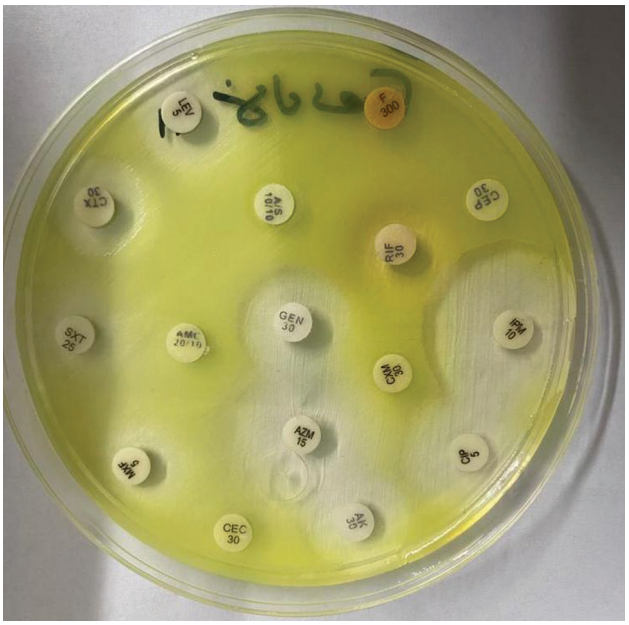
Figure 2



Colonial appearance of *Pseudomonas aeruginosa* isolates; a) alpha type of hemolysis on blood agar, b) pigment production on culture medium.

localities of Iran. For instance, in Tehran, the highest values of resistance were noticed for trimethoprim and ceftazidime 100% and 80%, respectively, while the

Figure 3

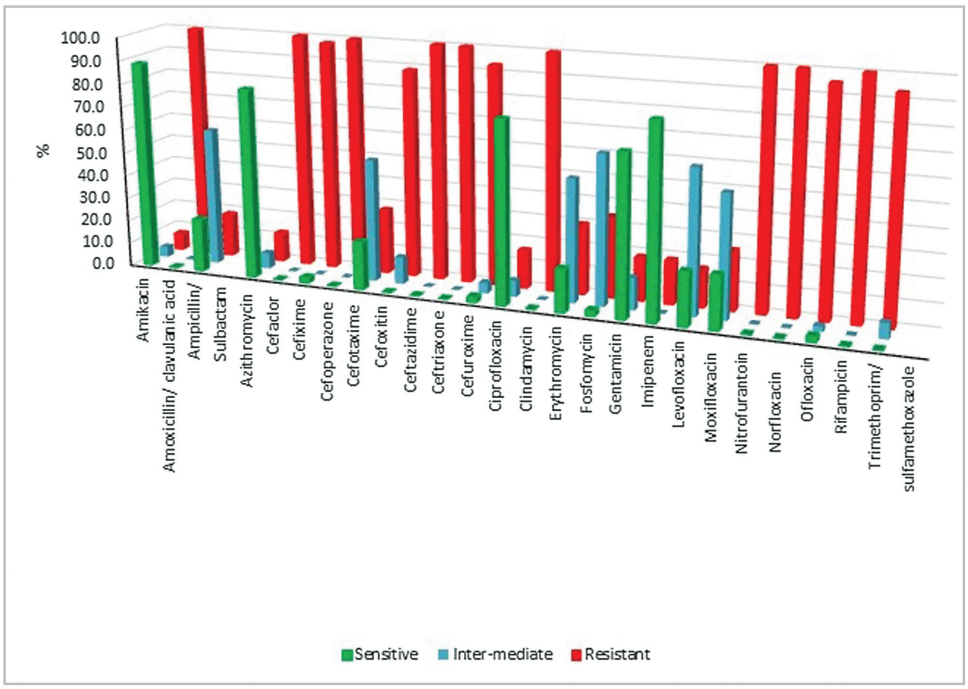


Antibiotic sensitivity testing of *Pseudomonas aeruginosa* isolates on Muller-Hinton agar.

lowest resistance was observed in imipenem (60%) and cefepime (52%).

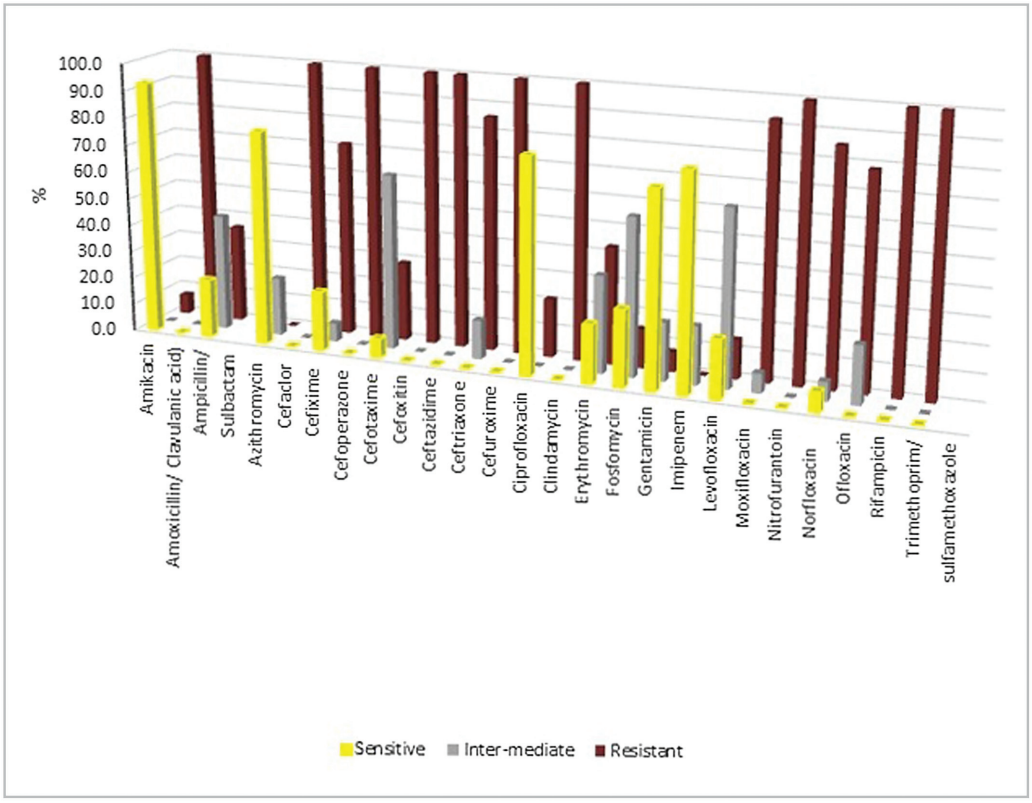
Concerning European continent; in an Italian study, *P. aeruginosa* looked to be the most multi-drug resistant Gram-negative bacteria obtained from canine specimens (79%). A resistance rate of 100% was recorded for amoxicillin–clavulanate and

Figure 4



Susceptibility of *Pseudomonas aeruginosa* canine isolates (n≈44) to the antibacterial agents.

Figure 5



Susceptibility of *Pseudomonas aeruginosa* feline isolates (n≈14) to the antibacterial agents.

trimethoprim–sulfamethoxazole. On the other side, gentamicin, and quinolones showed variable antibiotic resistance rates (10–33%) over the years of the study [7]. Another Italian study, 24 canine clinical isolates of *P. aeruginosa* showed no resistance to ceftazidime, gentamicin, and imipenem and low resistance values were encountered to quinolones (4.2%) [3]. Ludwig *et al.* [6] stated that *P. aeruginosa* canine isolates exposed resistance versus gentamicin by 18·8% while feline isolates showed resistance value by 18·2% for enrofloxacin in study conducted in 10 countries across Europe.

A novel study in Portugal, mentioned that resistance to different β-lactam cephalosporins was prevalent 74% out of 27 *P. aeruginosa* isolates obtained from urine, dermatitis, skin, ear and vaginal exudates. On the other hand, all isolates exhibited sensitivity to amikacin, while gentamicin resistance was noticed in 7% of the examined isolates. Furthermore, 30% of the isolates demonstrated resistance to imipenem while 7% exposed resistance to ciprofloxacin [24].

On the other side of the world; in Brazil, out of 106 rectal swabs from 81 dogs and 25 cats exposed isolation of *P. aeruginosa* from 73 (68.87%) of both healthy and ill animals. Assessment of antimicrobial resistance of the isolates revealed that 67 (91.78%) isolates were resistant to three or more antibiotic classes. Moreover, 13 (17.81%) isolates obtained from eleven ill dogs and two healthy cats were resistant to all assessed antimicrobial classes, indicated the probability of these pets to act as a reservoir of this zoonotic pathogen [25].

In Canada, a surveillance study was conducted on laboratory data collected over a 20-year period. *P. aeruginosa* was isolated from nasal cavity (20.7%), surgical (18.9%), abscess (10.3%) and wounds (9.3%). The antimicrobial sensitivity value was 75% and the multidrug resistance was noticed in 9% and 12% of the isolates from cats and dogs, respectively. Amikacin and gentamicin were the most effective antimicrobials versus *P. aeruginosa* isolates by 94.5% and 90.5%, respectively [26].

Generally it is derived that *P. aeruginosa* has intrinsic resistance mechanism to numerous antimicrobial agents; β lactamases, cephalosporins, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol and less resistant to imipenem, aminoglycosides and quinolones [27].

Conclusion

Overall, our study highlighted on the incidence of *P. aeruginosa* which is considered one of the important zoonotic pathogens among companion animals; dogs and cats in Great Cairo, Egypt. Consequently, the study may grape attention to these pets, as they can perform a role of reservoirs for these microorganisms, which are the prime pathogens of nosocomial infections globally. Also the study gives a spot on phenotypic antimicrobial pattern of *P. aeruginosa* isolates. The need for monitoring and minimizing the overuse and misuse of antibiotics among both human and pets is asserted to prohibit the emergence and dissemination of MDR strains of *P. aeruginosa*.

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Conflicts of interest

The authors declared that there is no conflict of interest and the manuscript has been read and approved by all the authors.

References

1 Grosso-Becerra MV, Santos-Medellín C, González-Valdez A, Méndez JL, Delgado G, Morales-Espinosa R, *et al. Pseudomonas aeruginosa* clinical and environmental isolates constitute a single population with high phenotypic diversity. BMC Genomics 2014; 15:318.

2 Behzadi P, Baráth Z, Gajdács M. It's Not Easy Being Green: A narrative review on the microbiology, virulence and therapeutic prospects of multidrug-resistant *Pseudomonas aeruginosa*. Antibiotics (Basel) 2021; 10:42.

3 Hattab J, Mosca F, Di Francesco CE, Aste G, Marruchella G, Guardiani P, Tiscar PG. Occurrence, antimicrobial susceptibility, and pathogenic factors of *Pseudomonas aeruginosa* in canine clinical samples. Vet World 2021; 14:978–985.

4 Fernandes MR, Sellera FP, Moura Q, Carvalho MPN, Rosato PN, Cerdeira L, Lincopan N. Zooanthroponotic transmission of drug-resistant *Pseudomonas aeruginosa*, Brazil. Emerg Infect Dis 2018; 24:1160–1162.

5 Saputra S, Jordan D, Mitchell T, Wong HS, Abraham RJ, Kidsley A, *et al.* Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia. Vet Microbiol 2017; 211:43–50.

6 Ludwig C, de Jong A, Moyaert H, El Garch F, Janes R, Klein U, *et al.* Antimicrobial susceptibility monitoring of dermatological bacterial pathogens isolated from diseased dogs and cats across Europe (ComPath results). J Appl Microbiol 2016; 121:1254–1267.

7 Nocera FP, Ambrosio M, Fiorito F, Cortese L, De Martino L. On gram-positive- and gram-negative-bacteria-associated canine and feline skin infections: a 4-year retrospective study of the university veterinary microbiology diagnostic laboratory of Naples, Italy. Animals (Basel) 2021; 11:1603.

8 Quinn PJ, Carter ME, Markey BK, Carter GR. In Clinical Veterinary Microbiology. Wolfe Publishing; London: Enterobactereaceae 1994; 109–135

9 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 30th ed. Supplement M100. 2020, Wayne, Pennsylvania

10 Overgaauw PAM, Vinke CM, Hagen MAEV, Lipman LJA. A One Health Perspective on the Human-Companion Animal Relationship with Emphasis on Zoonotic Aspects. Int J Environ Res Public Health 2020; 17:3789.

11 Wood SJ, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa*: Infections, Animal Modeling, and Therapeutics. Cells 2023; 12:199.

12 Bernal Rosas YE, Osorio K, Torres-García OA. *Pseudomonas aeruginosa*: An emerging nosocomial trouble in veterinary. Revista MVZ Córdoba 2015; (Suplemento-20):4937–4946.

13 Harada K, Arima S, Niina A, Kataoka Y, Takahashi T. Characterization of *Pseudomonas aeruginosa* isolates from dogs and cats in Japan: current status of antimicrobial resistance and prevailing resistance mechanisms. Microbiol Immunol 2012; 56:123–127.

14 Yukawa S, Tsuyukim Y, Sato T, Fukuda A, Usui M, Tamura Y. Antimicrobial resistance of *Pseudomonas aeruginosa* isolated from dogs and cats in primary veterinary hospitals in Japan. Jpn J Infect Dis 2017; 70:461–463.

15 Płókarz D, Czopowicz M, Bierowiec K, Rypuła K. Virulence genes as markers for *Pseudomonas aeruginosa* biofilm formation in dogs and cats. Animals 2022; 12:422.

16 Miller WH, Griffin CE, Campbell KL. In Muller and Kirk's Small Animal Dermatology. 7th ed. Toronto, Ontario: Elsevier 2013. 741–767

17 Saridomichelakis MN, Farmaki R, Leontides LS, Koutinas AF. Aetiology of canine otitis externa: a retrospective study of 100 cases. Vet Dermatol 2007; 18:341–347.

18 EFSA Panel on Animal Health and Welfare (AHAW), Nielsen SS, Bicout DJ, Calistri P, Canali E, Drewe JA, Alvarez J. Assessment of animal diseases caused by bacteria resistant to antimicrobials: Dogs and cats. EFSA J 2021; 19:e06680.

19 Eliasi UL, Sebola D, Oguttu JW, Qekwana DN. Antimicrobial resistance patterns of *Pseudomonas aeruginosa* isolated from canine clinical cases at a veterinary academic hospital in South Africa. J S Afr Vet Assoc 2020; 91: e1–e6.

20 Daodu BO, Amosun EA, Oluwayelu DO. 'Antibiotic resistance profiling and microbiota of the upper respiratory tract of apparently healthy dogs in Ibadan, south west Nigeria'. Afr J Infect Dis 2017; 11:1–11.

21 Yaovi AB, Sessou P, Tonouhewa ABN, Hounmanou GYM, Thomson D, Pelle R, *et al.* Prevalence of antibiotic-resistant bacteria amongst dogs in Africa: A meta-analysis review. Onderstepoort J Vet Res 2022; 89: e1–e12.

22 Hayashi W, Izumi K, Yoshida S, Takizawa S, Sakaguchi K, Iyori K, *et al.* 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. Microbiol Spectr 2021; 9: e0040821.

23 Shahini N, Shahini N, Ala S. Determining of resistance and sensitivity of *Pseudomonas aeruginosa* in Iran in 2010–2011. Res Pharm Sci 2012; 7:884.

24 de Sousa T, Garcês A, Silva A, Lopes R, Alegria N, Hébraud M, *et al.* The impact of the virulence of *Pseudomonas aeruginosa* isolated from dogs. Vet Sci 2023; 10:343.

25 Sfaciotte RAP, Parussolo L, Melo FD, Wildemann P, Bordignon G, Leitzke M, *et al.* Identification and characterization of multidrug-resistant extended-spectrum beta-lactamase-producing bacteria from healthy and diseased dogs and cats admitted to a veterinary hospital in Brazil. Microb Drug Resist 2021; 27:855–864.

26 Awosile BB, McClure JT, Saab ME, Heider LC. Antimicrobial resistance in bacteria isolated from cats and dogs from the Atlantic Provinces, Canada from 1994–2013. Can Vet J 2018; 59:885–893.

27 Feßler AT, Scholtzek AD, Schug AR, Kohn B, Weingart C, Hanke D, *et al.* Antimicrobial and biocide resistance among canine and feline *Enterococcus faecalis*, *Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* isolates from diagnostic submissions. Antibiotics (Basel) 2022; 11:152.