Antimicrobial resistance patterns of Pseudomonas aeruginosa isolated from canine clinical cases at a veterinary academic hospital in South Africa

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Although *Pseudomonas aeruginosa (P. aeruginosa)* can infect both animals and humans, there is a paucity of veterinary studies on antimicrobial resistance of *P. aeruginosa* in South Africa. Secondary data of canine clinical cases presented at the hospital from January 2007 to December 2013 was used. The following information was recorded: type of sample, the date of sampling and the antimicrobial susceptibility results. Frequencies, proportions and their 95% confidence intervals were calculated for all the categorical variables. In total, 155 *P. aeruginosa* isolates were identified and included in this study. All the isolates were resistant to at least one antimicrobial (AMR), while 92% were multi-drug resistant (MDR). Most isolates were resistant to lincomycin (98%), penicillin-G (96%), orbifloxacin (90%), trimethoprim-sulfamethoxazole (90%) and doxycycline (87%). A low proportion of isolates was resistant to imipenem (6%), tobramycin (12%), amikacin (16%) and gentamicin (18%). A high proportion of MDR-*P. aeruginosa* isolates was resistant to amoxycillin-clavulanic acid (99%), tylosin (99%), chloramphenicol (97%) and doxycycline (96%). Few (6%) of MDR-*P. aeruginosa* isolates were resistant to imipenem. *Pseudomonas aeruginosa* was associated with infections of various organ systems in this study. All *P. aeruginosa* isolates of *P. aeruginosa* exhibited resistance to β-lactams, fluoroquinolones and lincosamides. Clinicians at the hospital in question should consider these findings when treating infections associated with *P. aeruginosa*.

Keywords: antimicrobial resistance; *Pseudomonas aeruginosa;* dogs; multi-drug resistance; veterinary.

Background

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative, saprophytic and opportunistic pathogen capable of infecting both humans and animals (Alhazmi 2015). The organism is ubiquitous in moist environments such as water and soil (Iregbu & Eze 2015).

In human medicine, *P. aeruginosa* has been associated with nosocomial infections of the urinary tract, surgical wounds and bloodstream (Peleg & Hooper 2010). In addition, the organism has been isolated in patients with severe burn wounds, meningitis, brain abscesses and other underlying clinical conditions (Hauser & Ozer 2011; Strateva & Yordanov 2009; Türkyilmaz 2008). In veterinary medicine, *P. aeruginosa* has been isolated from dogs with chronic otitis externa, pyoderma, conjunctivitis, septicaemia, lower urinary tract infections, pneumonia and bacterial endocarditis (Dégi, Cristina & Stancu 2010; Petrov et al. 2013). Dogs with compromised immune systems and co-morbid conditions are at a higher risk of *P. aeruginosa* colonisation (Musser & Beamer 1961).

Although *P. aeruginosa* infections in human medicine are well documented in South Africa (Mudau et al. 2013; Odjadjare et al. 2012; Perovic et al. 2008), studies of *P. aeruginosa* infections in veterinary medicine could not be sourced. This is despite *P. aeruginosa* organisms having been reported as having high levels of resistance to commonly used antimicrobial agents such as penicillins, tetracyclines, fluoroquinolones and aminoglycosides (Prescott et al. 2003; Vingopoulou et al. 2018).

This study investigated the antimicrobial resistance patterns of *P. aeruginosa* from clinical samples obtained from dogs presented to a veterinary academic hospital in South Africa between January 2007 and December 2013. The results of this study will help guide empirical antimicrobial selection for the treatment of dogs infected with *P. aeruginosa* in veterinary medicine. In addition, the

information generated from this study will contribute to antimicrobial resistance surveillance programmes in animal health.

Methods

Study area

This study was conducted at a veterinary academic hospital located in Pretoria, South Africa. The veterinary hospital provides services for multiple veterinary disciplines, including internal medicine and surgical procedures. It offers training in companion, livestock and wildlife studies, and serves as a referral centre for complicated medical and surgical cases from other parts of the city, country and neighbouring countries.

Data collection

This study used secondary data of *P. aeruginosa* clinical isolates from dogs admitted to the veterinary academic hospital between January 2007 and December 2013. The hospital requires clients to sign consent forms granting the hospital permission to use information obtained from their animals for purpose of teaching and research. Information such as patient unique number, type of sample, date of sample collection, bacterial culture and antimicrobial susceptibility of the isolates was extracted from paper records submitted during the study period. The records of all the patients that yielded samples positive for *P. aeruginosa* (*n* = 155) were reviewed and included in this study.

Bacterial isolates and antimicrobial susceptibility testing

The bacteriology laboratory cultures all the submitted clinical samples to isolate *P. aeruginosa* using standard bacteriological methods as described by Quinn et al. (1994). Isolates were then subjected to a panel of 19 antimicrobial agents using the disk diffusion method to establish their susceptibility profiles. The bacteriology laboratory follows the Clinical Laboratory Standards Institute guidelines (Clinical Laboratory Standards Institute 2007, 2008, 2009, 2010, 2011, 2012) to isolate and conduct antimicrobial susceptibility testing.

Antimicrobials included in the test panel were the following: 30 μg-amikacin, 20/10 μg ampicillin, 100 μg carbenicillin, 30 μg ceftazidime, 30 μg cephalothin, 30 μg chloramphenicol, 30 μg doxycycline, 5 μg enrofloxacin, 10 μg gentamicin, 30 μg imipenem, 30 μg kanamycin, 2 μg lincomycin, 100 μg Lincospectin, 5 μg orbifloxacin, 10 μg penicillin-G, 100 μg piperacillin, 25 μg trimethoprim-sulfamethoxazole, 20/10 μg amoxycillin-clavulanic acid, 10 μg tobramycin and 15 μg tylosin.

The laboratory classifies the results of the antibiogram as intermediate, sensitive or resistant, following the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute 2007, 2008, 2009, 2010, 2011, 2012). However, for this study, isolates that had been classified as having intermediate susceptibility were reclassified as being resistant. Multi-drug resistance (MDR) was defined as

resistance to at least one antimicrobial in three or more antimicrobial categories (Magiorakos et al. 2011).

Antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and sulfamethoxazole-trimethoprim, which the organism showed inherent resistance to, were excluded from the MDR analysis (Pang et al. 2019). Lincospectin and lincomycin were also removed from the analysis because they are mainly efficacious against gram-positive bacteria (Farrington 2012). However, the newer generation β-lactams (imipenem) were included in the analysis because they have a broad-spectrum activity that allows them to be active against gram-negative organisms. Furthermore, amoxycillin-clavulanic acid was included in the calculation of MDR because clavulanic acid was shown to be effective against beta-lactamase enzymes.

Data management and analysis

The dataset was assessed for duplicates and missing information such as the lack of antibiogram results. None of the isolates had missing information and there were no duplications in the dataset.

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) (IBM SPSS statistics version 25). 'Specimen types' with a frequency of less than 4% were recategorised into a new category called 'others'. Thus, the category 'others' included specimen types such as aspirates, semen, prostate fluid, vaginal swabs, lung or pleural fluid, bile samples, foreign-object swabs, bone, nasal swabs, trans-tracheal aspirates and oral-cavity swabs. Descriptive statistics (i.e. frequencies and proportions) were computed and presented using tables.

Ethical consideration

This article followed all ethical standards for a research without direct contact with human or animal subjects.

Results

Thirty-four percent (34%, 52/155) of the *P. aeruginosa* isolates included in this study were recovered from ear canal samples followed by urine (22%, 34/155) and skin (10%, 16/155) samples. Abscesses contributed to the lowest proportion of isolates (4%, 6/155). Meanwhile, 30% (46/155) of the positive samples were categorised as 'others'.

Almost all *P. aeruginosa* isolates included in this study were resistant to lincomycin (98%, 150/153), penicillin-G (96%, 148/154), amoxycillin-clavulanic acid (93%, 142/152), carbenicillin (92%, 93/101), cephalothin (90%, 140/154) and doxycycline (87%, 134/154). However, lower levels of resistance were observed to imipenem (6%, 6/100), tobramycin (12%, 12/96) and gentamicin (18%, 29/154) (Table 1).

Multi-drug resistance

Almost all (92%, 142/155) *P. aeruginosa* isolates were MDR, with a high proportion of these MDR isolates exhibiting

TABLE 1: Antimicrobial resistance profile of *Pseudomonas aeruginosa* isolates from dog clinical samples tested at a veterinary academic hospital, South Africa.

Antimicrobial category	%	n/N	95% CI	
			Lower	Upper
Aminoglycosides				
Amikacin	16	26/156	11.64	23.3
Gentamicin	18	29/154	13.44	25.74
Kanamycin	89	134/150	83.38	93.33
Tobramycin	12	12/96	7.29	20.59
Penicillins				
Carbenicillin	92	93/101	85.14	95.93
Penicillin-G	96	148/154	91.76	98.20
Piperacillin	86	80/93	77.54	91.65
Amoxicillin/Ampicillin	92	133/144	86.84	95.68
Carbapenem				
Imipenem	6	6/100	2.78	12.48
Cephalosporins				
Cephalothin	90	140/154	85.32	94.51
Ceftazidime	77	78/101	68.93	85.00
Combination				
Amoxycillin-clavulanic acid	93	142/152	88.31	96.39
Tetracycline				
Doxycycline	87	134/154	80.79	91.43
Amphenicols				
Chloramphenicol	89	132/148	83.16	93.24
Fluoroquinolones				
Orbifloxacin	90	137/152	84.36	93.93
Enrofloxacin	73	113/154	65.89	79.73
Macrolide				
Tylosin-tartrate	92	143/154	87.66	95.96
Lincosamides				
Lincomycin	98	150/153	94.39	99.33
Lincosamide-aminoglycoside				
Lincomycin- spectinomycin	90	138/153	84.46	93.97

CI, confidence interval.

TABLE 2: Proportions of various antimicrobials that were involved in the multi-drug resistance combinations.

Antimicrobial agent	%	n/N	95% CI	
			Lower	Upper
Chloramphenicol	97.7	130/133	95.2	100
Doxycycline	96.4	134/139	93.3	99.5
Enrofloxacin	78.8	111/139	73.1	86.5
Imipenem	6.0	6/99	1.3	10.7
Orbifloxacin	96.3	132/137	93.2	99.4
Amoxycillin-clavulanic acid	99.2	132/133	97.7	100.0
Tylosin	98.5	137/139	96.5	100.0

CI, confidence interval.

Resistance of *Pseudomonas aeruginosa* to β-lactams

We observed that a high proportion (86%) of *P. aeruginosa* isolates was resistant to piperacillin. This is contrary to the 14% piperacillin resistance amongst *P. aeruginosa* isolated from human cancer, burn wounds and cardiac-neuro-paediatric surgical patients observed in Kuwait (Mokaddas & Sanyal 1999). A high proportion (92%) of *P. aeruginosa* resistant to amoxicillin-clavulanic acid was also observed in this study, which is consistent with the observation by Gad, El-Domany and Ashour (2008) who reported 95% amoxicillin-clavulanic acid resistance amongst *P. aeruginosa* isolated from human patients with respiratory tract, urinary tract and skin infections in Egypt. Human studies have also reported high proportions of *P. aeruginosa* isolates resistant to ceftazidime (Khan, Khan & Kazmi 2014; Oliveira et al. 2005; Papp-Wallace et al. 2011; Pintarić et al. 2017). This is consistent with the 77% resistance observed in this study.

The results of this study and other studies suggest that resistance against β-lactams is common amongst *P. aeruginosa* (Ansari et al. 2016; Mishra et al. 2012; Rafiee et al. 2014). This resistance is attributed to intrinsic resistance mediated by low membrane permeability and production of AmpC beta-lactamase amongst *P. aeruginosa* isolates (Pechère & Köhler 1999).

In contrast, we observed low levels (6%) of imipenem resistance amongst *P. aeruginosa* isolates. Our findings are consistent with the 10% imipenem resistance amongst *P. aeruginosa* from dogs with otitis externa in Brazil (Oliveira et al. 2005). In the light of these findings, imipenem remains the most effective drug for the treatment of β-lactam resistant *P. aeruginosa* and would most likely lead to a successful treatment outcome if used to treat *P. aeruginosa* exhibiting MDR at the veterinary hospital under study (Papp-Wallace et al. 2011).

Resistance to fluoroquinolones and tetracyclines

In this study, a higher proportion of resistance to enrofloxacin (73%) and orbifloxacin (90%) was observed amongst *P. aeruginosa* isolates from dogs as compared to 53% enrofloxacin resistant *P. aeruginosa* isolates from dogs reported by Pintarić et al. (2017). Similarly, Rubin et al. (2008)

resistance to amoxycillin-clavulanic acid combination (99%, 132/133), tylosin (99%, 137/139), chloramphenicol (97%, 130/133) and doxycycline (96%, 134/139). Only 6.06% (6/99) of MDR *P. aeruginosa* were resistant to imipenem (Table 2).

Discussion

We investigated the antimicrobial resistance patterns of *P. aeruginosa* in samples from canine clinical cases presented at a veterinary academic hospital in South Africa. Most *P. aeruginosa* isolates included in this study were isolated from ear-canal swabs and urine samples. Several other studies have also reported *P. aeruginosa* involvement in otitis externa (Dégi et al. 2010; Mekić, Matanović & Šeol 2011; Pye 2018; Steen & Paterson 2012) and urinary tract infections in dogs (Thompson et al. 2011; Wong, Epstein & Westropp 2015). However, given that *P. aeruginosa* is a secondary pathogen, more clinical and laboratory information is needed to determine the significance of these results (Weese et al. 2019). Although the presence of *P. aeruginosa* at these body sites could be attributed mainly to the increased sensitivity to infection because of the easy access (Cabassi et al. 2017), early diagnosis and implementation of the correct treatment are still important for improved prognosis (Marza et al. 2006).

reported lower proportions of *P. aeruginosa* from canine clinical isolates that were resistant to enrofloxacin (31%) and orbifloxacin (52%). Although we are not able to explain the difference between our results and those of other researchers, the resistance to fluoroquinolone observed in *P. aeruginosa* is generally attributed to the low permeability of the bacteria's outer membrane that limits the rate of penetration of antibiotic molecules into the cells (Nicas & Hancock 1983).

We also observed a high proportion (87%) of doxycycline resistant *P. aeruginosa*. This is comparable with the 91.07% and 99.6% resistance reported by Javiya et al. (2008) and Shah, Wasim and Abdullah (2015), respectively. Similarly, a high proportion (100%) of doxycycline resistant *P. aeruginosa* isolates from dogs with otitis externa was reported by Petrov et al. (2013). The high level of resistance to doxycycline observed in this study suggests that clinicians at the veterinary academic hospital under study might have to reconsider prescribing doxycycline for the treatment of *P. aeruginosa* infections in dogs presented at this the hospital.

Resistance to aminoglycosides

In comparison with resistance levels to other drugs observed and discussed above, low resistance levels to amikacin (16%), gentamicin (18%) and tobramycin (12%) were observed amongst *P. aeruginosa* isolates. This is consistent with the findings by Yukawa et al. (2017) who also reported low levels of *P. aeruginosa* resistance to amikacin (2.5%) and gentamicin (4.5%) in clinical cases of dogs and cats in Japan. Khan and Faiz (2016) also reported low proportions of *P. aeruginosa* isolates resistant to amikacin (7.4%) and gentamicin (11.6%) in various human clinical cases in Saudi Arabia. This is contrary to the view of some authors that *P. aeruginosa* tends to exhibit intrinsic resistance to aminoglycosides (Pang et al. 2019). The latter view is supported by studies that have reported very high proportions of *P. aeruginosa* isolates that are resistant to aminoglycosides. For example, Penna et al. (2011) in Brazil reported a high proportion of *P. aeruginosa* isolates from dog clinical cases that were resistant to amikacin (70%), gentamicin(71%) and tobramycin (65%). Javiya et al. (2008) in India also reported high proportions of *P. aeruginosa* isolates from human clinical cases that were resistant to amikacin (50%), gentamicin (67%) and tobramycin (66%). Similarly, 89% of *P. aeruginosa* isolates in this study were resistant to kanamycin. This is comparable with the 90% resistance to kanamycin amongst canine clinical isolates reported by Rubin et al. (2008) in the United States.

The higher proportion of resistance to kanamycin compared with other aminoglycosides that was observed in this study could be attributed to chromosomal aphA-encoded aminoglycoside phosphoryl transferase (APH(3′) IIb), which are enzymes that inactivate the action of antimicrobials, leading to resistance (Morita, Tomida & Kawamura 2013). Therefore, the results of this study support the theory of variations in the susceptibility of *P. aeruginosa* to different aminoglycosides based on their mechanism of action. In view of this, the observations cast doubt on the efficacy of

kanamycin in the treatment of *P. aeruginosa* infections amongst clinical cases presented at the veterinary academic hospital (Poole 2005).

Despite the widely accepted view that *P. aeruginosa* exhibits intrinsic resistance to aminoglycosides, available evidence suggests aminoglycosides such as amikacin or gentamicin are useful in the treatment of respiratory infections (Poole 2005). Furthermore, commercial topical preparations for treatment of ear infections also contain aminoglycosides that are known to be effective (Boyd, Santoro & Gram 2019).

Multi-drug resistance

Overall, 92% of *P. aeruginosa* isolates included in this study were MDR. Several other authors have also reported MDR levels of up to 97.9% amongst *P. aeruginosa* clinical isolates from humans (Shokri et al. 2016). In contrast, other authors have reported low proportions of MDR-*P. aeruginosa,* ranging from 14% to 29% in human studies conducted in Pakistan and Saudi Arabia (Gill et al. 2011; M.A. Khan & Faiz 2016; Tam et al. 2010; Ullah, Malik & Ahmed 2009).

Pseudomonas aeruginosa is known to exhibit intrinsic resistance against β-lactams, fluoroquinolones, tetracyclines, aminoglycosides and lincosamides (Iregbu & Eze 2015; Mekić et al. 2011; Steen & Paterson 2012; Türkyilmaz 2008). Intrinsic resistance is caused by factors such as low outer membrane permeability, the production of AmPc β-lactamase and the presence of efflux systems MexA-MexB-OprM, MexC-MexD-OprJ, MexE-MexF-OprN and MexX-MexY-Op (Morita et al. 2001, 2013). Because drugs against which *P. aeruginosa* exhibiting intrinsic resistance were not included in the determination of MDR, the high proportion of MDR-*P. aeruginosa* (92%, 142/155) observed in the present study is most likely explained by acquired resistance. This view is supported by the observation that antimicrobials that were frequently involved in MRD combinations such as enrofloxacin, imipenem, orbifloxacin and amoxycillin-clavulanic acid are drugs that *P. aeruginosa* is known not to exhibit intrinsic resistance to. Furthermore, because the veterinary hospital where the study was conducted is a teaching and referral hospital, it is also possible that by the time most of the dogs from which the samples were collected were presented at the hospital, they would already have been exposed to antimicrobial treatment. It is known that exposure to antimicrobials is a risk factor for the development of resistance. However, it is not possible to confirm this assertion because of lack of information on previous antimicrobial exposure amongst the dogs that were sampled.

Limitations of the study

The study was limited to only one veterinary academic hospital and did not include isolates from other veterinary hospitals in the vicinity of the study area. In view of this, findings reported in this study cannot be generalised to the whole of the Gauteng province. A history of previous antimicrobial usage amongst the dogs tested was also not available to the researchers; therefore, it was not possible to

associate the resistance patterns observed with antimicrobial usage patterns. Because intermediate resistant isolates were reclassified as resistant, there is a possibility that the proportions of resistance observed in this study were slightly overestimated. In addition, the susceptibility testing method used in this study was the Kirby Bauer Disk Diffusion (KBDD) method, which is reported to be limited when compared with the minimum inhibitory concentration (MIC) clinical application. Moreover, topical preparations appear to still be effective against *P. aeruginosa,* despite the KBDD method indicating a higher prevalence of resistance (Boyd et al. 2019). This notwithstanding, the KBDD method still provides accurate susceptibility testing to guide therapeutic options. Therefore, the results of this study contribute to baseline data for establishing the burden and patterns of antimicrobial resistance of *P. aeruginosa* from canine clinical isolates.

Conclusion

In this study, *P. aeruginosa* was isolated from dogs presented at the veterinary academic hospital with otitis externa, urinary tract infections and some skin infections. Furthermore, a high proportion of *P. aeruginosa* from these clinical cases was MDR. It is evident that *P. aeruginosa* from the study population tends to exhibit high resistance mainly to antimicrobials like β-Lactams, tetracycline, amphenicol, fluoroquinolone, macrolide and lincosamide. However, resistance against imipenem, amikacin, gentamicin and tobramycin in the same population, tends to be lower. In view of this, we recommend that clinicians at the hospital in question should take these findings into consideration when deciding on the treatment for cases associated with *P. aeruginosa* infections. The high level of resistance observed against kanamycin compared with other aminoglycosides in this study supports the theory of differences in *P. aeruginosa* susceptibility patterns within the aminoglycoside antimicrobial category.

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Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

D.N.Q and U.L.E. were involved in the study design, data analysis and interpretation, and preparations of manuscript. D.S. was involved in the study design as well as manuscript editing. J.W.O was involved in the study design and extensive editing of the manuscript. All authors read and approved the final manuscript.

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Data availability statement

The dataset that supports the findings of this study is available from Prof. Daniel Nenene Qekwana at the University of Pretoria and all the documentations have been approved and are in line with the regulations of the University of Pretoria.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official or position of any affiliated agency of the authors.

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