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Retrospective Analysis of Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* from Clinical Samples of Dogs in Grenada, West Indies

Jieun Hong[1], Wayne Sylvester[2], Andy Alhassan[3], Bhumika Sharma[3], Victor Amadi[3], Kamashi Kumar[4]

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ABSTRACT

Background: Clinical infections caused by multidrug-resistant pathogenic bacteria are associated with increased morbidity and mortality, imposing a severe economic burden. *Pseudomonas aeruginosa,* a gram-negative bacterium, had demonstrated a prominent level of acquired antimicrobial resistance to its selective antimicrobials. Currently, there is no updated information about the antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* in Grenada. Hence, the present study aimed to determine the antimicrobial susceptibility pattern of *P. aeruginosa* from the clinical isolates of dogs in Grenada.

Methods: This retrospective study used the diagnostic reports from January 2015 to December 2022. The diagnostic microbiology laboratory reports of canine patients at the Small Animal Clinic of St. George's University in Grenada were analyzed to study the antimicrobial susceptibility pattern of *P. aeruginosa*.

Result: Among the various bacterial clinical isolates, ninety-one clinical samples were positive for *P. aeruginosa*. The antimicrobial susceptibility test pattern of these isolates revealed susceptibility to gentamicin (95.6%), ceftazidime (96.7%), imipenem (97.7%) and ciprofloxacin (97.8%). The result of the present retrospective study illustrates that *P. aeruginosa* isolates from canine patients in Grenada are susceptible to the recommended antimicrobial drugs. However, this study also revealed the recent development of progressive antimicrobial resistance. This mandates the judicious use of antibacterial drugs against bacterial infections in Grenada.

Key words: Antimicrobial resistance, Dogs, Grenada, Multidrug-resistant, *Pseudomonas aeruginosa.*

INTRODUCTION

Antimicrobial resistance (AMR) has been a growing global threat to human, animal and environmental health worldwide. The World Health Organization (WHO) has declared AMR as one of the top ten global public health threats facing the human community (World Health Organization, 2023). *Pseudomonas aeruginosa* is a gram-negative, rod-shaped, encapsulated bacterium belonging to the family *Pseudomonadaceae* that can survive in a wide range of environments (Silby *et al*., 2010) and exists as a common bacterial contaminant in water and soil (Todar, 2020). *P. aeruginosa* is associated with respiratory infections, gastrointestinal infections, ear infections, urinary tract infections (UTI), skin and soft tissue infections and wound infections in domestic animals.

P. aeruginosa exists as an opportunistic pathogen and has been associated with nosocomial infections and ventilator-associated pneumonia in human patients (Barbier *et al*., 2013). In recent years, *P. aeruginosa* has developed antimicrobial resistance against various antimicrobial drugs, such as aminoglycosides, quinolones and beta-lactams (Kollef *et al*., 2014). The antimicrobial resistance mechanisms have gradually progressed over a period due to chronic/injudicious use of antimicrobials.

To minimize antimicrobial resistance and sustain antimicrobial susceptibility, it is mandated to keep track of *P. aeruginosa* susceptibility patterns. There are no currently available updated reports on the antimicrobial susceptibility pattern of *P. aeruginosa* in Grenada. Acknowledging the significance of emerging antimicrobial resistance, the present study was conducted to analyze the antimicrobial susceptibility pattern of *P. aeruginosa* from the clinical isolates of dogs from January 2015 through December 2022 in Grenada. Furthermore, the present study data could

[1]School of Veterinary Medicine, St. Georges University, Grenada, West Indies.
[2]Small Animal Clinic, School of Veterinary Medicine, St. George's University, Grenada, West Indies.
[3]Department of Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, West Indies.
[4]Department of Anatomy, Physiology and Pharmacology, School of Veterinary Medicine, St. George's University, Grenada, West Indies.

Corresponding Author: Kamashi Kumar, Department of Anatomy, Physiology and Pharmacology, School of Veterinary Medicine, St. George's University, Grenada, West Indies.
Email: kakumar@sgu.edu

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be compared with the previous antimicrobial susceptibility test (AST) analysis report of *P. aeruginosa* in Grenada, 2009 (Hariharan *et al*., 2009) to evaluate the susceptibility of *P. aeruginosa* against its recommended antimicrobials.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at St. George's University approved the study (IACUC-19007-R, 2019).

In this retrospective study, the diagnostic laboratory reports of canine patients presented to the Small Animal Clinic of St. George's University in Grenada, West Indies between January 2015 and December 2022, were retrieved from AVImark (Veterinary practice management software).

Inclusion criteria: In this study, the information of patient's details such as species, clinical condition, bacterial organism isolated and AST results were included.

The samples were collected from various clinical conditions of dogs such as otitis, abscess, infected wounds and skin and UTI using a culture swab. Clinical samples of feces and urine were also cultured for the isolation of bacteria. The samples were processed at St. George's University, School of Veterinary Medicine, Microbiology laboratory. The positive clinical samples of *P. aeruginosa* were then subjected to an antimicrobial susceptibility test using the Kirby-Bauer disk diffusion method, as outlined by Quinn *et al*. (1994) on Mueller-Hinton (MH) agar. The antimicrobial discs used were gentamicin (10 µg), ceftazidime (30 µg), imipenem (10 µg) and ciprofloxacin (5 µg). The zone of inhibition sizes was interpreted as susceptible, intermediate, or resistant, based on the guidelines provided by the Clinical Laboratory Standards Institute (CLSI, 2020) guideline.

The AST results were tabulated onto the Microsoft Excel sheet. The data were summarized and analyzed by Statistical Package for Social Sciences (SPSS) software. Descriptive statistics were used to analyze and derive the results of antibacterial susceptibility vs resistance of *P. aeruginosa* isolates of dogs in Grenada.

RESULTS AND DISCUSSION

Out of 1649 canine clinical samples submitted between January 2015 and December 2022, ninety-one clinical isolates (collected from clinical cases of otitis, UTIs, abscesses and wound infections) were found to be positive for *P. aeruginosa*. These ninety-one isolates were further studied for their antimicrobial susceptibility pattern using the Kirby-Bauer disc diffusion method. From the reports of these clinical isolates of *P. aeruginosa,* high susceptibility was recorded for gentamicin (95.6%), ceftazidime (96.7%), imipenem (97.7%) and ciprofloxacin (97.8%). One isolate showed an intermediate susceptibility to the ceftazidime and imipenem drug. This intermediate susceptibility could be categorized as an emerging resistant strain (Table 1).

Table 1 tabulates only the antimicrobials recommended for treating *Pseudomonas sp.* infections. Other antimicrobials such as amoxicillin+clavulanic acid, clindamycin, cephalexin, ceftriaxone, novobiocin, oxytetracycline and chloramphenicol used for the antimicrobial susceptibility test were not shown under this tabulation as these antimicrobials were not treatment choices for *P. aeruginosa* infections because of their inherent resistance.

During this study period, only seven *P. aeruginosa* isolates have been evaluated against amikacin (30 µg) antimicrobial drug. Out of this, only one isolate showed resistance to amikacin in 2021. Also, effective antibacterial drugs such as gentamicin, ceftazidime, imipenem and ciprofloxacin exhibited resistance patterns progressively since 2021 (Fig 1). This indicates that antimicrobial resistance has been triggered in a few *P. aeruginosa* isolates.

However, multidrug resistance was not documented in this study. This is a positive indication that the resistance development among *P. aeruginosa* is very minimal and could be well prevented by proper antibiotic use measures in Grenada.

P. aeruginosa is the most resistant bacteria and contributes major to nosocomial infections (Kollef et al., 2014). This bacterial pathogen exhibits multiple resistance mechanisms and is known for its high antimicrobial resistance pattern (Todar, 2020; Poole, 2011). The antimicrobial resistance mechanisms have gradually progressed over a period due to chronic/injudicious use of antimicrobials. The primary mechanisms to counter antibiotic efficacy in *P. aeruginosa* could be either intrinsic or acquired mechanisms. Intrinsic mechanisms include the expression of non-specific porin molecule (OprF) which limits the permeation of antibiotics, reduced expression of outer cell membrane porin channels and expression of efflux pumps that expel antibiotics out of the cell. Furthermore, *P. aeruginosa* could produce biofilm encapsulation, which prevents the antibacterial drug permeation (Breidenstein *et al*., 2011). The acquired resistance relates to the resistant gene

Table 1: Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolated from clinical samples of dogs in Grenada (January 2015 to December 2022).

Antimicrobial drugs (disc concentration, microgram, µg)	Number of *P. aeruginosa* isolates	Number of isolates (%)		
		Susceptible (S)	Intermediate (I)	Resistant (R)
Gentamicin (10)	91	87 (95.6%)	0	4 (4.4%)
Ceftazidime (30)	91	88 (96.7%)	1 (1.1%)	2 (2.2%)
Imipenem (10)	87	85 (97.7%)	1 (1.15%)	1 (1.15%)
Ciprofloxacin (5)	91	89 (97.8%)	0	2 (2.2%)

development acquired through horizontal gene transfer mechanisms such as transformation, transduction and conjugation. The resistant bacterium could synthesize antibiotic-inactivating enzymes to inactivate the antibiotic (Breidenstein *et al*., 2011; Okazaki *et al*., 2002; Mulcahy *et al*., 2010). The emergence of resistant *P. aeruginosa* strains is gradually increasing the challenge of treating resistant microbial infections (Giamarellou, 2002).

In this study, the antimicrobial susceptibility pattern of *P. aeruginosa* isolated from clinical samples of dogs in Grenada were evaluated. Under this retrospective study conducted from January 2015 to December 2022, ninety-one (91) isolates of *P. aeruginosa* were isolated from the clinical conditions of otitis, abscess, wound infections and UTI infections in dogs. This is in confirmation with the other research studies indicating *P. aeruginosa* as the cause of these clinical conditions. The results of this study report revealed that *P. aeruginosa* isolates of dogs in Grenada are susceptible to gentamicin, amikacin, ciprofloxacin, ceftazidime and imipenem. On comparing this retrospective study report to a similar study done for the period between 2005 through 2009 at St. George's University in Grenada (Hariharan *et al*., 2009), we found that *P. aeruginosa* isolates in Grenada have maintained a similar susceptibility pattern to aminoglycoside drugs, gentamicin and amikacin. The AST pattern of *P. aeruginosa* documented that the susceptibility of the bacterial isolates has been consistent over the last eleven years in Grenada. This is considered remarkable in the context of stabilized susceptibility patterns and emphasizes the importance of minimizing the possibilities of antimicrobial resistance development in the future.

The present study data from Grenada were compared with the global antimicrobial susceptibility pattern of *P. aeruginosa*, from South American, European and Asian continents. In Columbia, out of 89 Gram-negative bacterial isolates from canine patients collected between June 2013 and May 2014, at various clinics, 10 isolates were found to be *P. aeruginosa*. The antimicrobial susceptibility pattern of these isolates had documented susceptible responses to ceftazidime (70%), amikacin (70%), gentamicin (70%), ciprofloxacin (80%) and imipenem (90%) (Bernal-Rosas *et al*., 2015). Comparing this report with that of the present study data, the susceptibility pattern of canine *P. aeruginosa* isolates of Grenada revealed a higher sensitivity with gentamicin (95.6%), ceftazidime (96.7%), imipenem (97.7%) and ciprofloxacin (97.8%). The differences in antimicrobial susceptibility response rates between these two studies are of considerable significance and have demonstrated the sustained susceptibility pattern of *P. aeruginosa* in Grenada.

Multidrug resistance of *P. aeruginosa* has been demonstrated in France in canine patients, attributed to the high use of antimicrobials for otitis conditions in dogs. The antimicrobial susceptibility study conducted in France with 46 canine clinical isolates of *P. aeruginosa* that were collected between 2008 and 2011 showed a resistant response to gentamicin and ciprofloxacin of 56.5 % and 63% respectively (Haenni *et al*., 2015). On the contrary, this study revealed that there is no prevalence of multidrug-resistant strains of *P. aeruginosa*. In comparison with this study report, it became evident that a greater number of antimicrobials provides an effective susceptibility pattern against *P. aeruginosa* in canine species of Grenada.

In the present study analysis, out of eighty-seven (87) isolates of *P. aeruginosa* tested for imipenem, one isolate had shown intermediate resistance (1.15%) and one isolate exhibited complete resistance (1.15%). This is following the 6% imipenem resistance pattern reported for *P. aeruginosa* isolated from a veterinary academic hospital in South Africa (Eliasi *et al*., 2020). These reports are indicative of the progressive development of imipenem resistance among the *P. aeruginosa* bacterial strains. Carbapenem resistance has been reported for *P. aeruginosa* (Haenni *et al*., 2017) associated with increased expression of genes encoding efflux pumps to extrude the antimicrobial drug out of the bacterial organism (Poole, 2000). As imipenem is a broad-spectrum drug with effective action against *P. aeruginosa*,

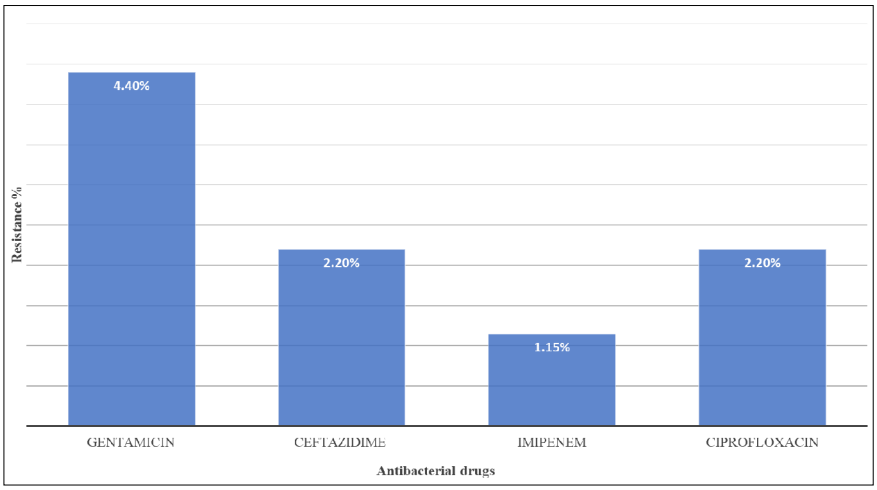

Fig 1: Antimicrobial resistance of *Pseudomonas aeruginosa* isolates of canines, Grenada (emerging from 2021).

it became imperative to prevent the further development of antimicrobial resistance.

This study report documented a lower proportion of antimicrobial resistance recorded for *P. aeruginosa* against aminoglycoside antibiotic, gentamicin (4.4%) in Grenada. This is following a similar study conducted in Japan with 200 clinical samples of *P. aeruginosa* isolated from dogs around seven major regions from September 2014 through February 2015. These isolates exhibited resistant responses to gentamicin and amikacin, occurring at 4.5% and 2.5% respectively (Yukawa *et al*., 2017). In this retrospective study, only seven isolates of *P. aeruginosa* are tested for amikacin antibacterial drug and this could be correlated to the reduced use of amikacin in Grenada.

P. aeruginosa demonstrated higher multidrug-resistant strains and was categorized as the main pathogen by WHO (Langendonk *et al*., 2021). Antimicrobial stewardship has been a challenge in recent years, as antibiotics use has increased to control common infections without any proper antimicrobial sensitivity test. This increased usage, along with the high level of prophylactic antibiotic use, the addition of antibiotics as a feed additive in animal feed and increased exposure to antiseptics and disinfectants globally, may further accelerate the development and spread of multidrug cross-resistance to antibiotics.

A recent study conducted in a veterinary teaching hospital testing the surface and liquid samples including sinks, rubber tubes and anesthesia breathing circuit had shown that the isolated *P. aeruginosa* isolates were highly resistant to gentamicin (47.4%), piperacillin/tazobactam (36.8%), levofloxacin (36.8%) and ciprofloxacin (36.8%). This study is authenticated proof of confirming the prevalence of *P. aeruginosa* strains in the animal clinic surroundings and is associated with the possible transfer of resistant organisms to the human population (Soonthornsit *et al*., 2023). Hence, continued antimicrobial surveillance in Veterinary hospitals is required to prevent the spread of resistant microorganisms.

In contradiction to the global reports, this study has demonstrated that *P. aeruginosa* isolates of Grenada are susceptible to antipseudomonal drugs used in Grenada. However, the smaller percentage of resistance level dictates that the resistance is emerging for the recommended antimicrobials. The probable reason for documented AMR could be correlated to the high level of antibiotic use in clinical conditions. Hence, it becomes mandatory to follow the essential surveillance pattern.

Furthermore, the antimicrobial susceptibility pattern exhibited by canine isolates of *P. aeruginosa* in Grenada revealed an effective susceptibility pattern compared to the global susceptibility pattern (Bourley *et al*., 2019). This is a significant update on the antimicrobial susceptibility pattern of *P. aeruginosa* isolates of canines in Grenada, West Indies.

CONCLUSION

From this retrospective study, it became evident that the *P. aeruginosa* isolates from canine patients in Grenada are highly susceptible to the antimicrobial drugs used for the treatment of *Pseudomonas* infection including gentamicin, amikacin, ciprofloxacin, ceftazidime and imipenem. Also, a recent lower percentage of the emergence of antibacterial resistance was documented in this study. These results indicate that the antimicrobial susceptibility pattern of *P. aeruginosa* remained consistent in Grenada between 2015 and 2022. This is a remarkable finding and dictates that clinicians mandate the appropriate use of antimicrobials in clinical conditions. Hence, it is imperative to adopt recommended measures to effectively maintain the antimicrobial susceptibility pattern of bacterial microorganisms with proper antimicrobial use and appropriate therapeutic strategies. The susceptibility pattern needs to be monitored crucially as the one health perspective is in line with the possible transfer of bacterial resistance to the human population.

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Conflict of interest

The authors declare that there is no conflict of interest about the publication of this article.

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