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Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas aeruginosa* isolated from canine infections

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Abstract

Infections with antimicrobial-resistant bacteria are a great challenge in both human and veterinary medicine. The purpose of this study was to determine antimicrobial susceptibility of 106 strains of *Pseudomonas aeruginosa* isolated from dogs with otitis and pyoderma from 2003 to 2006 in the United States. Three antimicrobial panels, including 6 classes and 32 antimicrobial agents, were used. A wide range of susceptibility patterns were noted with some isolates being resistant to between 8 and 28 (mean 16) of the antimicrobials tested. Among the β-lactams, all isolates were resistant to ampicillin, cefoxitin, cefpodoxime, cephalothin and cefazolin followed by amoxicillin/clavulanic acid (99%), ceftiofur (97%), ceftriaxone (39%), cefotaxime (26%), and cefotaxime/clavulanic acid (20%), whereas less than 7% of isolates were resistant to ceftazidime/clavulanic acid, ceftazidime, piperacillin/tazobactam or cefepime. Two isolates were resistant to the carbapenems. Among the quinolones and fluoroquinolones, the most isolates were resistant to naladixic acid (96%), followed by orbifloxacin (52%), difloxacin (43%), enrofloxacin (31%), marbofloxacin (27%), gatifloxacin (23%), levofloxacin (21%), and ciprofloxacin (16%). Among the aminoglycosides, the most resistance was seen to kanamycin (90%), followed by streptomycin (69%), gentamicin (7%), and amikacin (3%). Of the remaining antimicrobials 100% of the isolates were resistant to chloramphenicol followed by tetracycline (98%), trimethoprim/sulfamethoxazole (57%), and sulfisoxazole (51%). Point mutations were present in *gyr*A, *gyr*B, *par*C, and/or *par*E genes among 34 of the 102 naladixic acid-resistant isolates. Two isolates contained class 1 integrons carrying *aad*A gene conferring streptomycin and spectinomycin resistance. The findings suggest that many antimicrobial agents commonly used in companion animals may not constitute appropriate therapy for canine pseudomonas infections.

Keywords: *Pseudomonas aeruginosa*; Antimicrobial resistance; Fluoroquinolones; Canine; Class 1 integron; QRDR

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1. Introduction

Pseudomonas aeruginosa, a Gram-negative rod, is an important pathogen to both humans and animals (Hillier et al., 2006; Gales et al., 2001). The bacterium can be resistant to all classes of antimicrobial agents making it especially difficult to successfully treat patients with compromised immune defenses (Jalal et al., 2000; Pirnay et al., 2003). In dogs, *P. aeruginosa* is a common cause of pyoderma, otitis media/external and urinary tract infections (Cole et al., 2006; Gatoria et al., 2006; Hariharan et al., 2006; Hillier et al., 2006).

Due to the presence of several drug efflux systems and porins, *P. aeruginosa* is intrinsically resistant to a wide range of antimicrobials including benzylpenicillins, aminobenzylpenicillins, carboxypenicillins, first and second generation cephalosporins, chloramphenicol and tetracycline (Li et al., 1994; Nikaido, 1994). It also forms biofilms which are impervious to antimicrobials, further complicating therapy (Hall-Stoodley et al., 2004). The major classes of antimicrobials used for the systemic treatment of infections include the antipseudomonal penicillins, third and fourth generation cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. Unfortunately, resistance to these drugs is frequently encountered in clinical practice. Due to highly variable resistance patterns, empiric therapy may result in inappropriate treatment. Thus, antimicrobial susceptibility testing should be a crucial step in the selection of appropriate therapy.

With increasing utilization of fluoroquinolones in both human and veterinary medicine, emerging resistance is a concern (Hariharan et al., 2006; Linder et al., 2005). Resistance to fluoroquinolones is frequently due to point mutations in the DNA gyrase (*gyr*A and *gyr*B) and topoisomerase IV (*par*C and *par*E) genes. Plasmid-mediated resistance and efflux systems have been reported as alternate mechanisms of resistance. Class 1 integrons are important in the dissemination of resistance in Gram-negative bacteria (Hall, 1997). The objectives of this study were to define susceptibility patterns, characterize quinolone resistance and screen for class 1 integrons in 106 clinical dog isolates of *P. aeruginosa* from the United States.

2. Materials and methods

2.1. Bacterial strains

One hundred and six strains of *P. aeruginosa* isolated from dogs with soft tissue infections, e.g., otitis externa, otitis medius and pyoderma were used in this study. All strains were isolated from veterinary diagnostic laboratories from nine states in the U.S. between 2003 and 2006. History on prior antimicrobial chemotherapy was not available. Following the initial isolation and identification, the isolates were sent to the Center for Veterinary Medicine (CVM), the U.S. Food and Drug Administration (FDA). Upon receipt, the bacterial isolates were subcultured onto trypticase soy agar (TSA) plates supplemented with 5% defibrinated sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, MD). Isolates were confirmed as *P. aeruginosa* using VITEK Gram-negative identification cards (BioMerieux Inc., Hazelwood, MO) following the manufacturer's instructions and were then suspended in trypticase soy broth (TSB; Difco) containing 15% glycerol and stored at −80 °C until used.

2.2. Antimicrobial susceptibility testing

Antimicrobial minimum inhibitory concentrations (MICs) were determined using the sensititre automated antimicrobial susceptibility system in accordance with the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH). Initially, all isolates were tested using a panel designed by the National Antimicrobial Resistance Monitoring System (NARMS). This panel included ceftriaxone, ceftiofur, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ciprofloxacin, naladixic acid, amikacin, gentamicin, streptomycin, kanamycin, sulfamethoxazole, trimethoprim/sulfamethoxazole, tetracycline, and chloramphenicol. A panel containing only fluoroquinolones was also tested, including ciprofloxacin, levofloxacin, gatifloxacin, marbofloxacin, enrofloxacin, difloxacin, and orbifloxacin. In addition, all isolates were tested with a panel of β-lactam antimicrobials which included imipenem, meropenem, cefepime, piperacillin/tazobactam, ceftazidime, ceftazidime/clavulanic acid, cefotaxime/clavulanic acid, cefotaxime, ceftriaxone, cefoxitin, cefpodoxime,