In Vitro Antimicrobial Susceptibility of 183 Pseudomonas aeruginosa Strains Isolated from Dogs to Selected Antipseudomonal Agents

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Summary

During the years from 1993 to 2000, 183 strains of Pseudomonas aeruginosa were isolated from different pathological specimens originating from dogs. Antimicrobial susceptibility patterns against 10 antipseudomonal agents were obtained on 183 P. aeruginosa strains. In vitro antimicrobial susceptibility testing was performed using the disk diffusion method (Kirby-Bauer). Antimicrobial susceptibility profiles showed that aztreonam, ampicillin, cefoperazone, cefsulodin, nitrofurantoine and fluoroquinolones. Among the β-lactam antibiotics, new penicillins, including azlocillin, are more active than carbenicillin and some third-generation cephalosporins, notably cefoperazone, cefazidime and cefotaxim, also exhibit anti-Pseudomonas activity. Fluoroquinolones, especially enrofloxacin, have been used extensively during the past 10 years in veterinary dermatology, predominantly for the management of canine pyoderma and in the management of canine otitis externa and otitis media caused by P. aeruginosa. Approximately 40% of enrofloxacin is metabolized to ciprofloxacin and this active metabolite is further biotransformed to several additional compounds that are excreted primarily in urine (Cester and Toutain, 1997). Ciprofloxacin was shown to be the most potent quinolone against P. aeruginosa (Walker, 1999). Recently, marbofloxacin, was developed for veterinary medicine. Marbofloxacin has shown a broad-spectrum bacterial activity against numerous Gram-negative and Gram-positive bacteria, including mycoplasmas. It has also exhibited marked post-antibiotic effects, both in vivo and ex vivo, so it is suitable for use in wide range of clinical situations in dogs and cats (Spreng et al., 1995; Druegon and Thomas, 1997; Thomas et al., 1997; Weber et al. 2000; Barraza et al. 2000).

The present study investigated the in vitro antimicrobial susceptibility of 183 canine P. aeruginosa isolates against 10 antipseudomonal agents.

Introduction

Pseudomonas aeruginosa is a Gram-negative, glucose-nonfermenting aerobic bacterium. It is the epitome of an opportunistic pathogen of humans and animals and is rarely involved in primary disease. The pathogenesis of Pseudomonas aeruginosa infections is multifactorial, and connected by the number and wide array of virulence determinants possessed by the bacterium (Quinn et al., 1994). Pseudomonas aeruginosa can be cultured from the normal tissues of healthy animals, including gut, nasal cavity, mouth, tonsils, oral pharynx, distal urethra, prepuce, semen, vagina and conjunctiva (Greene, 1998). In animals, P. aeruginosa has been assigned as the distinct cause of infections such as otitis externa (Blue and Wooley, 1977; Mueller and Heusinger, 1994; Kiss et al., 1997; Colombini et al. 2000), cystitis, endocarditis, dermatitis, wound infections, conjunctivitis, equine metritis and ulcerative keratoconjunctivitis, mink haemorrhagic pneumonia, deep pyoderma, infections of the lower urinary tract (Rorich et al., 1983), prostatitis, osteomyelitis, chronic rhinitis, pleuritis, mastitis (Coats, 1998) septicaemia (Court et al., 1994), and bacterial endocarditis (Greene, 1998). It is notorious for its resistance to antibiotics and is therefore a particularly dangerous and dreaded pathogen. This bacterium is uniformly resistant to ampicillins, first- and second-generation cephalosporins, erythromycin and is also often resistant to streptomycin, tetracyclines, chloramphenicol, sulphonamides, cotrimoxazole, nitrofurantoine and fluoroquinolones. (Aires et al., 1999).

Materials and Methods

During the period from 1993 to 2000, 3184 specimens taken from dogs visiting The Clinics of the Veterinary Faculty at the University of Zagreb, were submitted to microbiological examination. Among them, 1230 swabs were taken from horizontal ear canals of dogs suffering from otitis. The rest of the specimens were taken from different body sites of diseased dogs. During that period, a total of 183 P. aeruginosa strains were isolated. Identification of P. aeruginosa was carried out according to the procedure described by Quinn et al. (1994). In the present study, 183 P. aeruginosa isolates were tested by the Kirby–Bauer's disk diffusion method on Mueller–Hinton agar (Prescott and Baggot, 1993) for antimicrobial susceptibility against 10 antipseudomonal agents: imipenem (10 µg), carbenicillin (100 µg), cefazidime (30 µg), cefotaxime (30 µg),...
amikacin (30 μg), gentamicin (10 μg), pipimedic acid (20 μg), enrofloxacin (5 μg) and ciprofloxacin (5 μg). Sensitivity to marbofloxacin (5 μg), was determined afterwards on all strains from our collection. A standard concentration of a pure culture of \textit{P. aeruginosa} was placed on Mueller–Hinton agar (Becton Dickinson, Cockeysville, USA), and individual filter paper disks containing known concentrations of individual antibiotics were placed on the pathogen. The culture was incubated for 18–24 h at 37°C. The zone of inhibition around each disk was measured, and the measurement was compared to the chart presented in Table 1. This classified the organism into one of three categories: sensitive, resistant and intermediate sensitive.

Results

In the period from 1993 to 2000, 3184 specimens originating from dogs were submitted to microbiological examination and a total of 183 \textit{P. aeruginosa} strains were isolated. The majority of the 183 \textit{P. aeruginosa} strains included in the present study originated from horizontal ear canals (106 isolates) and skin (40 isolates). More than half of the isolated strains originated from Cocker spaniels. The results of \textit{P. aeruginosa} isolations according to site of isolation are given in Table 2. Besides \textit{P. aeruginosa}, the most frequently isolated bacterial species was \textit{Staphylococcus intermedius} (487 isolates). Some other microorganisms were also found, e.g. \textit{Proteus sp.}, \textit{Streptococcus sp.}, \textit{Staphylococcus aureus}, \textit{Staphylococcus sp.} (coagulase-negative), \textit{Bacillus sp.}, \textit{Escherichia coli}, \textit{Malassezia pachydermatis} and \textit{Candida sp.} (226 isolates) which were not further investigated. Antimicrobial susceptibility profiles showed that among \textit{β}-lactam antibiotics, imipenem was the most active compound. Out of the 183 tested strains, 177 strains (96.7%) were sensitive and only four strains (2.2%) were resistant, while two strains (1.1%) were intermediately sensitive. A third-generation cephalosporin, cefoperazone showed a very good in vitro activity against 159 (86.9%) tested strains, 15 (8.2%) strains were resistant and nine strains (4.9%) had intermediate sensitivity. Against ceftazidime 141 strains (77.0%) showed

![Fig. 1. Results of in vitro sensitivity testing of 183 \textit{Pseudomonas aeruginosa} isolates against antipseudomonal agents.](image-url)
Antimicrobial Susceptibility of *P. aeruginosa* Strains in Dogs

sensitivity, 36 (19.7%) were resistant and six strains (3.3%) were of intermediate sensitivity. Carbenicillin was active against 131 strains (71.6%), 40 strains (21.9%) were resistant and 12 strains (6.5%) were intermediate. Sensitivity to amikacin was found in 160 (87.4%) isolates and in 152 strains (83.1%) to gentamicin. Against amikacin 14 strains (7.6%) showed resistance and 20 strains (10.9%) were resistant to gentamicin. To both aminoglycosides the intermediate sensitivity was similar, nine strains (4.9%) to amikacin and 11 strains (6.0%) to gentamicin. Against pipimedic acid 86 strains (47.0%) exhibited resistance, 82 strains (44.8%) were sensitive and 15 strains (8.2%) showed intermediate sensitivity. The *in vitro* sensitivity against enrofloxacin showed that 130 strains (71.0%) were sensitive, 48 (26.2%) were resistant and five (2.7%) were of intermediate sensitivity. The majority of all tested strains 171 (93.4%) were susceptible to ciprofloxacin, five strains (2.7%) showed intermediate sensitivity and seven strains (3.8%) were resistant. To marbofloxacin, 171 (93.4%) strains were sensitive, four (2.2%) showed intermediate sensitivity and eight strains (4.4%) were resistant. The results of *in vitro* sensitivity are given in Fig. 1.

**Discussion**

In the present study the antimicrobial susceptibility of 183 *P. aeruginosa* of canine origin was tested against three groups of anti-pseudomonal agents: β-lactams: imipenem, cefoperazone, ceftazidime and carbenicillin; aminoglycosides: amikacin and gentamicin; and fluoroquinolones: pipimedic acid, enrofloxacin, ciprofloxacin and marbofloxacin. Among the β-lactams imipenem was the most active compound. Out of 183 tested strains, 96.7% were sensitive and only 2.2% isolates were resistant. Similar results were obtained by Mueller-Premru and Gubina (2000), Panayotis et al. (1998) and Spencer (1996) in human *P. aeruginosa* isolates. In contrast, Watanabe et al. (1992) tested 334 clinical isolates against imipenem and found 23.4% resistant strains. Of the two third-generation cephalosporins in our study, cefoperazone showed higher activity than ceftazidime. Against ceftazidime 19.7% of tested strains showed resistance, while only 8.2% were resistant to cefoperazone. Relatively high resistance to ceftazidime was obtained by Watanabe et al. (1992), with 28% resistant strains. The susceptibility rates for ceftazidime *in vitro* sensitivity testing reported by other authors were much higher. Mueller-Premru and Gubina (2000) tested 208 isolates from humans and found only 9.1% strains resistant to ceftazidime, and Spencer (1996) showed that out of 29,425 *P. aeruginosa* isolates, 95% were sensitive. Higher resistance to ceftazidime was observed by Shawar et al. (1999), with 11.1% resistant strains of 1,240 tested, and Panayotis et al. (1998) who found 15% resistance against ceftazidime in 88 non-replicate nosocomial *P. aeruginosa* strains. The less active β-lactam was carbenicillin, against which 21.9% isolates were resistant. Colombini et al. (2000) investigated the *in vitro* sensitivity of 38 *P. aeruginosa* strains isolated from dogs suffering from otitis media and found a lower resistance rate to carbenicillin, with only 19%. There were no significant differences in sensitivity or resistance against the two tested aminoglycosides, amikacin and gentamicin, even though amikacin showed higher activity against *P. aeruginosa* than gentamicin. Sensitivity to amikacin was found in 87.4% isolates versus 83.1% sensitivity against gentamicin. Against amikacin, 7.6% strains showed resistance and 10.9% of them were resistant to gentamicin. Intermediate sensitivity to both aminoglycosides was similar: 4.9% to amikacin and 6.0% to gentamicin. Shawar et al. (1999) found 13.1% *P. aeruginosa* strains, isolated from humans, to be resistant to amikacin and 19.3% to gentamicin. Colombini et al. (2000) reported a higher gentamicin-resistance rate, out of 38 *P. aeruginosa* canine isolates, 32% were insensitive. The Vetoquinol group, Cedex, France (Charles-Eric Descotes, personal communication) in the *P. aeruginosa* epidemisurvey 2000, tested 15 canine isolates, of which 41% were resistant to gentamicin. The group showing *in vitro* sensitivity to quinolones gave some interesting results. Among four quinolone antibiotics, pipimedic acid, the oldest agent, was the least active compound of all those tested. Against pipimedic acid almost half of the tested strains, 47.0%, exhibited resistance, 44.8% were sensitive and 8.2% showed intermediate sensitivity. The *in vitro* sensitivity against enrofloxacin showed that 71.0% of tested strains were sensitive, 26.2% were resistant and 2.7% were intermediate in sensitivity. The majority of tested strains, 93.4%, were susceptible to ciprofloxacin, 3.8% were resistant and 2.7% showed intermediate sensitivity. The same results were obtained with marbofloxacin, to which 93.4% strains were sensitive, 2.2% showed intermediate sensitivity and 4.4% were resistant. Among the quinolone antibiotics, ciprofloxacin showed as the most active one. Ciprofloxacin activity against *P. aeruginosa* isolates by other authors exhibited a different activity pattern. Mueller-Premru and Gubina (2000) tested 208 strains and found 45.7% resistance to ciprofloxacin. Similar high resistance to ciprofloxacin, 20.7%, was reported by Shawar et al. (1999). In contrast, Spencer (1996) in an 8-year survey of 29,425 hospital *P. aeruginosa* isolates found 95% susceptibility to ciprofloxacin. The higher susceptibility to ciprofloxacin of our *P. aeruginosa* isolates is probably due to its very limited use in veterinary practice in Croatia. Enrofloxacin showed a relatively low activity against *P. aeruginosa* isolates compared to ciprofloxacin and marbofloxacin but also higher compared to the results of other authors. Those results are very interesting because enrofloxacin is in extensive use in the therapy of bacterial infections in small animals, especially dogs, suffering from otitis and/or skin disorders without prior antimicrobial susceptibility testing. A study by Cole et al. (1998) demonstrated that only 12.5% of *Pseudomonas* spp. isolates from the horizontal ear canal, and 35% of isolates from the middle ear canal were susceptible to enrofloxacin. Cole, [in a personal communication to Ihrke et al. (1999)], mentioned that 50% of canine isolates were resistant to enrofloxacin. Barrasa et al. (2000) tested 19 *P. aeruginosa* isolates and found 42.1% resistant strains to enrofloxacin. Colombini et al. (2000) tested 37 *Pseudomonas* isolates, of which 49% were resistant to enrofloxacin. The Vetoquinol group, Cedex, France (Charles-Eric Descotes, personal communication) in *P. aeruginosa* epidemisurvey 2000, tested 52 canine isolates and only 29% were susceptible to enrofloxacin. The *in vitro* sensitivity testing for marbofloxacin, included in the investigation afterwards, showed that 93.4% canine isolates were sensitive, 4.4% were resistant and 2.2% were intermediate sensitive. Barrasa et al. (2000) tested 19 *P. aeruginosa* isolates against marbofloxacin and found 89.9% sensitive strains, 9% were resistant and 1.1% showed intermediate sensitivity. Weber et al. (2000) investigated 31 *P. aeruginosa* and 27 *Pseudomonas* sp. isolates. They found that 83.9% of *P. aeruginosa* were susceptible to marbofloxacin and only...
19.4% were susceptible to enrofloxacin. Marbofloxacin not used in Croatia at all, and this is the first in vitro sensitivity testing of \textit{P. aeruginosa} isolates to this quinolone antibiotic. Vetoquinol group, Cedex, France (Charles-Eric Descotes, personal communication) in \textit{P. aeruginosa} epidemiosurvey 2000, tested 53 canine isolates of which 94% were susceptible to marbofloxacin. Even though the resistance of \textit{P. aeruginosa} strains of canine origin against enrofloxacin is relatively high (26.2%) it is much lower than reported by previously mentioned authors. The reason for the higher number of susceptible strains is probably because enrofloxacin was not in extensive use in Croatia as in other countries. The similar situation is with other quinolone antibiotics; use of ciprofloxacin is extremely rare in veterinary practice, and marbofloxacin is not yet registered in Croatia. A study by Frazier et al. (2000) showed that among marbofloxacin, enrofloxacin, (including its active metabolite ciprofloxacin) and difloxacin, marbofloxacin has greater \( C_{\text{max}} \) (maximum plasma drug concentration curve \( \mu g/\text{ml} \times h \)) and \( ACU_{0-\text{last}} \) (the area under the plasma drug concentration versus time curve \( \mu g/\text{ml} \times h \)) compared with enrofloxacin, ciprofloxacin, enrofloxacin plus ciprofloxacin combined, or difloxacin. Those results suggest that even though ciprofloxacin showed better activity than enrofloxacin and similarly activity to marbofloxacin, because of its pharmacokinetic properties, marbofloxacin should be the quinolone of choice. It is interesting to mention that one \textit{P. aeruginosa} strain, isolated from the horizontal ear canal of dog, showed a multiple resistance to all quinolone antibiotics as well as against imipenem, carbenicillin and cefazidime. Although not so common, multiple resistance of Gram-negative bacteria against fluoroquinolone antibiotics has already been noted (Thomson, 1999). It should also be pointed out that the majority of the isolated strains, 106 of them, originated from the horizontal ear canal and over half of the isolates originated from Cocker spaniels. We presume that some genetic background causes a predisposition to such a high rate of ear infections caused predominantly by \textit{P. aeruginosa}. Cole et al. (1998) evaluating otitis externa in 82 dogs found that Cocker spaniels represented 48.8% (40/82) of the dogs, while (1998) evaluating otitis externa in 82 dogs found that Cocker spaniels represented 48.8% (40/82) of the dogs, while (1998) evaluating otitis externa in 82 dogs found that Cocker spaniels represented 48.8% (40/82) of the dogs, while Cocker spaniels and otitis–media. Besides this mentioned study there were no data in the literature connecting Cocker spaniels and otitis–media. Besides this mentioned study there were no data in the literature connecting Cocker spaniels and otitis–media.

### References


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