Pantozzi y col.

Comunicación breve

ANTIMICROBIAL SUSCEPTIBILITY OF *Pseudomonas aeruginosa* ISOLATED FROM DISEASED MINKS

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ABSTRACT: *Bacterial susceptibility of 40 isolates of Pseudomonas aeruginosa to six antimicrobial agents ceftiofur (CFT), colistin (COL), danofloxacin (DNF), enrofloxacin (ENF), gentamicin (GEN), and trimethoprim:sulphamethoxazole (TMS) (ratio 1:19) were determined for the minimal inhibitory concentration (MIC) using the agar-dilution method. All strains were from ranch-raised mink (Mustela vison) origin, were considered clinically important (mostly with Pneumonia infection) and were collected during the past fourteen years. The MIC that prevented the growth of 90 % of the bacteria were in µg/ml, 100 for CFT, 100 for COL, 1.57 for DNF and ENF, 12.5 for GEN and > 100 for TMS. The fluoroquinolones showed higher activity in our strains than the classical antibiotics used against Pseudomonas aeruginosa. Based on the MIC values obtained and taking into account the administration form (mixed into the feed or water supply) of these drugs, we consider that they might be useful for treatment on this illness.*

Key Words: vison, antibiotics, *Pseudomonas aeruginosa*

SUSCEPTIBILIDAD ANTIMICROBIANA DE *Pseudomonas aeruginosa* AISLADAS DE VISONES ENFERMOS

RESUMEN: *La susceptibilidad bacteriana de 40 cepas aisladas de Pseudomonas aeruginosa a seis agentes antimicrobianos ceftiofur (CFT), colistin (COL), danofloxacin (DNF), enrofloxacin (ENF), gentamicin, (GEN), y trimethoprim:sulphamethoxazole (TMS) (razón 1:19) fue determinado para concentración inhibitoria mínima (CIM) usando el método del agar-dilución. Todas las cepas fueron aisladas de criaderos de visones (Mustela vison) de casos de neumonía hemorrágica y fueron recolectadas durante los últimos catorce años. La CIM que previno el crecimiento del 90% de las bacterias fue en µg/ml: 100 para CFT, 100 para COL, 1,57 para DNF y ENF, 12,5 para GEN y > 100 para las TMS. Las fluoroquinolonas mostraron actividad más alta en nuestras de cepas que los clásicos antibióticos usados contra Pseudomonas aeruginosa. Basado en los valores de CIM obtenidos y teniendo en cuenta la forma de administración de estas drogas (mezcla en el alimento o suministro de agua), consideramos que podrían ser útiles para el tratamiento de esta enfermedad.*

Palabras Clave: visón, antibióticos, *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is known worldwide to produce severe acute pneumonia in mink (*Mustela vison*) and is considered to be an important cause of economic loss in fur-bearing ranches. Hundreds of animals (up to the 80 % of the farm) may die within 12-24 hours (1, 2, 3, 4, 5). Nevertheless, its antimicrobial sensitivity status in mink has not been clearly defined.

Many different treatments have been proposed for control of *Pseudomonas aeruginosa* (chlorination of the water supply, disinfection with formalin of mittens and cages, drugs) but the disease is prevented well only by vaccination with autogenous formalinized vaccine (5, 6, 7).

There's no effective therapy for the disease but it would be possible to alleviate the condition by prompt and frequent administration of effective antibiotics.

In this survey, antibiotic susceptibility of 40 strains of *Pseudomonas aeruginosa* isolated from fatal pneumonic and septicemic cases in mink has been conducted.

MATERIAL AND METHODS

Collection of bacterial strains: Forty isolates of *Pseudomonas aeruginosa* were obtained from the Laboratorio de Investigaciones y Diagnóstico Bacteriológico (LADIB), Universidad Nacional de La Plata. Mostly of the specimens had been isolated from ranch-raised mink between 4 and 6 months old with sporadic or epizootic pneumonia infection (n = 31); the rest (n = 9) were from mink kits (1-4 week-old) with septicaemia (8). The strains were collected from different ranches (n = 7) of the southeastern region of the country, between April 1981 and May 1995. They had been stored at -70°C in trypteine soy broth with 20 % glycerine and/or lyophilized. All strains were characterized biochemically, and identification was carried out according to Lennette et al. (9) and Holt (10) (mainly with standard methods including serotyping (5) with a commercial *Pseudomonas aeruginosa* antisera test (Difco Lab., Detroit USA) following the manufacturer's instructions). Serotypes 1, 4 and 6 were the most commonly found among our strains (5). *Pseudomonas aeruginosa* ATCC (American Type Culture Collection) 27853 was used as reference strain.

Drugs used in this study were: ceftiofur (CFT, Upjhon Lab., USA), colistin (COL Argentia Lab., Argentina), danofloxacin (DNF, Pfizer Lab., Argentina), enrofloxacin (ENF, Bayer Lab., Argentina), gentamicin (GEN, Sigma Lab., USA), and trimethoprim: sulphamethoxazole (ratio 1:19) (TMS, Sigma Lab., USA).

Drug susceptibility testing was carried out by the agar-dilution method (NCCLS) (11). Each strain was grown on trypteine soy broth (Britania Lab., Argentina) at 37°C for 18 h. A broth culture of each strain was diluted to 10^6 colony-forming units/ml and spotted onto Mueller Hinton agar (Britania Lab., Argentina) supplemented with Cl_2Ca 50 mg/l and Cl_2Mg 25 mg/l, containing serial 2 fold dilutions of each drug by a multiplanter (Sakuma Seisakusho, Tokyo, Japan). Final concentrations of antibiotics tested ranged from 0.05 to 100 µg/ml. The minimal inhibitory concentration (MIC) was determined after aerobic incubation at 37°C for 18 h and was considered the lowest antimicrobial concentration that produced no visible bacterial growth.

RESULTS

The organisms were highly susceptible to DNF and ENF for each, (MIC 50: 0.78); (MIC 90: 1.57) (Table 1). COL and GEN showed low values to MIC 50 but high for MIC 90. The least-effective drugs were CFT and TMS.

The resistance pattern was homogeneous among the most recent and oldest isolations (1981-1995). Moreover, no particular association was observed between serotyping or case status (septicaemia, pneumonia epizootics) of the strains and their antimicrobial susceptibilities.

Table 1. Minimal Inhibitory Concentration (µg/ml) of 40 strains of *Pseudomonas aeruginosa* isolated from 40 mink.

Tabla 1. Concentración inhibitoria mínima (µg/ml) de 40 cepas de *Pseudomonas aeruginosa* aisladas de 40 visones.

MINIMAL INHIBITORY CONCENTRATION (µg/ml)

DRUGS	>100	100	50	25	12.5	6.3	3.15	1.57	0.78	0.39
ENF (enrofloxacin)		2						12	25	1
DNF (danofloxacin)		2						8	22	8
COL (colistin)	5					32	3			
GEN (gentamicin)	2				3	1	16	16	2	
CFT (ceftiofur)		10	25	5						
TMS (trimethoprim-sulphametoxazole)	35	5								

DISCUSSION

Literature on susceptibility of *Pseudomonas aeruginosa* isolates from mink is scarce. Chemotherapy with antibiotics is a commonly-used method for the control of mink pneumonia. However, treatment of this illness with these drugs is not effective owing possibly to its short incubation period which lasts 18-20 hours and the difficult to inject hundreds of animals at the same time during an epizootic (12, 13). Stress of handling and possible spread of the organism on mink-handling gloves are also possible reasons for poor results (14). When many mink are to be treated , the medication should be incorporated in the feed or water.

In The Netherlands and Denmark, the isolated *Pseudomonas aeruginosa* strains were highly resistant to most common antibiotics and chemotherapeutic drugs, therefore the disease couldn't be controlled satisfactorily by medication (4, 6).

Despite the fact that many isolates were reported as being antibiotic-susceptible "*in vitro*", response to treatment in field outbreaks was often ineffective. This was widely documented with sulfathiazole, the drug of "choice" in the past (12, 15, 16, 17), polimixin B and streptomycin (18), sulfadiazine-trimethoprim (4) and neomycin and dihydrostreptomycin (19).

Moreover, streptomycin and sulfonamides are not recommended for treating mink because of their toxicity and adverse local or systemic reactions in these animals (20, 21, 22).

Many ranchers rely on sulfathiazole to treat affected animals despite the fact that some of the *Pseudomonas aeruginosa* isolates are not susceptible to sulfathiazole using in vitro disc diffusion testing methods. Strains of *P. aeruginosa* virulent for mink vary in their susceptibility to sulfathiazole (15).

Long & Gorham (14) reported that most of the isolates have been susceptible to gentamicin but resistant to other antibiotics tested; nevertheless treatment with gentamicin by injection was variable and often not satisfactory.

In our study DNF and ENF were active in vitro against most of the strains suggesting that these drugs could be appropriate choices for initial treatment of infections. These 2 drugs have the additional advantage of oral administration and the post antibiotic effect (17, 23).

Seven strains were recovered from kits with general infection. Septicaemia is the most important cause of death among kits between 0-4 weeks-old of our ranches (8).

Two isolates showed values of MIC 100 and >100 µg/ml for all the drugs used in this survey. Although studies of multiresistant mechanisms were not realized, we suspected that this resistant mechanisms is due to impermeability .

In spite of the use of fluoroquinolones might be recommended administered in food or water for prevention and treatment on this illness, the only way of the correct use of antibiotics is the isolation of each strain in the outbreak and the choise of antibiotic of best performance with minor risk if generation of resistance in this type of bacteria. Moreover, it this pattern of antimicrobial susceptibility of *Pseudomonas aeruginosa* may be unique to our farms, and further studies from other countries are needed to determine general recommendations.

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