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Antibiotic Susceptibility of Bacteria Isolated from Virginia Opossum (*Didelphis virginiana*) in Hidalgo, Mexico

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Abstract: *Didelphis virginiana* is the only Mexican marsupial, in spite of to be a wild animal, it has been adapted to live near to human. We proceeded to evaluate the proportion of drug-resistant microorganisms isolated from *D. virginiana*, the study was done in the Mexican State of Hidalgo; were captured six adult animals, it were anesthetized. Samples were taken from the oral cavity, pharynx exudates, otic, optical and feces of all of them. The bacterial isolated were identified biochemically and evaluated by Disk Diffusion Susceptibility Testing. Were isolated Gram-negative bacteria, like *Escherichia coli*, *Proteus mirabilis*, *Citrobacter freundii*, *Edwardsiella* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica* and Gram-positive like *Staphylococcus aureus* coagulase-negative, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Corynebacterium* sp. and *Bacillus subtilis*. The results showed different levels of antibiotic resistance of bacteria isolated from wild opossums; multidrug-resistance was found in some of the strains in or at least one of them. *D. virginiana* could be a reservoir for a variety of microorganisms that have antimicrobial resistance and transfer it to other species as domestics like wild animal.

Key words: Tlacuache, antibiotic resistances, wild animal, tetracycline, reservoir, *D. virginiana*

INTRODUCTION

The marsupials are an important group of mammals found in Australia and America, they occupy a wide variety of ecological niches (Palhares *et al*., 2006). The Virginia Opposum (also known as tlacuache: *Didelphis virginiana*) is the only Mexican marsupial (Fig. 1). The offspring are born without being entirely developed and finishes its process in its mother marsupio (Krause *et al*., 1978, 1979a, b); species wont be variations since it appeared in our planet approximately, 60 million years ago. *Didelphis virginiana* is an omnivorous animal that feeds with fruits or insects, small reptiles and amphibians, regular size eggs and chickens, even with waste produced by humans (Flores-Vega, 1995; Hernandez-Huerta *et al*., 2002).

Since, the discovery and use of antibiotics for treating infectious diseases, the number of drug-resistant bacteria has been increasing at an alarming rate (Aarestrup *et al*., 2000). This problem has been extended to world of wildlife, where a significant increase of drug resistant microorganisms has been detected (Singh *et al*., 2004; Daly *et al*., 2006). In this context, some

Fig. 1: Picture of the Tlacuache captured for the present research

wild animals are part of the diet of different population groups in the central states of Mexico; the Virginia Opossum (tlacuache) being an example of such

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species, thus, constituting a health risk (Lira, 2004; Bautista-Urbano, 2008). Transfer of plasmid drug resistance to human pathogens might occur in this manner, if contaminated animals are not properly cooked (Nikolich *et al*., 1994; Bager *et al*., 1999; Aarestrup *et al*., 2000).

MATERIALS AND METHODS

Traps were placed at night and reviewed in the mornings; *D. virginiana*, after capturing them in the Mexican state of Hidalgo Situated around of 2168 msnm. The adults animals captured were anesthetized with ketamine 30 mg kg^{-1} (Anesket®Mexico)/Xylazine Hydrochloride 10 mg kg^{-1} (GAGSA®Mexico). Samples were taken from the oral cavity, pharynx exudates, otic, optical and feces of all of them. The samples were collected in Stuart medium and carry to the laboratory (Koneman *et al*., 2008); the Isolation of bacterial species was performed according to the Bergey's Manual of Systematic Bacteriology, the samples were grown in blood and MacConkey agar (BBL®) and incubated at 37°C for 24 h. Identification was done with biochemical tests. The method used in this study was the Disk Diffusion Susceptibility Testing with Mueller-Hinton agar (BBL, Sparks, MD) containing 5% blood of sheep; to each bacterial isolated was done a suspension in (Physiological Saline Solution) SSF and it was adjusted at 0.5 Nefelometro de Mc Farland, as indicated by Bauer *et al*. (1966). The results were interpreted according to National Committee for Clinical Laboratory Standards (2002).

RESULTS AND DISCUSSION

Six adult animals were captured (4 females/2 males) with an average weight of 1068 g. Five anatomical areas were sampled, making a total of 30 samples. Sixty one species were isolated (Table 1) and identified as (a) Gram-negative: *Escherichia coli* (7), *Proteus mirabilis* (9), *Citrobacter freundii* (6), *Edwardsiella* sp. (5), *Klebsiella pneumoniae* (5), *Pseudomonas aeruginosa* (6), *Mannheimia haemolytica* (1) and (b) Gram-positive *Staphylococcus aureus* coagulase-negative (6), *Staphylococcus epidermidis* (9), *Streptococcus faecalis* (3), *Corynebacterium* sp. (2) and *Bacillus subtilis* (2).

The isolated bacteria were evaluated for their susceptibility to antibiotics. For this, we used (BBL, Becton Dickinson) containing the following compounds/ amounts: nalidixic acid (30 g), amikacin (30 g), ampicillin (10 g), cefotaxime (30 g), erythromycin (15g), norfloxacin (10 g), penicillin (10 IU), sulfamethoxazole/trimethoprim (23.75/1.25 g), tetracycline (30 g).

The results showed different levels of antibiotic resistance of bacteria isolated from wild opossums; multidrug-resistance was found in some of the strains in or at least one of them, except in *B. subtilis*. *E. coli* was resistant to four out of ten antibiotics (4/10): nalidixic acid (4/7), amikacin (2/7), cefotaxime (5/7) and tetracycline (3/7). *P. mirabilis* (4/10), amikacin (1/9), ampicillin (4/9), sulfamethoxazole/trimethoprim (6/9) and tetracycline (2/9). *C. freundii* to nalidixic acid (2/6). *Edwardsiella* sp. to ampicillin (1/5) and tetracycline (2/5). *K. pneumoniae* (4/10): nalidixic acid (1/5), amikacin (1/5), ampicillin (4/5) and tetracycline (3/5). *P. aeruginosa* (4/10): amikacin (3/6), cefotaxime (1/6), norfloxacin (3/6) and tetracycline (3/6). *M. haemolytica* to erythromycin (1/1). *S. aureus* coagulase negative three; Erythromycin (2/6), penicillin (6/6) and tetracycline (3/6). *S. epidermidis* (3/10): erythromycin (1/9), penicillin (9/9) and tetracycline (4/9). *S. faecalis* to ampicillin (1/3) and penicillin (2/3). *Corynebacterium* sp. only to penicillin (1/2).

When resistance was analyzed, it showed a greater resistance in *P. mirabilis* and *S. epidermidis*, followed by *E. coli*. The antibiotics that showed more resistance were tetracycline to 20 strains resistant, penicillin and ampicillin with 19 and 10, respectively (Table 1).

Table 1: Anatomical source and occurrence of bacterial isolates

	Anatomical areas					
Bacteria	Oral cavity	Pharynx exudates	Otic	Optical	Feces	Total
Escherichia coli	0	0	0	0	7	7
Proteus miriabilis	0	2	0	0	7	9
Citrobacter freundii	0	0	2	0	4	6
Klebsiella pneumoniae	2	3	0	0	0	5
Edwardsiella sp.	0	0	1	1	3	5
Pseudomonas aeruginosa	2	2	2	0	0	6
Mannheimia haemolytica	0	1	0	0	0	1
Staphylococcus aureus coagulase negative	3	2	0	1	0	6
Staphylococcus epidermidis	3	3	1	2	0	9
Streptococcus faecalis	1	0	0	0	2	3
Corynebacterium sp.	1	1	0	0	0	2
Bacillus subtilis	0	0	0	0	2	2
Total	12	14	6	4	25	65

CONCLUSION

It is interesting to point out that *D. virginiana* is an animal that lives close to human population, dairy herds, pigs and poultry farms, many of these places often use antibiotics to treat diseases in their animals or as growth promoters (Aarestrup *et al*., 1998, 2000). Therefore, it is tempting to speculate that wild animals that drink run off water from these environments can thus, acquire these drug-resistant strains and locate them as normal flora.

Although, some of these species are not pathogenic to humans, it is important to know the distribution in wildlife as well as how resistance to chemotherapy is distributed among them. The *D. virginiana*, like any other wild species, could be a reservoir for a variety of microorganisms that have antimicrobial resistance and transfer it to other animals and even the men with a load of antibacterial resistance.

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