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Distribution of Multidrug Resistant Bacteria in Inanimate Objects Within Stalls in Hidalgo, Mexico

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Abstract: The emergence of multidrug resistant bacteria is a threat to veterinary and human health. The aim of this study was to determine what would be, if any, the contribution of inanimate objects present in stalls in Hidalgo, Mexico, as a possible reservoir for these microorganisms. About 72 strains are isolated from different locations within stalls, where 54.2% were gram positives and 45.8% were gram negatives and found that 38 out of 72 strains were resistant to at least one antibiotic (52.77%) and 13 out of 38 (34.2%) were resistant to more than 2 antibiotics. The present study underscores the need of carefully assessing what are the correct measures for using and disposing of antibiotics in the veterinary setting.

Key words: Multidrug resistance, antibiotics, stalls, inanimate objects, emergence, veterinary

INTRODUCTION

In recent years, there has been a worldwide increase of drug resistant bacteria; a routine approach is to evaluate antibiotic resistance of clinical isolates. However, considering the frequently reported appearance of pathogens that are able to resist a number of current antibiotics (Aarestrup *et al*., 1998; Wegener *et al*., 1997), we thought worth studying organisms isolated from environmental samples, aiming to ascertain whether or not they could be implicated in the rise and maintenance of bacterial drug resistance, possibly by its capacity to horizontally transfer it to acceptor organisms.

Antibiotics presence in the environment due to their production by competing bacteria (D'Costa *et al*., 2006; Wright, 2007) or as a side product of improper use by humans as well as the presence of naturally resistant saprophytic microorganisms could play a key role as sources for drug resistance transmission to other cells. From the veterinary point of view, it is necessary to consider that in general, antibiotic resistance might be the consequence of a production-oriented approach, whereby treatment is required to stop a spreading illness or from the perspective of improving profits, such as the use of antibiotics as promoters of growth in some species (Aarestrup *et al*., 2000; Bager *et al*., 1999). In this study, we would like to draw attention to the possible role for inanimate objects as reservoir for a persistent-like population which could infect animals or humans.

MATERIALS AND METHODS

We performed a sampling process on material from inert areas in 4 stalls located in Tulancingo Valley, Hidalgo, Mexico, a moderate climate zone with rains in summer and a dry winter; area handling and climate conditions allow maintaining bacterial viability (Makinson and Swan, 2006).

A total of 40 samples are obtained taken from utensils located in handling corrals, including water troughs, troughs, as well as from materials used for routine work like buckets water and milk tanks. We used the 2 most abundant isolated colonies of each sample for further analysis giving a total of 72 specimens.

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For the isolation and identification of bacterial species, we followed the protocols recommended in the Bergey's Manual of Systematic Bacteriology. Samples were inoculated onto Blood and MacConkey agar (BBL®) and incubated at 37°C for 24 h; species identification was carried out using biochemical tests.

For antibiotic susceptibility tests, used a diffusion disk assay (Bauer *et al*., 1966) where each bacterial strain was grown overnight and a suspension was made using 0.85% NaCl, adjusted to a 0.5 reading of Mc Farland's nephelometer (Murray and Zeitinger, 1983) and inoculated on Mueller-Hinton agar (BBL, Sparks, MD), supplemented with 5% defribinated sheep blood. The panel of antibiotics we used is shown in Table 1. The results were interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002).

RESULTS AND DISCUSSION

We isolated the following strains: *Escherichia coli, Citrobacter* sp. *Serratia marcescens*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus faecalis*, *Enterococcus* sp., *Bacillus subtilis*. A summary of the results is shown in Table 2. Out of the 72 isolated strains, 54.2% were gram positives and 45.8% were gram negatives.

From the first group, *Enterococcus* sp. (12.5%) and *Staphylococcus aureus* coagulase negative (11.11%) were the most commonly isolated species. In the gram negatives group, we frequently found *E. coli* (18%) followed by *P. aeruginosa* (15.2%).

Interestingly, 38 out of 72 strains were resistant to at least one antibiotic (52.77%) and 13 out of 38 (34.2%) were resistant to >2 antibiotics (Table 1). Species showing Multidrug Resistance (MDR) include *S. aureus* coagulase negative (5/8), *E. coli* (3/5) and *P. aeruginosa* (4/8).

It is relevant to note that several places in this area routinely use antibiotics for treatment of their ill animals or as growth enhancers, thus potentially providing a means for bacteria to develop drug resistance and then these organisms can be harbored in inanimate areas among the objects of daily work (Kramer *et al*., 2006).

Table 1: Detection of multi-resistant gram-positive and gram-negative bacteria

	Antibiotic concentration										
Bacteria	Amikacin 30 μg	Ampicillin 10 μg	Sulfamethoxazol/ trimethoprim 23.75/1.25 μg	Nalidixic acid 30 μg	Norfloxacine 10 mg	Cefotaxime 30 μg	Erytromycine 15 μg	Penicilline 10 UI	Tetracycline 30 μg	Gentamicine 10 μg	Total Isolations
Gram negative											
Escherichia coli	0	1	0	3	0	1	N/D	N/D	N/D	N/D	11
Citrobacter sp.	0	0	0	2	0	0	N/D	N/D	N/D	N/D	4
Serratia marcescens	0	2	0	0	0	0	N/D	N/D	N/D	N/D	5
Pseudomonas aeruginosa	3	1	0	0	2	2	N/D	N/D	N/D	N/D	13
Proteus vulgaris	1	2	1	0	0	0	N/D	N/D	N/D	N/D	6
Gram positive											
Sthaphylococcus aureus coagulase negative	N/D	N/D	N/D	N/D	N/D	0	3	2	1	0	8
Micrococcus sp.	N/D	N/D	N/D	N/D	N/D	0	1	1	1	0	6
Enterococcus faecalis	N/D	N/D	N/D	N/D	N/D	0	0	2	0	1	4
Enterococcus sp.	N/D	N/D	N/D	N/D	N/D	1	1	3	0	1	9
Bacillus subtilis	N/D	N/D	N/D	N/D	N/D	0	0	0	0	0	6
Total	4	6	1	5	2	4	5	7	2	2	38/72

N/D = Not Determined

Table 2: Origin and occurrence of bacteria isolated from different environmental sites

Subjects	*Escherichia coli*	*Citrobacter* sp.	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Sthaphylococcus aureus* coagulase negative	*Micrococcus* sp.	*Enterococcus* faecalis	*Enterococcus* sp.	*Bacillus* subtilis	Total
Water troughs	2	0	2	3	0	0	2	2	3	2	16
Troughs	3	1	0	2	1	2	1	0	2	1	13
Buckets	3	1	1	3	1	2	1	1	0	1	14
Water tanks	0	1	2	3	1	1	0	1	1	0	10
Milk tanks	0	0	0	1	0	2	1	0	1	0	5
Handling corral	3	1	0	1	3	1	1	0	2	2	14
Total	11	4	5	13	6	8	6	4	9	6	72

CONCLUSION

It is important to conduct a study that assess the distribution and permanency of bacteria as well as the resistance to the current therapeutically relevant agents and use it as an indicator of drug resistance transmission in areas where antibiotics are used as a preventive measure. In this manner, safety measures can be taken, to avoid transferring to other animals a variety of microorganisms to which directly or indirectly humans could have helped in becoming antibiotic resistant, remaining unnoticed in inert areas, as a potential source for widespread tolerance or resistance to multiple drugs.

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