Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 12, Page No: 183-187

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Prevalence of Vancomycin Resistant *Enterococci* among Pet Groomers and their Animals

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ABSTRACT

Introduction: Enterococcus species are found in the gut and feces of humans and different animals, with the ability to survive and grow under hard conditions carrying the antibiotic resistance genes especially in hospitalized patients.

Aims: Our study aimed to identify VRE carried in household animals' mouth and also on hands of their owners.

Methods: Swabs from pet's mouths and the hands of their groomers were taken and the vancomycin resistant Enterococci were isolated and identified by classical, automated and molecular techniques, as well as their antibiogram according to CLSI protocols.

Results: Thirty-three Enterococcus faecium and Enterococcus gallinarum, with 66,67% Vancomycin resistance were identified by the classical microbiological and Vitek-2 and DNA-based methods. The isolates were distributed as: 25 isolates from animal's mouths; 7 and one isolate from the animal owners' hands and mouth respectively. The isolated enterococcus spp. possesses the gene Van-B with product size 635 bp.

Conclusion: As in many researches in different countries, Vancomycin resistant Enerococcus faecium still the most prevalent VRE, with its great importance to cause infections and to transfer the resistance gene to other infectious bacteria.

Key words: Pets, Vancomycin resistant *Enerococcus faecium*, Van-Agene, Van-B gene.

HOW TO CITE THIS ARTICLE: Ghada Younis Abdulrahman, Prevalence of Vancomycin Resistant *Enterococci* among Pet Groomers and their Animals, J Res Med Dent Sci, 2022, 10 (12): 183-187.

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Received: 19-November-2022, Manuscript No. jrmds-22-84150;

Editor assigned: 21-November-2022, PreQC No. jrmds-22-84150(PQ);

Reviewed: 05-December-2022, QC No. jrmds-22-84150(Q);

Revised: 09-December-2022, Manuscript No. jrmds-22-84150(R);

Published: 16-December-2022

INTRODUCTION

Serious infections caused by the Enterococcus spp., such as bloodstream infections, are often treated with gentamicin and penicillin or vancomycin. But since the Enterococcus genus is known to be resistant to several medicines, there are few possibilities for a successful treatment. When ampicillin-resistant *Enterococci* are discovered, we often choose medicines that are associated with greater side effects, such as vancomycin, quinopristin, and linezolid. Additionally, an effective cure may not be achievable if ampicillin, gentamicin, or high dosages of vancomycin resistance are present [1]. Because of the zoonotic potential of domestic animals, the prevalence of antibiotic-resistant bacteria is a significant and global public health problem [2]. Household pets are animals that people keep in their homes for entertainment, companionship, or psychological support. These pets may be infected or colonized with various pathogenic bacterial species that are harmful to both people and animals, and the transmission of resistant bacteria can happen either directly through physical contact (such as petting or close contact), licking, or injuries, or indirectly through tainted food. Additionally, the frequent transmission of skin microbes between humans and their pets emphasizes the significance of touch. In recent years, the number of home animals has increased (there are now around 127 million dogs and cats in the EU nations alone), and it has expanded beyond the usual house pets of dogs and cats to include rats, rabbits, birds, reptiles, and more. Due to the numerous genetic similarities found between Multi Drug Resistance isolates from humans and from household pets, as well as recent studies that suggest contact with pets as a zoonotic risk factor for human infections by resistant bacteria, the rapid emergence and spread of Multidrug Resistance bacteria among household animals is cause for concern [3,4]. VanA-type glycopeptide resistance is the mechanism behind

vancomycin resistance in VRE strains. Glycopeptides impede the formation of the peptidoglycan and trans-glycosylation processes in gram-positive bacteria by binding to the C-terminal of the pentapeptide (D-Ala-D-Ala) precursors. The rate of resistant *Enterococci* to vancomycin increased from 0.3% to 40% in the United States within the past two decades, despite the lengthy time it takes for the (vancomycin-resistant gene-vanA) to spread to other bacterial species. As a result, it is crucial to diagnose bacteria, determine their antibiotic susceptibility pattern, and manage VRE strains in order to prevent infections [5-7].

Drug resistance in *Enterococci*, in particular *E. faecalis* and *E. facium*, and their contribution to horizontal gene transfer to the species isolated from the oral cavity, have received a lot of attention recently.

MATERIALS AND METHODS

In this research, samples were collected from 25 domestic animals and their owners (n=25), who visited veterinarian clinics for various animal-related recommendations. These were the animals: Using sterile cotton swabs, swabs were obtained from the mouths of 10 cats, 10 dogs, and 5 rabbits and their owners. The swabs were then transported to the lab in a vial containing 4ml Brain Heart Infusion broth. These samples were used to inoculate Petri dishes with M-Enterococcus agar (HIMEDIA) and MacConkey agar (LAB045), which were then incubated at 37C0 for 24 hours before being checked for colonies exhibiting the traits of Enterococcus spp. and Escherichia coli, respectively. With the use of the Bio-Me'rieux Vitek-2 technology, the identity was verified.

The Clinical and Laboratory Standards Institute (CLSI): The Clinical and Laboratory Standards Institute guidelines, were followed in determining the antibiotic sensitivity of the isolated bacterial colonies against Vancomycin using the disk diffusion method (Kirby-Bauer method [8,9], which is a standardized technique for testing rapidly growing pathogens. Antibiotic discs containing vancomycin (VA 30g-Bioanalyse) were used.

PCR in multiplex The acquired Vancomycin Resistant Enterococcal isolates were submitted to DNA extraction by the Bacterial DNA kit, kept at 70 C0 before testing, in accordance with the manufacturer's instructions (Macrogen South Korea). The collected DNA was submitted to Multiplex PCR on a Thermal Cycler using specified primers (Table 1). The analysis of amplified results included electrophoresis on 1% agarose [10-12].

RESULTS AND DISCUSSION

Numerous bacteria that are part of the natural flora act as a natural defense for the body by boosting the host's immune system [13]. Swabs from the mouths and owner's hands and mouths were obtained from 11 domestic pets (ten cats, ten dogs, and five rabbits). Figure 1 illustrates the isolation and identification of thirty-three (33) enterococcal isolates on two distinct culture media: Seventeen (17) pink to red-colored isolates on M-Enterococcus agar and sixteen (16) pink-colored isolates on MacConkey agar. In Table 2, the number and origin of the isolates were shown. Gram-positive bacteria made up every isolation.

E. faecalis is not currently thought to be a normal component of the oral microbiota. It has been noted as the most frequent species collected from teeth that had unsuccessful endodontic therapy and as the main infectious agent linked to subsequent endodontic infections. It has been shown that *E. faecalis* may live in multiple levels of the oral biofilm, causing endodontic

Table 1: Vancomycin resistance genes used, (Microgen, South Korea public biotechnology company).

Oligo	**Van A-F**
Sequence	5- GGGAAACGACAATTGC-3 (17 mer)
Oligo	Van A- R
Sequence	5-GTACAATGCGCCGTTA-3 (16mer)
Oligo	Van B -F
Sequence	5-ATGGGAAGCCGATAGTC-3(17mer)
Oligo	Van B -R
Sequence	5-GATTTCGTTCCTCGACC-3 (17mer)

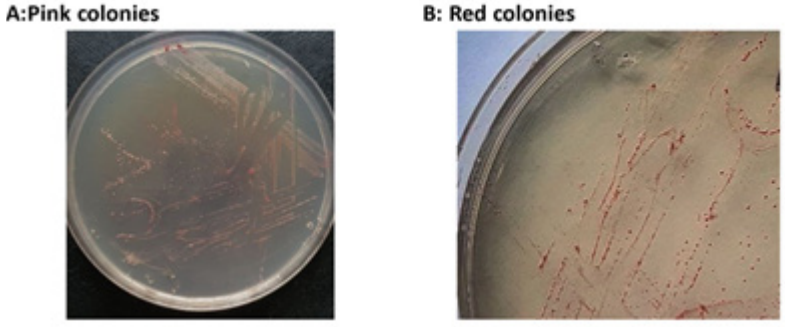

Figure 1: Growth on M Enterococcus agar.

Table 2: The isolates bacteria from pets and their owners.

Type of medium	Sources and numbers of the isolates			
	Mouth animal	Mouth human	Hand human	Total no.
M-*Enterococcus* agar	15		2	17
MacConkey agar	10	1	5	16
Total	25	1	7	33

Table 3: Vancomycin sensitivity pattern of the isolates.

No.of isolate	Name of isolate	Source of isolate	Diameter of inhibition zone mm	Sensitive	Resistant
1	1EG	Dog mouth	23	S	
2	2EG	Dog owner mouth	29	S	
3	3EG	Cat owner hand	8		R
4	4EG	Cat owner mouth	8		R
5	5EG	Cat mouth	30	S	
6	6EG	Cat owner hand	8		R
7	7EG	Cat owner mouth	20	S	
8	8EG	Cat mouth	30	S	
9	9EG	Cat owner mouth	13		R
10	10EG	Cat mouth	18	S	
11	11EG	Cat mouth	18	S	
12	12EG	Cat mouth	10		R
13	13EG	Cat mouth	29	S	
14	14EG	Rabbit mouth	10		R
15	15EG	Rabbit mouth	10		R
16	16EG	Cat mouth	35	S	
17	17EG	Dog mouth	11		R
18	1MG	Cat owner hand	35	S	
19	2MG	Cat mouth	13		R
20	3MG	Cat mouth	14		R
21	4MG	Cat owner mouth	12		R
22	5MG	Cat mouth	9		R
23	6MG	Cat owner hand	36	S	
24	7MG	Dog mouth	9		R
25	8MG	Dog owner hand	9		R
26	9MG	Dog owner mouth	11		R
27	10MG	Dog mouth	10		R
28	11MG	Dog owner hand	12		R
29	12MG	Dog owner mouth	10		R
30	13MG	Cat mouth	14		R
31	14MG	Cat owner hand	12		R
32	15MG	Cat owner mouth	10		R
33	16MG	Dog mouth	9		R
Total &%	33(100%)			11 (33.33%)	22 (66.67%)

treatment to fail [7]. In this section, we will discuss the significance of Enterococcus faecalis isolates from animal sources, particularly in relation to human health. This bacterium is isolated from animals and their meat, as well as from feces samples and blood samples in cases of blood stream infections in some people [14]. These bacterial strains were crucial to community health because they were linked to serious and challenging diseases, were multi-resistant and endemic, and had a remarkable potential to produce and spread resistance determinants [15,16]. We found that 22 (66.67%) of the enterococcal isolates were resistant to vancomycin, as demonstrated in Table 3. The inhibition zone diameters according to CLSI (Clinical and Laboratory Standard Institute; 31st edition) (8) were equal or more than 17 mm for sensitive strains and equal to or more than 20 mm for resistant strains.

When the Vancomycin-resistant Enterococcus bacteria was discovered and recognized as a serious health issue in Europe in 1986, this germ was first isolated. Later, with the discovery of the type A resistance genes' transmission to methicillin-resistant bacteria like Staphylococcus aureus MRSA, the problem's significance increased and complexity increased. According to the Saudi study, 6.1% of the 246 *Enterococci* isolates tested positive for vancomycin resistance. Additionally, data from the Disease, Dynamic, Economic and Policy Centre (CDDEP) shows that the highest prevalence of *E. facium* is found in Argentina and the United States, followed by Australia and Ireland. Romania and Vietnam also rank highly among Asian countries, with Vietnam and India topping the list by 27% for each. 28 samples out of 298 samples, or 9.39 percent, tested positive for vancomycin-resistant *Enterococci*, with the most common isolates

coming from poultry and pig workers: *E. facium* (43%), Enterococcus faecalis (32%), and Enterococcus galinirum (25%) [12]. One of the last alternatives for treating bacterial alpha caused by multiresistant bacteria is the antibiotic vancomycin [17]. *Enterococci* are the most widely distributed multi-resistant bacteria right present. Infection with these bacteria, particularly Enterococcus faecalis and Enterococcus facium, has drawn a lot of interest owing to the creation and manufacturing of strains with many antibiotic resistances, which explains the reason of the spread of infection.

Clinical and epidemiological studies have supported the idea that commensal bacteria from humans and animals serve as a reservoir for vancomycin-resistant (VanR) *Enterococci*. However, few of these studies have examined the in vivo transfer of vancomycin resistance genes, and animal models are still required to assess the transfer potentialities between different *Enterococci* species [18].

Vitek-2 was used to confirm the enterococcal isolates' identities, which are Enterococcus facium and Enterococcus gallinarum, and Multiplex-PCR was used to find the vancomycin-resistant genes. The findings are presented in Figure 2. The findings demonstrate the strong resistance of the detected *Enterococci* to vancomycin as many researchers also remark since the isolates had the gene Van-B with a product size of 635 bp [5,9,15,18].

Particularly those with the vancomycin resistance gene operons with distinct variation, which lead to a large number of resisting phenotypes, the Enterococcus spp. possess exceptional adaptive ability to develop and transmit the resistance genes. Different Vancomycin resistant genes (A, B, C, D, E, G, L, M and N) are distinguished by the degree of resistance to glycopeptides, their transfer ability, and their inducibility [16].

Dog and cat bites account for a significant portion of patients who are referred to accident and emergency services (e.g., 1% in America and Europe), and while the

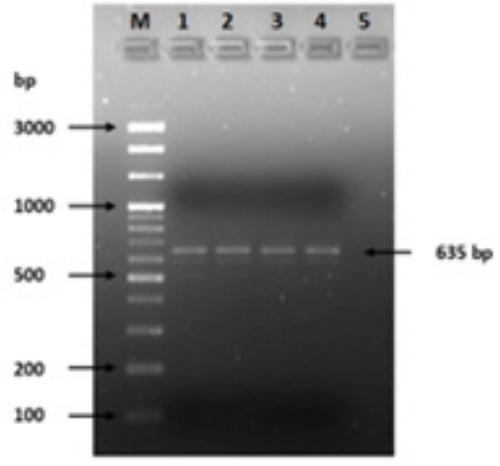

Figure 2: Agarose gel electrophoresis showing polymerase chain reaction for detection of Van-B gene with product size 635 bp. M: Marker 100 bp. Wells 1-4 are positive samples, well 5 is negative control.

location and severity of the bite are undoubtedly factors that determine the type and number of bacteria in, as well as other factors, such as wound care, general health, and patient immunity, all of which have an effect on the injury, severity, and treatment, It is crucial to monitor the presence of this bacterium in domestic animals and their owners due to the resistance of bacteria to antibiotics [3]. We must aid medical professionals in the quick and accurate identification of these bacteria, as well as in the prevention and management of VRE infection [19].

CONCLUSION

It is obvious that domestic animals have an impact on population health, particularly for those who live with their pets. A significant screening study is required in order to identify potentially dangerous pets or their owners who are carrying *Enterococci* strains that are Vancomycin resistant. This information is crucial for maintaining public health.

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