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Association of High Prevalence of Vancomycin Resistant Enterococci With Unsafe Waste Disposal Practices in Poultry Shops

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Abstract: The occurrence of antibiotic resistant bacteria in human consumables like poultry is a major concern to be addressed. Fecal samples from broilers were analyzed for the presence of Vancomycin resistant Enterococci. A total of 100 broiler fecal samples obtained from different slaughterhouses of Lahore were spread on Kanamycin Aesculin Azide agar and incubated for overnight at 37ºC. The Vancomycin concentrations used during this study were 16µg/ml and 64µg/ml of the medium for enumeration of Vancomycin resistance and superbugs Enterococci, respectively. Chicken feces samples were contaminated with VRE and superbugs were 79% and 25%, respectively. It was observed that chicken feces collected from Dharampura, Islampura, Shadbagh, Garhi Shahu and Data Nagar had a heavy load of VRE. These congested and highly populated areas where there are inappropriate waste disposal systems and poor management practices contained high number of Vancomycin resistant Enterococci in chicken feces. Chicken feces samples collected from posh areas *i.e.*, Lahore Cantt, Gulberg, Model Town, Samanabad, Walton Cantt and Allama Iqbal Town did not show any prevalence of Vancomycin resistant Enterococcus because of best hygienic practices. The prevalence of Vancomycin resistant Enterococci was high in all localities of northern division of Lahore as compared to other divisions. Results have confirmed that areas with unhygienic conditions and poor waste disposal practices promote the prevalence of Vancomycin resistant Enterococci. So, it is recommended to manage safe waste disposal practices in poultry shops to reduce the risk of food borne diseases.

Key words: Vancomycin resistant Enterococci (VRE), poultry waste, chicken feces

INTRODUCTION

The use of intricate intensive poultry production systems have led to marked increase in the production of poultry meat and eggs throughout the world (Armstrong, 1986). One of the results of this development has been the discovery and widespread use of number of additives. The main objective of adding these additives has been to boost animal performance by increasing their growth rate, better feed conversion efficiency, greater livability and lowered mortality in clinically healthy and nutritionally normal poultry birds and other animals.

The use of antimicrobial drugs has become significant in human and veterinary medicine, animal husbandry, aquaculture, agriculture and food technology (Barbosa and Levy, 2000). Acquired resistance against antibiotics is closely related to the amount of drug used, a fact observed ever since these agents were introduced into human and veterinary medicine. However, the rate of development of resistance appears to have accelerated in the past decade (Abbas *et al*., 2007). In animals, antibiotics are not only used for therapy and prevention of bacterial infections, but are also added to animal feed to act as growth promoters. In some countries, antibiotic use for growth promotion is greater than veterinary usage (Van Den Bogaard and Stobberingh, 1999).

Avoparcin is a glycopeptide antibiotic with a gram positive spectrum of activity produced by fermentation of a strain of *Streptomyces candidus*. It has been in continual use in Australian livestock feeds since 1978 for growth promotion and improved animal feed conversion efficiency in broiler chickens, growing pigs, calves and beef cattle. Avoparcin is also used as an aid in the prevention of necrotic *enteritits* in broiler chickens. Concerns were raised regarding the continual usage of in-feed antibacterials in animals leading to the likelihood of acquired bacterial resistance development in the gut of the animals (Nra, 2001). Infection in humans by the antibiotic resistant bacteria is being associated with poultry and could be a source of Vancomycin resistant Enterococcus (VRE) in humans through food chain. VRE was first described in Britain in 1988 and soon afterward was

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reported in the USA and other European countries (Leclercq *et al.*, 1988). The only attributable effect on humans has been some diminution in Vancomycin resistance in enterococci isolated (VRE) from human faecal carriers (Van Den Bogaard *et al.*, 2000). Survival of Vancomycin resistant microbes in poultry environments has, however, been studied by Davies and Wray. They showed that *S. enteritidis* persisted for at least one year inside poultry houses and that this persistence was associated with dust particles in food troughs, as well as with incomplete disinfection and cleansing procedures, and presence of rodents and wild bird populations on the farms (Davies and Wray, 1996a, 1996b). The objective of the present study was to the access the resistance of *Enterococci* against Vancomycin in chicken feces samples and to correlate the hygienic conditions of food shops with incidence of microbial threats.

MATERIALS AND METHODS

Sample collection and preparation

A total of 100 chicken feces samples were collected from local poultry markets. The city of Lahore was divided into four parts: east, west, south and north. Samples were collected from each part. The samples were collected from cages in and outside of the shops, and from the vehicles that carry chickens from poultry farms to the markets. Samples (approximately 7-10 g of each sample) were taken by spatula from cages and transferred to a clean zip lock bag. Then dried feces samples were taken in sterile aluminum foil (2×2cm) and weighed 0.1g of dried sample were crushed with the help of spatula. Each sample was suspended in 1ml of autoclaved distilled water in an eppendorf tube and was then placed on vortex mixer for half an hour to produce homogeneous suspension.

Preparation of culture medium

KAA agar is a selective medium for the isolation and enumeration of Enterococci in fecal samples (http://www.oxoid.com/UK/blue/prod detail/prod_detail.asp=CM0591). Kanamycin and azide largely inhibit the accompanying bacterial flora. Enterococci are, however, slightly senitive to these substances, so they can grow almost normal and hydrolyse the glucoside esculin to give glucose and esculetin. Esculetin forms an olive green to black complex with iron (III) ions (http://fr.vwr.com/frFR/content/thematics/microbiol ogy/pdf/KanamycinEsculinAzide, Agar.pdf). Culture medium was prepared and sterilized. After the medium was sterilized, it was allowed to cool to 55°C before adding Vancomycin. For the resistant Enterococci, 16µg/ml of Vancomycin was added. For the superbugs 64µg/ml of Vancomycin was added in the medium.

Microbial processing of the samples

Each sterile Petri plate was poured with about 25ml of medium. Sample was spread on each petriplate with the help of spreader. All experiments were performed in triplicates. The plates were incubated at 37˚C for 24 hours. Sodium azide and Kanamycin provide the selective inhibition required, whilst aesculin and iron salts form an indicator system for the presumptive identification of Enteroccoci (http://sms home.com/pages/ Kanamycin %20 Aesculin%20 Azide %20 Agar_jpg.htm). Round, white or grey colonies about 2mm in diameter, surrounded by black zones of at least 1cm diameter are considered to be Enterococci (presumptive) (Corry *et al.*, 2003). Colony count method was used to estimate the microbial load of chicken feces samples. The counting of colonies was facilitated by the use of colony counter.

RESULTS

Microbial analysis of resistant and superbugs VRE of this study indicated high levels of Enterococci contamination in chicken feces collected from different localities of Lahore. Percentage occurrence of VRE (resistant and superbugs) in different parts of Lahore are shown in Figure 1.

Samples collected from the eastern part of Lahore contained high number of resistant Enterococci especially the areas of Dharampura, Baghbanpura and Jallo but the localities like Lahore Cantt and Defence Housing Authority (DHA) have minimal or negligible number of Vancomycin resistant Enterococci (Table I). Samples collected

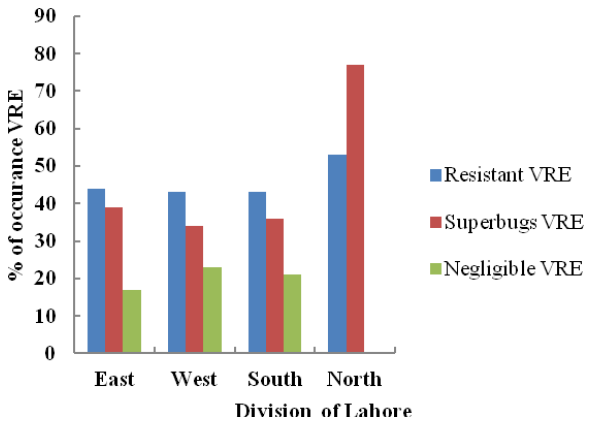

Fig 1. Percentage occurrence of VRE (resistant and superbugs) in different parts of Lahore

from the western part of Lahore contained highest number of resistant Enterococci especially the areas of Shadman, Islampura and Gulshan-e-Ravi but the localities like Allama Iqbal Town and Samanabad had minimal number of resistant Enterococci (Table I). Samples collected from the southern part of Lahore contain high number of resistant Enterococci especially the area of Township but the localities like Gulberg, Model Town, Walton Cantt and Johar Town had relatively low number of resistant Enterococci (Table I). Samples collected from the northern part of Lahore contained highest number of resistant Enterococci in comparison to other parts of Lahore. The chicken feces samples collected from different chicken shops of North Lahore contained highly resistant and superbug Enterococci (Table I).

DISCUSSION

The study comprised of analytical research work in which microbial contamination of Vancomycin resistant Enterococci in broiler chicken feces collected from different chicken shops of Lahore was detected. The results of this study indicated high levels of Enterococci contamination in chicken feces collected from different localities of Lahore. 79% chicken feces samples were contaminated with Vancomycin resistant Enterococci and 25% chicken feces samples were contaminated with Enterococci that were superbugs to Vancomycin.

Table I.- Prevalence of vancomycin resistant enterococci in chicken feces collected from different areas of Lahore

Location	Area	Vancomycin Resistant Enterococci (CFU/g ±SEM)	
		Resistant	Superbugs
East Lahore	Lahore Cantt	0 ±0	0 ±0
	Defence Housing Authority	10.47±1.9	4.07±0.9
	Dharampura	30±0	29.50±0.32
	Baghbanpura	16.8±1.8	1.7±0.17
West Lahore	Jallo	18.2±0.47	7.80±1.0
	Shadman	22.7±0.32	16.2±1.09
	Isslampura	25.6±0.27	16.0±4.27
	Gulshan-e-Ravi	23.4±0.69	8.0±0.65
	Allama Iqbal Town	1.42±0.19	0.63±0.07
	Samanabad	16.1±2.55	8.07±1.08
South Lahore	Gulberg	2.47±0.51	1.60±0.54
	Model Town	5.44±0.8	1.8±0.53
	Walton Cantt	12.9±1.6	3.77±0.72
	Township	24.76±0.81	11.0±0.60
	Johar town	13.8±0.74	4.50±0.60
North Lahore	Shahdara	22.5±1.32	7.22±0.66
	SHADBAGH	26.44±1.0	11.9±0.8
	Data nagar	25.0±1.9	7.9±1.1
	Gari shahu	27.36±0.27	17.0±0.3
	Bata pur	22.0±0.42	12.4±0.49

These findings indicate an increase in Vancomycin resistance in Enterococci species. Brown *et al.* has reported that during 2008, the prevalence of Vancomycin resistance in Cyprus, Germany, Greece, Italy, Portugal, and Israel were 32.1% for *E. faecium* and 2.8% (Brown *et al.*, 2008) .The prevalence of Vancomycin resistant Enterococci in chicken feces collected from Lahore and its surroundings indicates that in poultry farms Vancomycin is used as growth promoter. Bager *et al.* (1997) found an association between glycopeptide use in animal production and the occurrence of *E. faecium* with high-level resistance to Vancomycin in poultry as well as in pigs (Bager *et al.*, 1997). The similar findings were published by Aarestrup (1995) and Klare *et al. (*1995), where they pointed out that the use of Avoparcin as a growth promoter is associated with the occurrence of Vancomycin resistant Enterococci in domestic

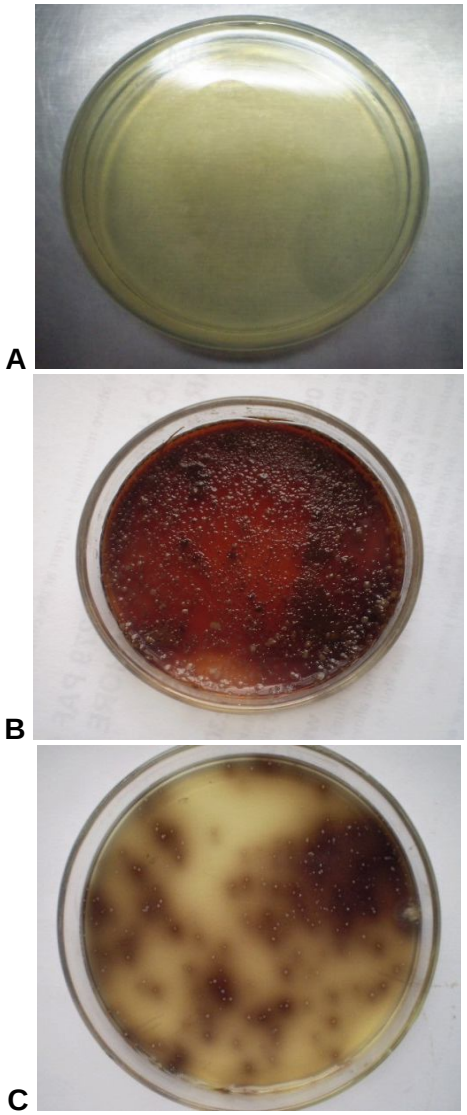

Fig. 2. Kanamycin Aesculin Azide agar plate (Van 16µg/ml) incubated at 37 °C for overnight as negative control (A), showing VRE resistant colonies (B) and showing VRE Superbugs (C).

animals (Aarestrup, 1995; Klare *et al.*, 1995). The analysis of the resistance patterns suggested, that the broiler feed and the farm environment as the most probable external sources, their resistance patterns were often equivalent to those found in feces (Costa *et al.*, 2010). Costa *et al.* (2010) isolated 13 different phenotypes of multiresistant Enterococci from environmental swabs and suggested that there was substantial carry-over of resistant *Enterococci* from previous batches of broilers housed in this shed. The recovery of resistant Enterococci from broiler houses after depopulation and cleaning has also been observed (Heuer *et al.*, 2002). As Enrofloxacin, Gentamicin and Amoxicillin had been used to treat three previous batches of chickens in the shed, this could have contributed to the high frequency of resistance to Ciprofloxacin, Gentamicin and Ampicillin among farm resident Enterococci.

In this study it was observed that chicken feces collected from Dharampura, Islampura, Shadbagh, Garhi Shahu had a heavy load of VRE. It was observed that these areas are thickly populated and because of that unhygienic conditions were not monitored and managed. In addition to that, use of Avoparcin as a growth promoter enhances the prevalence of Vancomycin resistant Enterococci in the environment. While feces samples collected from posh areas i.e., Lahore Cantt, Gulberg, Model Town, Samanabad and Allama Iqbal Town did not show any prevalence of Vancomycin resistant *Enterococcus* because of best hygienic practices. Chicken wastes are disposed off on regular basis, shops have efficient ventilation system and cages are washed and disinfected on daily basis. The prevalence of VRE in these areas was negligible. The present study showed that unhygienic areas with poor management contained higher number of Vancomycin resistant Enterococci as compared to clean areas. Chicken feces samples collected from congested and highly populated areas where there are inappropriate waste disposal systems and poor management practices contained high number of Vancomycin resistant Enterococci. The similar results of Borgen *et al* (2001) revealed that incomplete cleansing and disinfection procedures on three of the five farms, and the walls and floors inside the broiler houses often had a rough surface where microorganisms easily hide and that make possible survival of VRE in chicken environment. This showed that unhygienic conditions promote the prevalence of vancomycin resistant Enterococcus and can be a source of VRE in human food. Consumers get exposure to VRE when handling raw chicken meat. These VRE contaminated chicken products represent a risk for human exposure to VRE. Eating food contaminated by VRE may lead

intestinal colonization and emergence of VRE carriers outside hospital environments (Borgen *et al.*, 2001). Thus it was demonstrated that chicken shops and their environment should be monitored by frequent testing of pathogens. The use of antimicrobial drugs should be minimized to reduce the risk of antibiotic resistant microorganisms.

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