Original Article

Prevalence of vancomycin resistance and multiple drug resistance in enterococci in equids in North India

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Abstract

Introduction: Vancomycin resistant and multi-drug-resistant enterococci are the major emerging pathogens in surgical, neonatal, and tertiary care units.

Methodology: In this study, 267 enterococci from different clinical and non-clinical samples of equine origin were tested for their antimicrobial drug sensitivity against 19 antimicrobials using disc diffusion method.

Results: A total of 80.2% enterococci tested were resistant to vancomycin and 99.6% to multiple-drugs. There was a significant association between haemolytic potential and vancomycin resistance (χ^2, 0.00). Enterococci isolates from healthy equids were significantly (χ^2, 0.04) less resistant to vancomycin than the isolates from clinically sick animals. Besides vancomycin sensitivity, isolates were also tested for 18 more antimicrobial drugs; maximum numbers of isolates were sensitive to imipenem (75%) followed by tetracycline (60%), amoxicillin+clavulanic acid (54%), and minimum for cefdinir (4%).

Conclusion: More than 80% strains of enterococci of equine origin were found resistant to vancomycin and 99.6% were multiple-drug resistant in Northern India. High prevalence of VRE and MDRE in healthy equids might be a potential danger for the health of persons in equine contact.

Keywords: VRE, enterococci, Vancomycin-resistance, MDR, MDRE, equine

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Introduction

Although considered benign and opportunistic pathogens, enterococci have long been known to cause endocarditis in hospitalized patients [1,2]. Recently vancomycin resistant enterococci (VRE) and multi-drug-resistant (MDR) enterococci (MDRE) have emerged as cause of nosocomial super infections in surgical and in tertiary care units, particularly after organ transplants [3]. Besides nosocomial infections of surgical wounds, VRE and MDRE are emerging causes of meningitis in neonates, as well as infections of the central nervous system, osteomyelitis, lung infections, and urinary tract and pelvic infections in adults [2,4-7,8].

Although little is known about the actual reservoir of VRE and MDRE, animals, humans, food and inanimate objects are the most potential sources [9]. Enterococcal colonization in animals is poorly understood but similar MDRE and VRE strains causing infections in human beings have often been isolated from dogs, deer, pigs, cows, horses, chickens, ducks, geese, turkeys and other gallinaceous birds [9-14]. Close contact with horses was once associated with streptococcal meningitis but enterococcal infections directly contracted from animals are rarely reported [15]. VRE from waste water were recently reported to have a similar genotypic profile as that of the clinical isolates from human cases [16], indicating a probable role of faecal contamination of water in the spread of VRE. The study [16] indicated an epidemiological link between the isolates from the reservoirs and human urinary tract infections, and suggested a possible transmission route for a VRE clone from hospital patients via urban sewage to surface water.

Epidemiology of VRE infections has been studied in several hospitals in different parts of India [3,4,7]; however, there is dearth of information on VRE from animals, including equids. The present study was undertaken to assess the extent of vancomycin resistance and MDR in enterococci isolates of equine origin.

Material and Methods

A total of 269 isolates of enterococci (Table 1) from different samples of healthy and clinically sick

Table 1. Multiple drug resistance in enterococci isolated from equids and their environment.

Resistant to No. of Drugs	Sources of enterococci strains studied							
	Healthy Equids	Infertile mares	Foal Diarrhoea	Poly Arthritis	Abortion	Abscess	Stable Environment	Total
2	1	0	0	0	0	0	0	1
4	1	0	0	0	0	0	0	1
5	4	1	0	0	0	0	1	6
6	9	0	0	1	0	0	1	11
7	5	1	0	0	0	0	0	6
8	8	1	0	0	0	0	1	10
9	9	0	0	0	1	0	0	10
10	18	3	0	0	0	0	0	21
11	9	2	0	0	0	0	0	11
12	11	7	0	0	2	0	0	20
13	17	9	0	2	2	0	3	33
14	13	3	0	0	0	1	5	22
15	16	9	2	0	0	1	8	36
16	14	6	2	0	0	0	0	22
17	16	3	6	0	0	0	1	26
18	8	1	5	0	0	0	4	18
19	4	0	5	0	0	1	3	13
Total number of isolates	163	46	20	3	5	3	27	267

equids received in last 12 months for microbiological investigation at the Bacteriology Laboratory of the Institute were examined. The isolates were received as frozen stocks from the Bacteriology Laboratory. A total of 267 enterococci strains could be revived and tested for purity on blood agar and for specificity through growth on *Enterococcus* selective agar, bile salt esculin azide agar, and M-*Enterococcus* agar (Hi-Media, Mumabi).

Antimicrobial drug sensitivity assay of all the isolates was determined through the disc diffusion method [17] on brain heart infusion agar (BHI, Hi-Media) using amoxyclav (amoxycillin+clavulanic acid, 10µg), ampicillin (10µg), carbenicillin (100 µg), cefdinir (5 µg), cefotaxime (30 µg), cephalexin (10µg), chloramphenicol (30 µg), ciprofloxacin (10µg), clindamycin (2 µg), cloxacillin (1 µg), co-trimoxazole (25 µg), co-trimazine (25 µg), erythromycin (15 µg), gentamicin (10µg), imipenem (10µg), norfloxacin (10µg), oxacillin (5 µg), tetracycline (30 µg) and vancomycin (30 µg) discs. Tests were conducted in triplicate using antimicrobial discs from two producers (Hi-Media and Oxoid India) because of the observed high rate of drug resistance in isolates and confirmation of results was desirable. A Reference strain of *Enterococcus faecalis* ATCC29212 was used as a control. Results were interpreted per the guidelines of the antimicrobial disc manufacturer (Hi-Media, Oxoid).

Results and Discussion

Out of 267 *Enterococcus* spp. strains tested for antimicrobial drug sensitivity, 265 were resistant to five or more antimicrobial drugs. Such a high resistance rate has rarely been reported in enterococci [9-13]. Multiple drug resistance (MDR) was detected in all but one strain of enterococci from a healthy mare (Table 1). All the strains of diarrhoeic foal origin and the majority of environmental isolates (21 of 27) were resistant to 14 or more antimicrobials.

The greatest number of *Enterococcus* spp. strains from equids and their environment were sensitive to imipenem (75.3%), followed by tetracycline (59.6%) and amoxycillin+clavulanic acid (53.9%). For other antimicrobials, more than 55% of the strains were resistant, with the highest number resistant to cefdinir (96.5%), followed by oxacillin (90.6%), cefotaxime (89.1%), ampicillin (88.4%), cloxacillin (88.4%), co-trimazine (87.3%), and vancomycin (80.2%). For other drugs, resistance was detected in 55-80% of the strains (Table 2).

The presence of vancomycin resistance in 80.2% and MDR in more than 99% of enterococci isolates of equine origin indicated that VRE and MDRE in

Table 2. Antimicrobial drug resistance in Enterococci isolates from equids and their sources

Antimicrobials used for sensitivity assay (active antimicrobial agent in each disc)	Number (%) of isolates resistant to different antimicrobials							
	All strains	Non-haemolytic	Beta-haemolytic	Alpha-Haemolytic	Infertile mares	Healthy equids	Foal Diarrhoea	Other isolates
	n=267	n = 129	n = 80	n = 58	n = 46	n = 163	n = 20	n = 38
Amoxyclav (10µg)	123 (46.1)	119 (92.2)	53 (66.3)	25 (43.1)	21 (45.7)	76 (46.6)	13 (65.0)	13 (34.2)
Ampicillin (10µg)	236 (88.4)	128 (99.2)	58 (72.5)	55 (94.8)	42 (91.3)	137 (84.0)	20 (100.0)	38 (100.0)
Carbenicillin (100 µg)	214 (80.1)	127 (98.4)	57 (71.3)	50 (86.20	39 (84.8)	122 (74.8)	20 (100.0)	34 (89.5)
Cefdinir (5 µg)	258 (96.6)	126 (97.7)	74 (92.5)	56 (96.6)	44 (95.7)	154 (94.5)	20 (100.0)	38 (100.0)
Cefotaxime (30 µg)	238 (89.1)	127 (98.4)	76 (95.0)	53 (91.4)	41 (89.1)	145 (89.0)	20 (100.0)	33 (86.8)
Cephalexin (10µg)	191 (71.5)	127 (98.4)	38 (47.5)	41 (70.7)	36 (78.3)	109 (66.9)	19 (95.0)	28 (73.7)
Chloramphenicol (30 µg)	158 (59.2)	123 (95.3)	39 (48.8)	38 (65.5)	30 (65.2)	80 (49.1)	20 (100.0)	29 (76.3)
Ciprofloxacin (10µg)	192 (71.9)	124 (96.1)	66 (82.5)	38 (65.5)	33 (71.7)	118 (72.4)	13 (65.0)	29 (76.3)
Clindamycin (2 µg)	196 (73.4)	126 (97.4)	54 (67.5)	36 (62.1)	31 (67.4)	123 (75.5)	17 (85.0)	26 (68.4)
Cloxacillin (1 µg)	236 (88.4)	128 (99.2)	59 (73.8)	53 (91.4)	39 (84.8)	138 (84.7)	20 (100.0)	38 (100.0)
Co-trimoxazole (25 µg)	205 (76.8)	127 (98.4)	42 (52.5)	47 (81.0)	34 (73.9)	114 (69.9)	20 (100.0)	38 (100.0)
Co-trimazine (25 µg)	233 (87.3)	128 (99.2)	59 (73.8)	51 (87.9)	39 (84.8)	140 (85.9)	20 (100.0)	35 (92.1)
Erythromycin (15 µg)	176 (65.9)	126 (97.7)	33 (41.3)	43 (74.1)	36 (78.3)	95 (58.3)	20 (100.0)	26 (68.4)
Gentamicin (10µg)	188 (70.4)	127 (98.4)	38 (47.5)	43 (74.1)	38 (82.6)	102 (62.6)	20 (100.0)	29 (76.3)
Imipenem (10µg)	66 (24.7)	30 (23.3)	13 (16.3)	23 (39.7)	29 (63.0)	29 (17.8)	14 (70.0)	13 (34.1)
Norfloxacin (10µg)	149 (55.8)	123 (5.3)	26 (32.5)	42 (72.4)	23 (50.0)	83 (50.9)	20 (100.0)	23 (60.5)
Oxacillin (5 µg)	242 (90.6)	128 (99.2)	65 (81.3)	55 (94.8)	43 (93.5)	142 (87.1)	20 (100.0)	38 (100.0)
Tetracycline (30 µg)	108 (40.4)	122 (94.6)	21 (26.3)	24 (41.4)	18 (39.1)	61 (37.4)	13 (65.0)	16 (41.1)
Vancomycin (30 µg)	214 (80.2)	86 (66.7)	71 (88.8)	50 (86.2)	40 (87.0)	115 (70.6)	20 (100.0)	34 (89.5)

equines are much more common in North Indian horses than reported in other parts of world [9-13,18]. The high prevalence of VRE and MDRE might be either due to more intimate contact between pets and humans in India, or it might be due to the high prevalence of VRE and MDR in the Indian environment. Further studies are needed to confirm these hypotheses. VRE and MDRE have been commonly reported in animals and birds from different parts of world [9-14] but rarely from India. de Niederhäusern and coworkers [10] isolated enterococci from 104 equine and 64 swine rectal swabs and from 93 clinical samples; comparison of their antimicrobial susceptibility in tandem with genotype patterns showed that enterococci strains circulated in different populations. Although nosocomial and community acquired VRE and MDRE infections in humans are reported from India [3,4,7], there is little information about their prevalence in Indian equids.

Frequency of vancomycin resistance was very high in enterococci isolates of equine origin (> 80%) but the reasons for such a high resistance rate are not clear. In contrast, using similar methodology, in studies on enterococci from human infections in North India, only 2-3% of enterococci isolates were found resistant to vancomycin using 30μg discs [3,7]. High prevalence of MDRE in equids might be even more serious than the occurrence of VRE. As evident from the results, 13 (4.9%) strains of enterococci were resistant to all 19 antimicrobials tested, and the outcome of infections with such poly-drug resistant *Enterococcus* strains might be fatal. In most of the earlier reports, such a high MDR or vancomycin resistance is rarely observed in enterococci either of animal or of human origin [7-10,12]. However, a study conducted in 1995 in Pennsylvania indicated that MDRE and VRE might be more common in horses than in other pet animals. The study reported that in a small number of *Enterococcus* strains of equine origin, about 50% were VRE and more than 75% were MDRE [9].

In our study, out of 267 strains of enterococci tested, 80 and 58 strains caused β and α-haemolysis respectively on horse blood agar, which is in concurrence with earlier reports on the haemolytic potential of many of the enterococci isolates belonging to different species [19-21]. However, any association of haemolytic potential with clinical infections could not be established except that many of the isolates from foals with diarrhoea were α-haemolytic (χ^2, 0.08). Earlier studies [22] have also found no specific correlation of haemolytic patterns with isolation of enterococci from different systemic infection in human beings.

Although the β-haemolytic potential of enterococci had no association with any specific kind of infection in equids, comparatively fewer numbers of β-haemolytic strains were resistant to oxacillin (χ^2, 0.00), ampicillin (χ^2, 0.00), carbenicillin (χ^2, 0.07), co-trimazine (χ^2, 0.00), gentamicin (χ^2, 0.00), norfloxacin (χ^2, 0.00), cephalexin (χ^2, 0.00), tetracycline (χ^2, 0.11), cefdinir (χ^2, 0.02), cloxacillin (χ^2, 0.00), co-trimoxazole (χ^2, 0.00) and erythromycin (χ^2, 0.00). A significantly larger number of strains were resistant to vancomycin (χ^2, 0.00), ciprofloxacin (χ^2, 0.11), amoxyclav (χ^2, 0.01) and cefotaxime than non-haemolytic and α-haemolytic strains. Additionally, more α-haemolytic strains were resistant to vancomycin (χ^2, 0.00), carbenicillin (χ^2, 0.08), norfloxacin (χ^2, 0.00), cotrimoxazole (χ^2, 0.02) and erythromycin (χ^2, 0.00), than non-haemolytic strains. A higher prevalence of VRE in diarrhoeic foals than in isolates associated with infertility, abortion, or other infections in equids indicated further that a major source of VRE might be the feces of foals with diarrhoea than the the feces from normal healthy equids. Feces as major source of VRE have also been suspected in earlier studies [1,3,9,11].

Relatively high numbers of strains from diarrhoeic foals were resistant to chloramphenicol (χ^2, 0.01), norfloxacin (χ^2, 0.04), erythromycin (χ^2, 0.10), vancomycin (χ^2, 0.04) and imipenem (χ^2, 0.00) as compared to isolates from healthy equids. Additionally, the majority of enterococci isolates from abortion, abscess, and poly-arthritis cases were also resistant to vancomycin (Table 2). Although the majority of strains from diarrhoeic cases and from equids with other health problems were resistant to imipenem, most of the strains from infertile mares were sensitive to the drug. Resistance to chloramphenicol, norfloxacin, erythromycin, vancomycin and imipenem in a large number of enterococci isolated from foals with diarrhoea indicated that pathogenic enterococci may also be resistant to those antimicrobials, which are rarely used in equids [23]. Thus selective pressure for antimicrobial-drug resistance might not be the only reason for the emergence of VRE and MDRE in

equids; rather, their selection elsewhere in the environment might be more important.

Both the clinical use of vancomycin in equids and the use of avoparcin (a feed antibiotic linked with the origin of vancomycin resistance in feedlot animals) [24] have rarely been reported in equids in India [23]. Moreover, Indian horses are rarely fed on feeds of animal origin except for the occasional use of milk for curative purposes; thus acquiring VRE from foods of animal origin cannot be considered an important source of VRE in equids as it might be in other pet animals [12]. While there is always a possibility that equids may acquire VRE and MDRE from the environment, such as from contaminated water, feed and fodder, more studies are needed to confirm this hypothesis. Although it is not clear how VRE and MDRE emerge in equids, it can be concluded that equids might be an important source of VRE or MDRE.

This study concludes that although a high prevalence of VRE in equids could not be explained exactly, it indicates that, as observed in other countries [9], equids might be an important reservoir of VRE and MDRE strains in India. However, transmission of VRE from animals to humans has rarely been established, and several reports suggest that VRE contaminated animal products might be hazardous to humans.

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