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Isolation and Identification of Vancomycin Resistance Enterococci From Clinical Specimens

KEYWORDS: Adolescents ; structured teaching program; social network addiction .

Mr.Harsha Reddy Kolli	Dr.K.Swarnalatha	Dr.B.Sreekanth Reddy
Department of Microbiology, SVS Medical College & Hospital, Mahabubnagar	Associate Professor KURNOOL MEDICAL COLLEGE, KURNOOL	Assistant professor KURNOOL MEDICAL COLLEGE, KURNOOL

Dr Manisha Singh	Dr Vasanti Kabra
Department of Microbiology, SVS Medical College & Hospital, Mahabubnagar	Department of Microbiology, SVS Medical College & Hospital, Mahabubnagar

ABSTRACT *INTRODUCTION: Enterococci are normal inhabitants of the intestinal tract of humans. Enterococcus faecalis and E.faecium are the two most important species of human infections. vancomycin was the only drug that could be consistently relied on for the treatment of infections caused by multi drug resistance enterococci. Currently, vancomycin resistance among enterococcus isolates is a major problem worldwide.*

AIM OF THE STUDY:

- *To isolate and identify the enterococci from various clinical samples.*
- *To detect the resistant patterns for the different species of enterococci.*
- *Detecting the vancomycin resistance by disc diffusion method.*
- *To determine the minimum inhibitory concentration for these resistant isolated by using the vancomycin E-strips.*

METHODS: A total of 2427 samples were processed during the study period out of which 108 strains of enterococci were grown in pure culture. All these enterococcal isolates were speciated and antibiotic susceptibility testing was done by Kirby Bauer method. Vancomycin resistance was determined by using the E-strips of vancomycin.

RESULTS: A total of 108 Enterococci were obtained from various samples during the study period. Majority of the isolates were obtained from urine specimens. The most common species of enterococci isolated was Enterococcus faecalis accounting for 61(56.5%) followed by Enterococcus faecium 45(41.6%) and Enterococcus avium 2(1.9%). In the present study majority of the isolates were resistant to ciprofloxacin (62.03%) followed by doxycycline (49.07%). High level gentamicin resistance was seen in 44.44% of the isolates. Resistance to ampicillin and amoxyclav was almost equal accounting to 38 and 37% respectively. Resistance to vancomycin was seen in 6.5% of the total isolates and no resistance was observed for linezolid (0%).

CONCLUSION: Vancomycin resistance was observed in (6.5%) isolates. Glycopeptide resistance among our isolates was high, probably reflecting the increased use of vancomycin in our hospital over the past few years. This fact highlights the importance of strict enforcement of antibiotic policies coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria.

INTRODUCTION

Enterococci are normal inhabitants of the intestinal tract of humans. More than a dozen species of enterococci are currently recognized, of these only two species Enterococcus faecalis and Enterococcus faecium are the most important causes of human infections.

Enterococci considered as the opportunistic pathogens, are an important global cause of nosocomial infections. Of the two most common enterococcus species Enterococcus faecalis has been found to be responsible for 80-90% of cases and Enterococcus faecium for the remaining human enterococcal infections1,2. Most frequent infections caused by these organisms include, urinary tract infections followed by intra-abdominal or intra pelvic abscess3.

Other infections include blood stream infections, wound infections, endocarditis, osteomyelitis, respiratory tract infections and CNS infections. Enterococci are currently ascending nosocomial pathogens, having become the second most common organisms recovered from nosocomial, UTI, wound infections and the third most common cause of nosocomial bacteremia4.

The prolonged survival of the organisms in the hospital environment is because of their intrinsic resistance to several commonly used antibiotics and more important is their ability to acquired resistance to all currently available antibiotics either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons.

Most enterococci are tolerant to the bactericidal activity of β lactam antibiotics, that's why these antibiotics are used synergistically with aminoglycosides to treat serious enterococcal infections like endocarditis. Though low level resistance to aminoglycosides is a normal occurrence, high level resistance is usually mediated by aminoglycosides modifying enzyme which is also not uncommon.

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Vancomycin has been in clinical use for more than 30 years, until recently vancomycin was the only drug that could be consistently relied on for the treatment of infections caused by multi drug resistance enterococci. Currently, vancomycin resistance among enterococcus isolates is a major problem worldwide. Emergence of vancomycin resistance and easy dissemination of this resistance to other clinical isolates has left with only a very limited number of antibiotics for the treatment, thus increasing the toll of mortality and morbidity along with the increase cost of treatment. With the proper identification, appropriate isolation and control measures the burden of these infections can be reduced to a minimum.

The present study was aimed at detecting the antimicrobial resistance pattern among enterococcal isolates obtained from clinical specimens at tertiary care hospital with a special emphasis on vancomycin resistance in enterococci.

AIMS AND OBJECTIVES:

- To isolate and identify the enterococci from various clinical samples.
- To detect the resistant patterns for the different species of enterococci.
- Detecting the vancomycin resistance by disc diffusion method.
- To determine the minimum inhibitory concentration for these resistant isolated by using the vancomycin E-strips.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology from August 2013 to August 2014. All the samples obtained were processed according to the standard protocols. A total of 2427 clinical samples were obtained during the study period. Out of total 2427 samples, 897 samples of urine, 626 samples of pus, 609 samples of blood, 225 samples of pleural fluid and 70 samples of ascitic fluid were processed.

Out of total 2427 samples processed for study, 108 strains of enterococci were grown in pure culture. Confirmation and Identification of the enterococci up to the species level was done by Gram staining, Catalase test, Bile esculin hydrolysis, Carbohydrate fermentation test, Pyruvate test, Arginine dihydrolyse test

Enterococcus isolates were identified to the species level by following (Table no:1) phenotypic characterization scheme for Enterococci 5.

Sugar	E.faecalis	E.faecium	E.durans	E.avium
Arabinose	-	+	-	+/-
Mannitol	+	+	-	+
Raffinose	-	+/-	-	+/-
Sorbitol	+	+/-	-	+
Arginine dihydrolase	+	+	+	-
Pyruvate	+	-	-	-

All isolates of Enterococcus were tested for their antimicrobial susceptibility by Kirby-bauer disk diffusion technique using the standard guidelines according to the Clinical Laboratories Standards Institute (CLSI)6.

The antibiotics tested were ampicillin (10 µg), amoxyclav, ciprofloxacin (5 µg), gentamicin high potent (120 µg), doxycycline (30 µg), vancomycin (30 µg), linezolid (30 µg). For urine isolates, susceptibility testing for nitrofurantoin (300 µg) was also done.

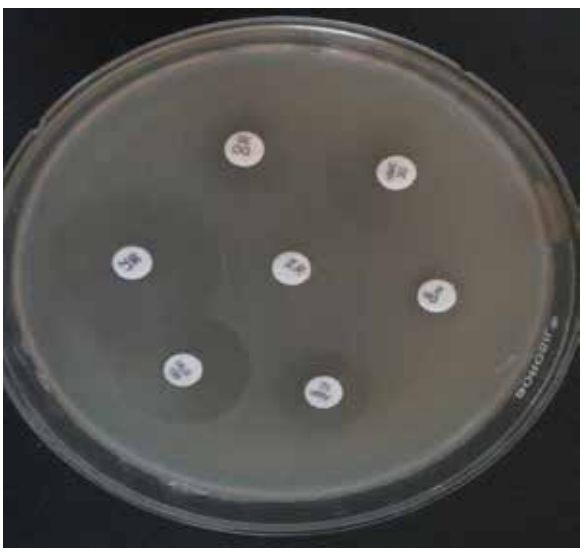

Fig No 1: MH agar with antibiotics

Vancomycin MIC testing:

All the isolates which have shown resistance by disc diffusion testing were further tested to identify the minimum inhibitory concentration by using the e-strips of vancomycin (Hi-media).

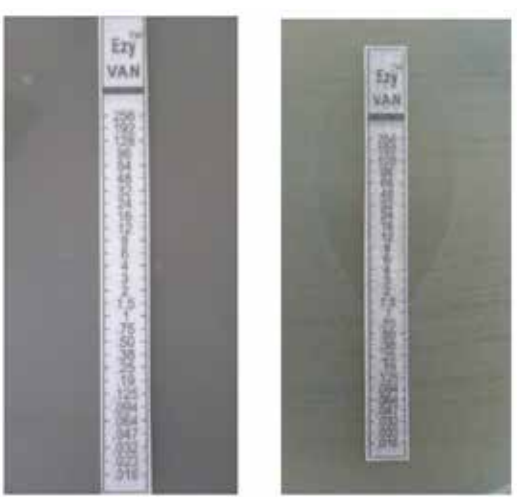

Fig No 2: Vancomycin E-strip showing MIC of >256µg/ml (left) and 1.5 µg/ml (right)

RESULTS

A total of 108 Enterococci were obtained from various samples during the study period. Majority of the isolates were obtained from urine specimens accounting for 62(57.4%) out of 108 samples. This is followed by pus samples 28(25.9%), blood samples 12(11%), pleural fluid 4(3.7%) and ascitic fluid 2(1.8%).

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Table No 2 : showing number of isolates obtained from various samples:

Sample	No: of isolates
Urine	62(57.4%)
Pus	28(25.9%)
Blood	12(11.1%)
Pleural fluid	04(3.7%)
Ascitic fluid	02(1.8%)
Total	108(100%)

In the present study majority of the isolates were obtained from the male patients (55.6%) and the remaining 44.4% from the female patients

Majority of the isolates were obtained in the age group of 31-45 years accounting to 27.8% of the total isolates followed by 16-30 years age group(25%) and below the age of 15 years(25%) patients.

Table No 3: showing percentage of different species of Enterococci

Species	Number & percentage
E.faecalis	61(56.5%)
E.faecium	45(41.6%)
E.avium	02(1.9%)

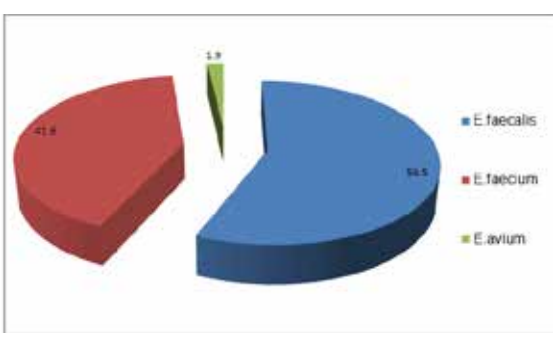

Fig No 3: Percentage of different species of Enterococci

The most common species of enterococci isolated was Enterococcus faecalis accounting for 61(56.5%) out of 108 enterococci followed by Enterococcus faecium 45(41.6%) and Enterococcus avium 2(1.9%).

Table No 4: showing sample wise distribution of species

species	Urine	Pus	Blood	Body fluids	Total
E.faecalis	37(60.6%)	15(24.5%)	4(6.5%)	5(8%)	61
E.faecium	25(55%)	12(27%)	8(18%)	0(0%)	45
E.avium	0(0%)	1(50%)	0(0%)	1(50%)	2
Total	62	28	12	6	108

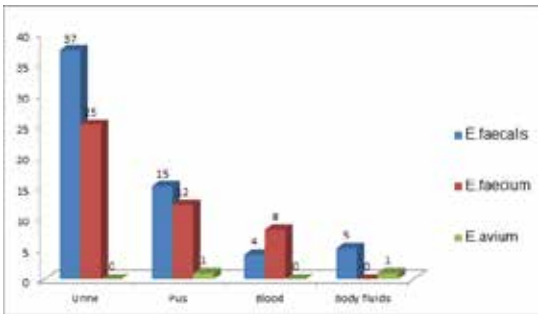

Fig No 4: sample wise distribution of species

In the present study, majority of the enterococcus faecalis were isolated from urine specimens accounting to 37(60.6%) out of 61 isolates. The remaining E.faecalis isolates were isolated predominantly from pus (24.5%) followed by blood (6.5%) & body fluids (8.2%).

Majority of the E.faecium were isolated from the urine specimens accounting to 25(55.5%) out of 45 isolates followed by pus (26.6%) & blood (17.7%) E.avium were isolated only from pus & body fluids (50% each) only.

Though E.faecalis was predominantly isolated in majority of the specimens, E.faecium was the dominant organism in the blood isolates accounting to 2/3rds of the isolates (67%).

Table no 5: showing antibiotic resistance

Antibiotic	Resistance percentage
Ampicillin	38%
Gentamicin	44%
Ciprofloxacin	62%
Amoxyclav	37%
Doxycycline	49.%
Vancomycin	6.5%
Linezolid	0%

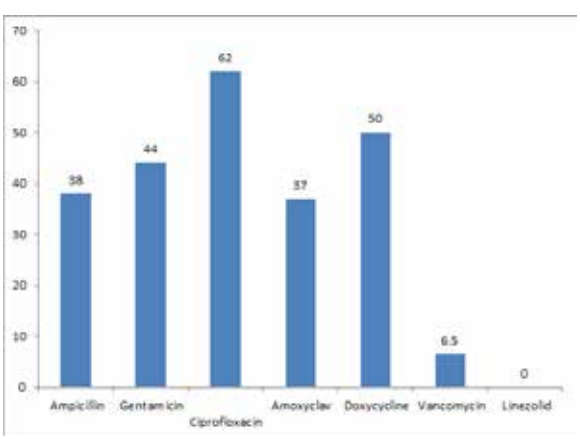

Fig No 5: Antibiotic resistance

In the present study majority of the isolates were resistant to ciprofloxacin (62.03%) followed by doxycycline (49.07%). High level gentamicin resistance was seen in 44.44% of the isolates. Resistance to ampicillin and amoxyclav was almost equal accounting to 38 and 37% respectively.

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Resistance to vancomycin was seen in 6.5% of the total isolates & no resistance was observed for linezolid (0%).

Table no 6: showing antibiotic resistance in Enterococci species

Species	Ampicillin	Doxycycline	Amoxyclav	Ciprofloxacin	Gentamicin	Vancomycin	Linezolid
E.faecalis	18 (30%)	27 (44%)	17 (28%)	33 (54%)	22 (36%)	3 (5%)	0 (0%)
E.faecium	23 (51%)	25 (56%)	23 (51%)	33 (73%)	26 (58%)	4 (9%)	0 (0%)
E.avium	0 (0%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)

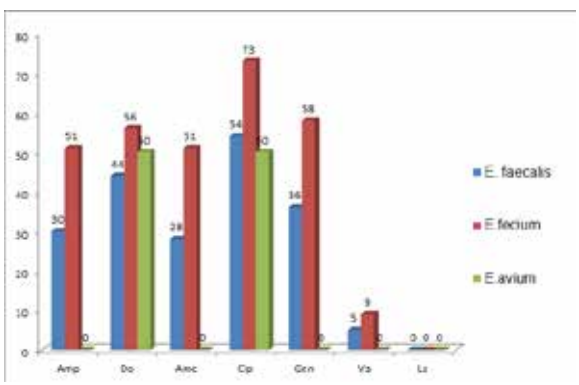

Fig No 6: Antibiotic resistance in Enterococci species

Ampicillin resistance was predominantly seen in E.faecium (51%) followed by E.faecalis (30%) and no resistance to ampicillin was seen in E.avium. High level gentamicin resistance was observed commonly in E.faecium (58%) followed by E.faecalis (36%). Ciprofloxacin resistance was also more common in E.faecium than E.faecalis (73% & 54% respictively).

Vancomycin resistance was observed in 7 (6.5%) isolates out of 108 isolates. 4 (57%) were E.faecium and the remaining 3 (43%) were E.faecalis. vancomycin resistance was not observed in E.avium.

With respect to the number of resistant isolates in accordance with the species, vancomycin resistance was considerably higher in E.faecium isolates with 4 out of 45 (8.9%) E.faecium isolates being resistance to vancomycin whereas only 3 out of 61(5%) E.faecalis isolates were resistant to it.

DISCUSSION

Enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. Serious enterococcal infections are often refractory to treatment and mortality is very high.

Table No 7: showing number of species isolated from various samples

Study	E.faecalis	E.faecium	E.avium	Others	Total
Taneja et al (2004)[7]	80 (55%)	17(11.8%)	5(3.5%)	42(29%)	144
Shah et al (2012)[8]	69(75%)	21(23%)	---	2(2.2%)	92
Fernandez et al (2013)[9]	84(56%)	51(34%)	3(2%)	12(8%)	150
Present study(2014)	61(56.5%)	45(41.6%)	2(1.9%)	---	108

In the present study, E.faecalis (56.5%) was the predominant species isolated from the culture followed by E.faecium (41.6%). This is similar to Fernandez et al study where E.faecalis was the predominant isolate (56%) followed by E.faecium(34%). Other studies conducted by Shah et al and Taneja et al have also observed similar findings where E.faecalis was the predominant isolate (75% and 55% respectively). Though Shah et al study isolated E.faecium as the second common isolate (23%), In Taneja et al study, E.casseliflavus (23.6%) was the second common organism followed by E.faecium which was the third common organism(11.8%).

E.avium was the only species isolated apart from E.faecalis and E.faecium accounting to only 1.9% of the total isolates. Fernandez et al and Taneja et al have also isolated E.avium to an accounting of 2% and 3.5% of the total isolates respectively, which is similar to our study.

Table No 8: showing antibiotic resistance in various studies

Antibiotic	Anbumani et al[10] (2011)	Shah et al[8] (2012)	Fernandez et al[9] (2013)	Present study (2014)
Ampicillin	31%	40%	37%	38%
Gentamicin	56%	53%	53%	44%
Ciprofloxacin	58%	62%	---	62%
Amoxyclav	---	---	---	37%
Doxycycline	---	---	---	49%
Vancomycin	0%	8%	8.6%	6.5%
Linezolid	0%	0%	---	0%

In the present study majority of the enterococcal isolates were resistance to ciprofloxacin(62.03%) this is similar to Anbumani et al and Shah et al studies where ciprofloxacin resistance accounted for 58% and 62% respectively.

High level gentamicin resistance(HLGR) was observed in 44.44% of the entrococcal isolates in our study. This findings correlates with the Anbumani et al, Shah et al and Fernandez et al studies where HLGR was 56%, 53% and 53% respectively.

Ampicillin resistance in our study was 37.96% which is similar to Fernandez et al study with 36.9% of isolates showing resistance to ampicillin. Other study with similar observations includes Anbumani et al and Shah et al studies (31% and 40% ampicillin resistance respictively).

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

In the present study all the isolates were uniformly sensitive to linezolid (0% resistance) which is similar to other studies Anbumani et al, Shah et al and Fernandez et al where no resistance was observed against linezolid.

Vancomycin resistance:

In the last two decades, the emergence of VRE and their increasing prevalence worldwide has made it difficult to treat serious enterococcal infections. VRE was first reported by Uttley et al in 1989 from Great Britain and after that has been reported from many countries of the world. It is especially a big problem in the western world. Studies from the United States in the past decade reported vancomycin resistance in as many as 17 per cent of all Enterococcus strains and in up to 28 per cent of all nosocomial Enterococcus species strains.

Although the prevalence of VRE infections in India is much lower than in the western world, it has been increasing in the past one decade. Mathur et al from New Delhi were the first to report VRE from India in 1999. Another study from north India reported vancomycin resistance in only 1 per cent of the Enterococcus species strains, followed by a study from Chandigarh in which 5.5% of 144 Enterococcus isolates from urine specimens were identified as VRE. However, all isolates of VRE in their study showed a low-level vancomycin resistance ranging from 8-32 µg/ml. In a study done on enterococcal isolates from blood in New Delhi, only four isolates of E. faecium showed low degree resistance to vancomycin. In another study from north India, 2 per cent of all enterococcal isolates were vancomycin resistant.

Table No 9: showing vancomycin resistance in various studies

Study	Vancomycin resistance %
Praharaj et al (2013)[11]	8.7%
Fernandes etal (2013)[9]	8.6%
Mathur et al (2003)[12]	1%
Goshal et al (2006)[13]	1.4%
Shah etal (2012)[8]	8%
Karmarker et el (2004)[14]	23%
Present study (2013-14)	6.5%

In the present study vancomycin resistance was observed in 6.5% of the total isolates. This finding is similar to many other studies conducted by various authors. In Shah et al study vancomycin resistance was observed in 8% of the total isolates. In Fernandez et al and Praharaj et al the resistance percentage was almost similar accounting to 8.6% and 8.7% respectively. In contrary to our study, Mathur et al and Goshal et al have isolated vancomycin resistance organisms only in 1% and 1.4% of the total isolates respectively. In Karmarker et al study vancomycin resistance was quite high accounting to 23% of the total isolates.

In our study vancomycin resistance was observed predominantly in E.faecium isolates where 4 out of 7 vancomycin resistance isolates belonged to this species. The remaining 3 vancomycin resistance isolates were E.faecalis isolates.

Table No 10: showing Vancomycin resistance according to species

Species	Shah et al (2012)[8]	Fernandz et al[9] (2013)	Taneja et al (2004)[7]	Present study (2014)
E.faecalis	6(8.7%)	4(4.7%)	1(1.2%)	3(4.9%)
E.faecium	2(9.5%)	6(11.7%)	5(29.4%)	4(9.7%)
E.avium	---	1(33.3%)	0(0%)	0(0%)
Others	0(0%)	2(16.6%)	2(4.7%)	---
Total	8(8.7%)	13(8.7%)	8(5.5%)	7(6.5%)

When compared with respect to the individual species E.faecium has shown the highest vancomycin resistance with 4 (9.7%) out of 45 E.faecium isolates being resistant to vancomycin. When compared to E.faecium, E.faecalis isolates have shown less resistance towards vancomycin with only 3(4.9%) out of 61 E.faecalis isolates being resistant to it. Our findings are similar to shah et al and Taneja et al studies. In Shah et al study vancomycin resistance was slightly higher in E.faecium isolates (9.5%) when compared to E.faecalis (8.7%). Where as in Taneja et al study E.faecium isolates have shown very high resistance (29.4%) when compared to E.faecalis (1.2%).

In Fernandez et al study resistance percentage in E.faecium and E.faecalis was 11.7% and 4.7% respectively which is similar to our study but the highest resistance was shown by E.avium (33.3%) in their study while none of the E.avium isolates were resistance to vancomycin in our study.

Table No 11: showing MIC of vancomycin resistance in various studies

Study	Vancomycin MIC range
Praharaj et al (2013)[11]	8 to > 128µg/ml
Shah et al (2012)[8]	8 to 32µg/ml
Mathur et al (2003)[12]	8 to 512µg/ml
Goshal et al (2006)[13]	62 to 25 6µg/ml
Present study (2014)	>256µg/ml

Minimum inhibitory concentration (MIC) of the vancomycin resistance isolates varies from study to study, in the present study all the 7 vancomycin resistance isolates have

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

shown an MIC of more than 256µg/ml. In Mathur et al study the MIC range was 8µg/ml to as high as 512 µg/ml, where as in Shah et al study the MIC range was only from 8 to 32 µg/ml.

In Goshal et al study the MIC range was 62 to 256µg/ml . In Praharaj et al study out of 32 vancomycin resistance isolates 31 were having an MIC of >128µg/ml only 1 isolate showed MIC of 8µg/ml.

With respect to the sample wise distribution of vancomycin resistance isolates 3 out of 7 isolates were obtained from urine specimens the remaining 4 isolates 2 were obtained from the blood and two from pus samples.

Our results correlates with Shah et al study where 5 out of the 8 isolates were from urine samples and 2 from blood samples. In Taneja et al study all the 8 vancomycin resistance enterococci were obtained from urine samples. In contrary, Praharaj et al isolated majaroty of the vancomycin resistance isolates were isolated from pus samples(21.8%) followed by urine samples(18.7%).

Vancomycin resistant enterococci can be expected to be a major problem in the coming years and hence it is essential that adequate measures be taken in all health care settings to contain the dissemination of the resistant strains. Routine testing of all enterococcal isolates for vancomycin resistance at least by vancomycin agar screen test, judicious use of vancomycin, rapid isolation of patients suspected to have VRE infections and effective surveillance mechanisms will go a long way in limiting the spread of VRE. Further studies are necessary to characterize the virulence factors and the drug resistance genes of enterococcal isolates by molecular methods to know their role in the pathogenesis of nosocomial infections

CONCLUSION:

Glycopeptide resistance among our isolates was high, probably reflecting the increased use of vancomycin in our hospital over the past few years. This fact highlights the importance of strict enforcement of antibiotic policies coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria.

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