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Prevalence of Various Genotypes of Vancomycin Resistant Enterococci in Neonatal Intensive Care

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Abstract After they were first identified in the mid-1980s, vancomycin-resistant enterococci (VRE) spread rapidly and became a major problem in many institutions both in Europe and the United States. Since VRE have intrinsic resistance to most of the commonly used antibiotics and the ability to acquire resistance to most of the current available antibiotics, either by mutation or by receipt of foreign genetic material, they have a selective advantage over other microorganisms in the intestinal flora and pose a major therapeutic challenge. The possibility of transfer of vancomycin resistance genes to other gram-positive organisms raises significant concerns about the emergence of vancomycin-resistant Staphylococcus aureus. Multiple drug–resistant organisms such as vancomycin –resistant enterococci (VRE),cause serious infections especially among high –risk patients in NICU, we started active surveillance cultures to determine their efficacy in detecting and controlling the speed of VRE among high risk infants active surveillance cultures other infection control measures, and mandatory in service education is the module for preventing multiple drug resistance organisms transmission which were performed on NICU on admission and then weekly during their stay, molecular DNA extraction from rectal swab specimen of VRE isolates then amplification and genotyping by PCR using 3 primers Van A, Van b,VanC1. **Results:** active surveillance cultures identified forty nine patients with VRE colonization or infection among 500 admitted to the NICU. PCR was done on this 49 identified plus 16 detected from reculture after 1 week. Two genes clusters appeared 36 were identified biochemical as E.faecium and were shown to contain Van A. 10 were identified as E. gallinum and contained Van C.1 specimens contained both E. faecium and E.gallinurm and 2 specimens were shown to contain Van B and identified as E.faecium. 16 VER isolates were identified from patients examined after 1 week 9 of them was contained Van A and identified as E.faecium 5 was contained Van C1 and identified asE.gallinurm.2 was contained Van B. **Conclusions**: VRE is often passed from person to person by the contaminated hands of caregivers. VRE can get onto a caregiver's hands after they have contact with other people with VRE or after contact with contaminated surfaces. VRE can also be spread directly to people after they touch surfaces that are contaminated with VRE. VRE is not spread through the air by coughing or sneezing, Control transmission of multi colonel VRE stains can be achieved by active surveillance cultures together with complementation of other infection control measures. The risk of VRE infection can be reduced by minimizing the use of indwelling devices such as intravenous lines and urinary catheters. The risk is also reduced by eliminating inappropriate use of antibiotics control of transmission of multiple drug resistance colonel VER strains active surveillance cultures together with implementation of other infection control measures, were instrumental in controlling VER transmission in NICU.

Keywords: *enterococci, vancomycin, neonatal, prevalence*

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1. Introduction

After they were first identified in the mid-1980s, vancomycin-resistant enterococci (VRE) spread rapidly and became a major problem in many institutions both in Europe and the United States. Since VRE have intrinsic resistance to most of the commonly used antibiotics and the ability to acquire resistance to most of the current available antibiotics, either by mutation or by receipt of foreign genetic material, they have a selective advantage over other microorganisms in the intestinal flora and pose a major therapeutic challenge. The possibility of transfer of vancomycin resistance genes to other gram-positive organisms raises significant concerns about the emergence of vancomycin-resistant Staphylococcus aureus [30].

Enterococci are gram positive bacteria found in the bowel, female genital tract and the mouth, Some degree of drug resistance occur naturally in these organism, Enterococci are typically resistant to commonly used antibiotics such as penicillin and treatment has depended on more recently discovered antibiotic during 1990. Vancomycin was one of the few antibiotics available for

treatment of infections due to enterococci. Vancomycin resistance in enterococci was initially reported in 1986 in Europe. In the last decade enterococci have become recognized as a leading cause of nosocomial bacterial infection more than a 20 fold increase in the incidence of infection and colonization with vancomycin resistant enterococci VER have been reported from U.S.A. hospital from 1986 through 1993 from 0.3% to 7.9% [7]. Enterococci are member of the normal flora in the gut of humans and animals but have become increasingly important as hospital acquired pathogens. The y have been associated with infections of the urinary tract, post surgical wound, septicemia, endocarditis and meningitis [6] they have a remarkable ability to adapt to exposure to antibacterial maintaining intrinsic resistance to penicillin and low resistance to amino glycosides and a tremendous capacity to acquire resistance to other antibacterial including high level resistance to amino glycosides and glycopeptides [13,28] and are recognized as an important resistant pathogen in Europe [16]. Infections by VER are associated with sever adverse outcomes such as extended length of hospital stay increased cost and increased mortality [7]. Vancomycin – resistant enterococci (VRE) are antibiotic – resistant colonizers of the gastro intestinal that cause nosocomial outbreak of both colonization and the gastrointestinal tract and infection at various sites [8] have become a common pathogen responsible for nosocomial infection, with incidence rate increases significantly over the past decade [15]. This is result of an increased incidence of enterococci resistance to many antimicrobials [19]. Vancomycin –resistant enterococci (VRE) cause serious infections, especially among high – risk patients in NICUS, [23] PCR methods for rapid detection of vancomycin – resistant enterococci are now available [21]. Many hospital have initiated surveillance program for VRE, however, these programs have proven to be time-consuming for infection control personal and expansive for microbiology laboratory to conduct. [12] Active surveillance culture (ASC) programs for RVE and aggressive implementation of infection control measures reduce VRE transmission among adult and pediatric patients. Indeed, enteroccoci are the second most common nosocomial pathogens in the U.S, E. faecalis account for 80 – 90% of human enteroccal infection and E. faecalis 10 -15%. The emergence of Vancomycin resistant enterococci (VRE), mandates rapid identification. Risk factor for VRE colonization in children include young age, use of invasive devices, antimicrobial drug administration, immunosuppression, low birth weight and underlying malignancy [22] Indeed, enterococci are the second most common nosocomial pathogens in the U.S, E. faecalis account for 80 – 90% of human enterococcal infection and E. faecalis 10 -15%. The emergence of Vancomycin-resistant enterococci (VRE), mandates rapid identification. More over VRE can transfer the Van A gene for Vancomycin resistance to more virulent pathogen as staphylococcus aureus both in vitro and vivo [10].

The genus enterococcus includes more than 17. enterococcus feacalis and enterococcus faecium account for most clinical infections in hospital increasing antibiotic resistance. Enterococcus species are hardly facultative anaerobic organisms that can survive and grow in the laboratory Enterococci are distinguishly the morphologic appearance on gram stain positive cocci that grow in chain and their ability to hydrolyze esculin in the presence of sodium chloride. They were formally known as group D streptococci until assigned their owned genus [27].

For patients with Vancomycin resistant enterococci in stool, treatment with ant anaerobic antibiotic promotes high density colonization, limiting the use of such agents in these patients may help decrease the spread of Vancomycin resistant enterococcus [26].

VRE is often passed from person to person by the contaminated hands of caregivers. VRE can get onto a caregiver's hands after they have contact with other people with VRE or after contact with contaminated surfaces. VRE can also be spread directly to people after they touch surfaces that are contaminated with VRE. VRE is not spread through the air by coughing or sneezing [31].

2. Aim of the Study

The potential emergence of vancomycin resistance in clinical isolates of S. aureus or S. epidermidis is a serious public health concern. The vanA gene, which is frequently plasmid borne, can be transferred in vitro from enterococci to a variety of gram-positive S.aureus.

Aim of this study was to survey of stool and rectal swab as recommended by hospital infection control to allow for the early identification of colonized patients so that prevent transmission of VRE & Determine the prevalence of Vancomycin-resistant enterococci (VRE) colonizing the intestinal tract of NICU and define risk factors & Determined the gene typing of VRE, To minimize hospital acquired infection transmission of VRE, hospitals must use a multidisciplinary approach that requires participation by a variety of departments and personnel.

3. Material and Methods

This study was carried out in three phases each of which used a slightly different set of selective media. Rectal swabs were collected from 500 neonates in ICU (Dacron-typed swabs were moistened with sterile trypticase soy broth before rectal sampling), placed in Amies transport media and processed within 8 hours of collection. The fecal material from swab was suspended in 350 ul of sterile water and the mixture was vortexed vigorously for 5 seconds, 100 micro liters of this suspension was inoculated into 3ml of enrichment broth, and two 50 ul sample of the suspension were inoculated selective agar plate media the agar plates were incubated at 35C while the broth was incubated at 35 to 45C depending on phase of the study.

Ten micro liter samples from the enrichment broth were sub-cultered onto selective agar plated after 18 hours incubation. The agar plates were examined after 24hr, 48hr and 5 days of incubation. Colonies resembling enterococci by colonial morphology and Gram stain were subculture onto trypticase soy agar with 5% sheep blood, and were identified by standard microbiology methods according to motility and xylose production., Brain heart infusion agar containing 6 ug of Vancomycin per ml also inoculated for the detection pf VRE MIC of Vancomycin were determined by culture on campylobacter blood agar plates containing different concentration of Vancomycin

VRE were identified by using standard laboratory procedures, species identification and Vancomycin susceptibility were determined by using micro scan gram positive. Breakpoint combo panel [Dade Behring, USA] with a 24 hours incubation. Vancomycin susceptibility results were categorized according to the standard by clinical and laboratory Susceptible isolates had vancomycin MICS <4ug/mL, intermediate isolates had MICS 8-16ug/ml. and resistant isolates had MICS 32 ug/ml. Enterococcus faecuim and E. gallinarum were differentiated by motility and detection of acid production. from xylose Dacron typed swabs were moistened with sterile trypticase soy broth before rectal sampling.

All neonates with VRE were placed on contact isolation during their NICU hospitalization rectal swab specimens were added, quality control strains for susceptibility testing included Escherichia coli, Staphylococcus aureus, and E. faecalis.

3.1. DNA Extraction from Rectal Swabs Specimens

Fifty micro liter samples of stool suspension were centrifuges at 16,000 xg f or 5 minutes. The pellet was resuspended in 180 ul of distilled water, and 4 ug lysozyme per ul was added to the suspension, which was their incubated at 37 for 30 minutes. The lysate was then treated with 0.5mg of proteinase K at 70C for 30 minutes. Then at 95Cfor additional 30 minutes, two volumes of ethanol were added to precipitate the nucleic acids the ethanol mixture was applied directly to QIA amp in the tissue kit column [QIAGEW, Canada] and washed twice in buffer as described by the manufacturer, and the nucleic acid was elute with 50ul of 10mm Tris HCL (ph 9.0). 20 micro liters of the elute was applied to centrisep gel Filtration column and the elute containing purified nucleic acid was used in the PCR assays. DNA sample were frozen -20c until they were required.

3.2. Application and Detection of Van Genes by PCR

Multiplex PCR Oligonucleotide primary directed to the Van A, Van B, Vanc 1, were used in conjunction with PCR to amplify DNA from fecal material. 4 up samples of DNA purified to 21ul of chilled PCR mixture containing 10mm Tris (ph8.3) 50mm KCl, 1.5 mm mg cl2, 0.2mm each, primer 200 ul each d ATP, GTP, TTP CTP, and 2.5u of Taq DNA polymerase DNA thrombocycler was programmed as follow 10 minutes at 95c, 30 cycles of 30secondes at 94C, 30secondes at 58c, 30 seconds at 72C and 10 minutes at 72C.). Known positive and negative controls (including multiple controls consisting of sterile water [i.e., no DNA] were included in each PCR run by multiplex PCR (Aperkin –Elmer cetus model 9600DNA theromocycler Norwalk, conn.) Samples were held at 4C until the product could be analyzed. Sixteen microliter samples of the PCR products were electrophorized through l 1.5% agrose gel for 45 min at 150 V. The gel were stained with ethodiem bromide and photographed under UV light PCR was simultaneously performed with three pairs of primer. (Van A, Van B, Van C) some reaction to identify genotype of vancomycin resistance or intermediate or sensitive enterococci.

4. Results

500 rectal swabs were taken from neonatal ICU, the maternity age 1-45 days with mean 14 days. 49 of which were culture positive and 44 1of which were VER culture negative were examined for the presence of Van A or van B or Van C by PCR following enrichments broth culture.

Forty-nine isolates were recovered from stool rectal specimens of ICU by at least one culture method, (direct plating on agar or after broth enrichment).

Forty-four were identified biochemical as E. faecium and were shown by PCR. to contain Van A. 5 were identified as gallinarum and contained Van C, 2 specimens contained both E. gallinarum and Van A – containing E. faecium. Many of the specimens positive only by broth enrichment showed <10 colonies of VRE on subculture. These patients were followed after 1 weak or until will discharge, 16VRE isolate were recorded from rectal swab after 1 week.

Table 1. Age of selected groups

Patient	Range	Mean
500	1-45	14

Table 2. Characteristics of VRE ((Vancomycin resistance enterococci) genotype on culture and 1 week cuture

Parameter	Sample detected by culture	Sample detected after 1week of culture of sample
Van A		
Detecting by plate	13	3
Detecting by enrichment broth	23	6
Total Van A	36	9
Van C		
Detecting by plate	3	2
Detecting by enrichment broth	7	3
Total	10	5
Van B		
By plate	2	0
Detection by broth	0	0
Total	2	2

Table 3. Correlation between Risk factor &PCR genotype

Parameter	Van A	Van C	Van B
1. Brighweigh >1500 (Wo 300)	32	11	2
2. Hyperalimentation > 2 weeks (275) < 2 weeks (125)	 29 5	 9 2	 2 0
3. Receiving Vancomycin 5mg/Kg I.V Twice (231)	16	9	2

Table 4. Characteristics of phenotyping with antibiotics (The relation of VER and use of different type of antibiotics)

Antibiotic	Resistant Isolate %	
	E.Faecalis	**E.Faecium**
Ampicillin	11	2
Gentamycin	10	3
Streptomycin	11	2
Erythromycin	8	2
Ciprofloxacin	3	1
Chloramphenicol		
Vancomycin	36	2
Teicoplanin	7	2

Table 5. Correlation of MIC &No. of isolates (Susceptible isolates to vancomycin MIC)

Parameter	No. of cases
Vancomycin MIC 4 ug	0
Vancomycin MIC 8 ug	21
Vancomycin MIC 16 ug	15
Vancomycin MIC 32 ug	13
Total	49
After one week culture	
Vancomycin MIC 4 ug	9
Vancomycin MIC 8 ug	0
Vancomycin MIC 16 ug	6
Vancomycin MIC 32 ug	1
Total	16

5. Discussion

Because VRE can colonize the gastrointestinal tract for a prolonged period without progressing to clinically apparent disease, early recognition of colonization is essential for preventing patient – to patient transmission [17].

Vancomycin – resistant enterococci (VRE) are a cause of nosocomial infections in U.S hospitals. The national Nosocomial infections surveillance system of the center for disease control and prevention reported Vancomycin resistance in 28.5% of nosocomial enterococcal intensive care unit infections in 2003 [14].

PCR has the potential to reduce both time and cost of detecting VRE and can provide information on the vancomycin resistance genotype, which may be useful for epidemiological studies [2].

In this study surveillance program started from 2015-2016 on NICU and rectal swab was cultured on different selective media, then the specimen culture positive we used for strain typing. by PCR. PCR assays were performed with samples taken from nucleic acid extracted from fecal material from rectal swabs suspended in distilled water. We use a simple and en inexpensive column purification system for isolation of nucleic acid. This study we use combination Van A and Van B primer set and Van C. In our study we do surveillance culture for VNSE (Vanccomyicn non-susceptible enterococci) included rectal swab culture perfumed upon admission to NICU. Repeated culture were collected weekly from the patient with negative admission cultures unless they became colonized or were discharge to campylobacter blood agar plates containing 10ug/ml Vancomycin VRE were identified by using standard laboratory procedures, species identification and Vancomycin susceptibility were determined by using micro scan gram positive. Breakpoint combo panel [Dade Behring, USA] with 24 hours incubation. Vancomycin susceptibility results were categorized according to the standard by laboratory.

Susceptible isolates had vancomycin MICS < 4ug/mL, intermediate isolates had MICS 8-16ug/ml. and resistant isolates had MICS 32 ug/ml. Enterococcus faecuim and E. gallinarum were differentiated by motility and detection of acid production from xylose. The genetic relatedness of 49 VNSE was determined with PCR after DNA was extracted. The extracted DNA was amplified by PCR of 500 NICU patients admitted a total of 49 were colonized with VNSE, yielding a colonization rate of 9.8 %.

The median age of these natures was 14 ranges 1 – 45days. All of this isolate were available for DNA analysis. 36 [73%] of the 49 isolates were E gallinarium and all had intermediate susceptibility to Vancomycin. And 9(56.2%) of 16 detected from reculture were contain Van A. 12 isolate (24.4%) were E. Faecium and all were vancomycin resistant And 5 (31.2%). Of 16 detected from reculture were Van C and 2 contain Van B, anorher2 detected after reculture.65 infants colonized with VNSE identify by surveillance program in 2005-2006, all isolates were available for DNA extraction there PCR genotyping. 17 cases out of 65 (25.1%) of patients harbored vancomycin-resistant E. faecium and 45 out of 65 63.3% had vancomycin intermediate E. gallinarum, although gallinarum has lower level intrinsic vancomycin resistance and same result reported by [24].

Wile 2000 who studied on 333 swabs for rectal than these swab were culture on several selective agar medium before broth enrichment. DNA was extracted from the fecal material and was analyzed by PCR by using of four primer sets only 59 (67.8%) of the samples were positive for Van A whish was enterococcus faecium and (Wil et al 2000) who studied nosocomial infection spread in premature infant in nosocomial intensive care unit(NICU) developed coagulase negative staph and found 48 isolates of 216 proved to hetero resistant to vancomycin and Achillaes et al [1] who isolate 266 Vancomycin-resistant enterococci strain from 1,246 specimens collected from 13 hospital Van A phenotype was 30.8%, Van C phenotype was 57.1%. and Singh et al, 2005 who studied VRE among 1820 use admitted to NICUs and found 65 VRE colonization

Suzanne et al., [25] which use direct multiples PCR assay using Van A and Van B prizers, which provides rapid results and they reported that PCR was more sensitive, more rapid than culture and selective media for samples collected by rectal swab 20 of 46 versus 8 of 46 $p<0.001$ or perineal swab 17 of 58 versus L of 58 $p = 0.059$ for the detection of gastrointestinal colonization by Vancomycin and M. Leven et al., [9] who studied the prevalence of glycopeptides resistant enterococci (GRE) in the intestine by different media, and different methods, stool samples results was n= 2/3 rectal swab n= 123 were plated directly on enterococcal agar and after enrichment in enterococcol broth the prevalence of GRE was 12.8% direct plating recovered 53.4% of GRE isolates, and broth enrichment recovered 46.5%. The author reported that GRE species isolated included E. gallinarum 44.2%, E francium 30.2% E. faecalis 13.9% E. casseflavus all faecalis 11.6% isolates and faecium carried van A gene Sotake et al., [18] who are PCR for detection of Vancomycin resistant enterococci in fecal samples by PCR for surveillance instead of culture as it instinct consuming and expensive for labeling this study revealed that 67.8% samples were positive for Van Padiglone 2013 who investigated the prevalence of entrococcus resistant vancomycin isolated from stool and rectal swab in acute surveillance program for VWSE. ASC culture Vancomycin non-susceptible enterococci included rectal swab culture performed upon admission to NICU (neonatal I.C.U) repeated culture were collected weekly from patients with negative admission cultures unless they became colonized or were discharge rectal swab specimens were added to campylobacter blood agar plates

containing 10ug/ml Vancomycin. Rapid detection of VRE colonization may have important infection control implications in C where VRE colonized patients are separated C cohorts to minimize nosocomial transmission (Padiglone et, al., 2003).

Elhanan et al., 2013 reported one case of VRE of 23 children treated with Vancomycin which comes with our result. Although high prevalence of Vancomycin and Ampicillin resistance in Europe and USA [13]. The detection of high level Gentamycin resistance in 14.7% and 15.6% of faecium is the cause of concern it may signify the problem [5]. Our study revealed that 2 cases isolates was used Gentamycin and 2 only for teichoplanine. Charnow J.A. [4], reported that 69 of cases of VRE found that 42 different combination of antibiotics had been used our result revealed that significant relation with birth weight and hyperilumination reported 2 cases with Van B had birth weight less than 1.5.which comes with Grimsley 1999 who reported that gestational age significance was achieved only when infants under 35 week and weight significantly influence and similar concern were associated with influence of form of nutrition, infants who received intravenous feeding higher than nasogastric but all this factor had little effect on overall date.

Claeys [29] reported that susceptibility testing is essential for all enterococcal isolates that require antimicrobial therapy. Stool specimens, perirectal cultures that grow resistant *Enterococcus*, or both are the criterion standard for evaluating VRE colonization. Multiple blood cultures that are positive for enterococci are associated with increased inpatient mortality.

6. Conclusion

VRE is often passed from person to person by the contaminated hands of caregivers. VRE can get onto a caregiver's hands after they have contact with other people with VRE or after contact with contaminated surfaces. VRE can also be spread directly to people after they touch surfaces that are contaminated with VRE. VRE is not spread through the air by coughing or sneezing, Control transmission of multi colonel VRE stains can be achieved by active surveillance cultures together with complementation of other infection control measures.

The risk of VRE infection can be reduced by minimizing the use of indwelling devices such as intravenous lines and urinary catheters. The risk is also reduced by eliminating inappropriate use of antibiotics.

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References

[1] Achillaes.G, Athanasia Christidou, Efstathia Scouilica, Paulos Nikolaidis and Yannis Tselentis (2005): Epidemiological and molecular analysis of intestinal colonization by Vancomycin resistant enterococci in Greek hospitals.

[2] Clark, N.C.R.C. Cooksey, B.C. Hill, J.M. Swenson and F.C.Tenover.1993.characteriszation of glycopeptides resistant enterococci from US hospitals. Antimicrob. Agents Chemother. 37. 2311-2317.

[3] Centinkaya, y.,Kalk,P.& Mayhall, c.g.(2000). Vancomycin-resistant enterococci. Clin Microbiol Rev 13, 686-707.

[4] Charnow J.A.Studies expand found of epidemiology data on VRE, Inf.Dis.Nawes.1994.10.1-5.

[5] Edet E.Udo, Noura Al sweih, Oludotun A.Phillins and Tulsi D.2003: Speices prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals 1.J.Med.Microbiol. 52. (2003)163-168.

[6] Jone, R.N., Marshall, S.A., Pfaller M.A., Wilke, W.W., Hollis, R.J., Erwin, M.E., Edmoned., M.B & Wenzel R.P. (1997): Nosocomial enterococcal blood stream infections in the scope program: antimicrobial resistance, species occurrence, molecular testing results and laboratory testing accurately. Diag. Microbiol. Infec.Dis.29, 95-102 (Medline).

[7] Huycke, Mark, Sahm DF, Gilmore M.S; Multiple drug resistant Enterococci the nature of problem and an agenda for the future. Emerging infectious diseases 1998., 4(2); 239-49.

[8] Low, D.E., N.Keller, Barth and R.N.Jone. 2001. Clinical prevalence antimicrobial susceptibility, and geographic resistance patterns of enterococci:resultes from the antimicrobial surveillance program 1997.

[9] Lavan, M., Vercauteren. E. Descheemaeker. P. VanLaer and Goossens H., (1999): Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide- resistant enterococci among hospitalized patients. j. Clin. Microbiol. 37: 1436-1440.

[10] MMWR 2002 morb mortal wkil rep. 2002. 51: 565-7: Staphylococcus aureus resistant to vancomycin –United States.

[11] Millar B, Moore J, Mallon P.et al., Molecular diagnosis of infective endocarditis anew Duke's criterion Scand J Infect 2001. 26; 163-71., 26; 163-71.

[12] Morris J.G Shay J.Hebden, Mc Carter, c. Dowling and R.S. Shwallbe 1995: enterococci resistant to multiple antimicrobial agents including Vancomycin Ann.Intern.med.123:250-259.

[13] Nelson R.R.S., Mcgrrogor, k.f. Brown, A.R., Amyes. S.G.B & Young, H-K (2000). Isolation and characterization of glycopeptides –resistant enterococci from hospitalized patients over a 30 month period J.Clin Micrbiol 38, 2112-2116.

[14] NNIS National Nosocomial Infection Surveillance system Report data summary from JAN 1992 through June 2004, issued October 2004 Am, J, Infec. Control 2004, 32., 470-85.

[15] Mundy L.M., Sham.D.F and Gilmore M200: Relationships between enterococcal virulence and antimicrobial Resistance. clin. Micrbiol. Rev. 13; 513-522.

[16] Reinert, R.R., Conrad, G., Schaeger, J.J., Werner, G., Witte, W., & Klare, I. (1999). Survey of antibiotic resistance among enterococci in North Rhine –Westphalia Germany. J. Clin Microbiol.37, 1638-1641.

[17] Rebecca C, Bruce M, Sprague, Joseph M, Sumathi Nambiar, and Nalini Singh (2005): characterizing vancomycin resistant enterococci in neonatal intensive care.Emerging infectious diseases. www.cdc.gov/eid.vol.11,no.9,September 2005.

[18] Sotake 5.1 Clask N, Rimland, Wolfe f.s. and turnover f.c 1997. Detection Vancomycin resistant enterococci in fecal samples by PCR. Journal of clinical microbiology (35). No 9. 23 25-30.

[19] Reina M.Flores, PharmD James A. & Thomas W M.S.RPH 2006: Vancomycin resistant enterococci approach to treatment and control http://www.moffitt/. Cancer Control Journal (3) NO.1:1-7.

[20] Petrich, A.k.,. k.E.Luinstra, D. Groves, M,A. Chenesky and J.B.Mahony.1999; direct detection of Van A and Van B genes in clinical specimens for rapid identification of vancomycin resistant enterococci using multiplex PCR. MOL. Cell. PROBES. 13; 275-281.

[21] Perez-Hernandez, Mendez-Alvarez s, Claverie-Martin f; PCR assay for rapid detection of vancomycin resistant enterococci. Diagn. Microbiol. Infect Dis 2002., 42; 273-7.

[22] Singh-Naz N, Sleemi A, Pikis A, Patel K, Compos J. (1999): Vancomycin resistant Enterococci faecium colonization in children. J CLIN MICROBIOL.37; 413-6.

[23] Singh N,Leger MM, CampbellJ, Short B, Campos JM (2005): Control of vancomycin resistant enterococci in the neonatal intensive care uint.Infection control hosp epidemiol 2005 jul; 26(7) :646-9.

[24] Sachiko Satake, Nancye clark, David Rimland, and Fred C. (1997): DETECTION OF Vancomycin resistant enterococci in fecal Samples by PCR. Journal of clinical microbiology sep. 1997,

2325-233024-Suzanne M, William E, Trick, Fred C. and lance p. Peterson: Composition of PCR assay to culture for surveillance detection of vancomycin resistant enterococci. Journal of clinical microbiology 2013 Oct. 4 and 05-4807. (41) No. 10.

[25] Suzanne M, William E, Trick, Fred C. and lance p. Peterson: Composition of PCR assay to culture for surveillance detection of vancomycin resistant enterococci. Journal of clinical microbiology 2013 Oct. 4 and 05 – 4807. (41) No. 10.

[26] Schleifer.K, H.and Kilpper Balz 1984: Transfer of Streptococcus feacalis and Streptococcus francium to the genus Enterococcus now. rev.as Enterococcus feacalis comb nov.Int.J.Syst. Bacteriol 34:31-34.

[27] Susan L Fraser, col., Juilia lim, Donskey M.D and David H.,: enterococcal infections:
http://www medicine.com/med/topic.680.htm.

[28] Quale, J., Laudman, D. &, Atwood E. (1996) Experience with a hospital –wide outbreak of vacomycin-resistant enterococci Am J Infection control 24,372-379(MEDLIN).

[29] Claeys KC, Zasowski EJ, Lagnf AM, Rybak MJ. Comparison of outcomes between patients with single versus multiple positive blood cultures for Enterococcus: Infection versus illusion. *Am J Infect Control*. 2016 Jan 1. 44 (1):47-9.

[30] Yesim Cetinkaya, Pamela Falk, and Glen Mayhall (Vancomycin-Resistant Enterococci)Clin. Microbiol. Rev. October 2000 vol. 13 no. 4 686-707 1 October 2000.

[31] Siegel JD, Rhinehart E, Jackson M, et al. 2011: The Healthcare Infection Control Practices Advisory Committee (HICPAC). Management of Multidrug-Resistant Organisms In Healthcare Settings, CDC-INFO.