Vancomycin Resistance Genes in Various Organisms- An *Insilico* Study

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Abstract

Vancomycin, a glycopeptide antibiotic is widely used to treat many serious infections caused by gram positive bacteria particularly, methicillin resistant Enterococci and Staphyllococcus sp. Emergence of commonly acquired resistance in bacterial pathogens has lead to clinical complications and threat to human health. The resistance in these organisms is conferred by several vancomycin resistance genes (Van) like VanA, VanB, VanD, VanC and VanM. Knowledge of presence of Van genes in various organisms was felt necessary prior to molecular investigation. Therefore, in this *insilico* study screening was carried out for presence of Van gene like sequences using the DNA sequence information available in biological databases. Results indicated that many pathogenic and non-pathogenic bacteria, fungal, viral and protozoan pathogens, other organisms like fishes, mammals possess Van like sequences. The present findings provide first hand information on extent of presence of Van sequences in many organisms and their phylogenetic relationship with each other. Further investigation on the significance of these genes is emphasized using molecular and bioinformatics tools to help minimize acquired resistance in antibiotics.

Keywords : Van Gene, Similarity, Sequence Analysis, Antibiotics, Enterococci, Phylogenetic Analysis

1. INTRODUCTION

The post surgical and hospital acquired infections have become life threatening diseases and lead to many clinical complications [1]. The development of antibiotic resistance in community acquired bacterial pathogens has become matter of great concern. Some opportunistic human pathogens like Entrococci, Staphylococci, and Clostridium sp. cause many diseases like skin infections, cellulitis, folliculitis, pneumonia, meningitis, endocarditis, bacteremia and sepsis. Infections that occur during hospitalization are called as nosocomial infections. These infections are dangerous as they exhibit resistance to methicillin and glycopeptide antibiotics.

Vancomycin and teicoplanin are glycopeptide antibiotics effective against majority of gram positive bacteria, particularly against multiple drug resistant Enterococci and Staphylococci which are resistant to β-lactum antibiotics [2]. These pathogens have acquired resistance to these compounds by virtue of their intrinsic property especially in clinical isolates. This leads to severe complications in immuno compromised as well as surgical patients. vancomycin resistance enterococci (VRE) was first reported in 1988 in Europe [3,4] and USA [5]. In Asia nosocomial infections in hospitals have been reported from China, Japan, South Korea. Taiwan and Thailand [6,7,8,9]. Similarly, resistance to glycopeptides have been reported in *S. aureus* in Japan [10] and

USA [11]. In India emergence of vancomycin resistance *S.aureus* was analysed [12] and the Vancomycin resistant gene in *S.aureus* was detected first time [13].

Vancomycin binds with the C-terminal D-alanyl-D-alanine (D-Ala-D-Ala) residue of pentapeptide and blocks the addition of precursors by transglycosylation to peptidoglycan chain and inhibits cross linking of cell wall by transpeptidation[14]. Resistance to vancomycin is caused by synthesis of precursors with low affinity for these antibiotics conferred by operons present on Van gene clusters that encode enzymes which synthesize low affinity precursors wherein C-terminal D-Ala residue is replaced by D-lactate (D-Lac) or D-serine (D-Ser) that modify vancomycin binding site. These genes confer high or low level resistance to vancomycin and teicoplanin which may be inducible or constitutive. VanA type gene confers inducible high levels of resistance to vancomycin and teicoplanin. Inducible various levels of resistance to vancomycin and susceptibility to teicoplanin is conferred by type VanB [15]. VanC confined to *Enterococcus gallinarum* is intrinsically resistance to low levels of vancomycin but susceptible to teicoplanin [16]. VanC resistance expressed constitutively or inducibly by production of peptidoglycan precursor ending in D-Ser [17]. VanD type confers resistance to intermediate levels of vancomycin and low levels of teicoplanin that is expressed constitutively. VanD is not transferable to other Enterococci and that distinguishes it from VanA and VanB [18,19].

Van A has a Tn3-like transposon with a cluster of seven genes (VanR, vanS, VanH, Van A, VanX, VanY and VanZ) [20]. VanA is ligase of broad substrate specificity [21] responsible for production of dipeptidase which is incorporated in peptidoglycan precursor in place of D-alanyl – D alanine. VanA is produced by VanH dehydrogenease [22]. The VanR and VanS genes encode VanR-VanS regulatory system which activate transcription of VanH, VanA, VanX in presence of vancomycin. These proteins are necessary for vancomycin resistance where the vancomycin binding site is altered. VanY encodes an accessory resistance protein D, D-carboxypeptidase, insensitive to the activity of β-lactams like penicillin G that hydrolyses the C-terminal residue thereby preventing the translocation of D-Ala D-Ala containing precursors to cell surface [23]. VanZ-is likely teicoplanin resistant protein encoding gene [2].VanB ligase gene cluster is divided in to three sub types $VanB_1$, B_2, B_3 located on transposon T_n 5382 resulted from conjugation of plasmids [24,25]. Another glycopeptide resistance gene ,VanM reported from China [8] encodes D-Alanine:D-Lactase ligase and is related to VanA, VanB and VanD gene and transferred by conjugation.

As there is increase in emergence and rapid dissemination of resistance to vancomycin that has become challenge to treat human diseases, it was felt necessary to screen presence of vancomycin resistance or vancomycin resistance like DNA sequences that are present in various organisms prior to carry out molecular characterization. Therefore, in this *Insilico* study presence and extent of Van genes has been analysed with the sequence information available in biological databases and also by construction of phylogenetic tree. Possibly, this would provide important clues before initiating any treatment with glycopeptide or other antibiotics and understand the nature of dissemination of antibiotics resistance in various organisms.

2. PRELIMINARIES

2.1 Methodology

Vancomycin resistance genes from *Enterococcus faecalis* viz.,VanA (VanR, VanS, VanH, VanA, VanX, VanY and VanZ), VanB, VanC, VanD and VanM were selected using National Centre for Biotechnology Information (NCBI) genome search with following accession numbers. VanA (VanR, VanS, VanH, VanA, VanZ, VanY)-M97297.1 and (for VanY &VanZ) -AB563188.1, Van B-U00456, VanC - AF162694.1, VanD-AF130997.1, and VanM-FJ349556. The individual genes were screened using Basic Local Alignment Search Tool (BLASTn) tool [26] against NCBI's nonredundant nucleotide database for pairwise alignment in the month of December, 2010 (BLASTn is nucleotide sequence similarity search algorithm that aligns matches with query sequence with the existing nucleotide sequence database). The sequences produced with significant alignments with higher score, percent identity ≥ 70% and percent query coverage ≥

4% were considered for the study. Further, the sequence information of *E.faecalis* and *S.aureas* were mainly used for comparison. The vancomycin resistance like gene sequences found in various organisms were selected after pairwise alignment with help of BLASTn tool [27].

Phylogenetic analysis is an evolutionary investigation to carry out comparison in various sequences especially during the evolution among different organisms. The closely related sequences are placed in neighboring branches while distantly related sequences are placed far in phylogenetic tree. In our present study,a total of 28 sequences from various organisms were used for the analysis. (Only few organisms representing from various groups, that were significantly homologous to van resistance genes were considered for analysis). Hence the selected sequences of all organisms were analyzed phylogenetically to determine how Van genes have been associated during evolution and compared. The Phylogenetic tree was constructed using distance based Neighbor-Joining method using percent identity through CLUSTALW tool[28]. Flow chart (Fig.1) depicts the method of screening of vancomycin genes in various organisms.

2.2 Results and Discussion

Results of screening of all the Van genes is presented in Table No.1-5 ,Graphs 1-5 and Fig.2. The results indicated that there were around 37 various organisms that were homologous to Van genes. Most of the genes were identical in around 7 organisms around (93-100% identity) in *Streptococcus sp.*, *Bacillus sp.* and *Paenibaillus sp., Eggerthella lenta, Clostridium sp., Ruminococcus sp*. apart from *E.faecalis and S.aureus*. Van R found homologous in around 11 species viz., (Table -1-4, Graphs-1- 5). VanA, VanH, VanS, VanX, VanY and VanZ which are components of VanA gene clusters individually were homologous (70-100%) in different organisms like *Streptococcus gallolyticus*, *S.haemolyticus, Paenibacillus thiaminolyticus, Paenibacillus apiaris*, *Bacillus cereus, B.circulans, B.thurungiensis, B.clausil, Eggerthella lenta,* and *Ruminococcus sp.*. VanC and VanD recorded homologousness only in 4 species. Van M was recorded in 14 organisms apart from Enterococcus species while *Streptococcus suis, Lactococcus lactis, Lactococcus garvieae, Lactobacillus salivarius, Tetragenococcus halophilus* displayed homology with VanY and VanM genes (Table- 1-4, Graphs-1,2 and 3). Further, VanC, VanD and VanZ showed comparatively lesser similarity with VanA and VanB genes. Interestingly, VanZ was present in *Mus musculas, Rattus norvegicus,* Human Immuno deficiency virus-1(HIV-1) isolates from Belgium and USA envelope glycoprotein and *Schistsoma masoni* (Table-4-5,Graphs 4-5). Surprisingly, the part of DNA sequences of various organisms which were homologous to Vancomycin genes also ranged from Thermopiles to *Aspergillus sp.*, *Haemophilus influenzae*, *Plamodium vivax* and *P.falciparam, Trypanosoma brunie*, *Salmonella enterica* and *Danio verio* (zebra fish), *Taeniopygia guttata* (zebra finch), *Gallus gallus* (domesticated fowl) and *Monodelphis domestica* (marsupial).

Clostridium species, Streptococcus sp., *Paenibacillus thiaminolyticcus, Paenibacillus apiarius, Paenibacillus popillae, Ruminococcus sp, Eggerthealla lenta, Bacillus circulans, Staphylococcus gallolyticus* showed identity with VanA, VanB, VanM and VanD genes(Table- 3-4, Graphs 3-4). Other organisms like *Monodelphis domestica, Rattus novegicus, Mus musculas, Aspergillus nidalans, Gallus gallus, Staphylococcus bovis, Anophelis gambaei, Trypanosoma brunei gambiense, Plasmodium falcipuram, P.Vivox, Salmonella enterica, Haemophilus influenzae, Schistosoma masoni, Bacillus thuringiensis*, (Table- 3-5, Graphs 3-5) showed similarity with VanA, VanY, VanZ and VanB. Presence of VanC was limited only to Streptococcus sp. and clostridium sp. apart from Enterococcus sp. (Table- 1and 3, Graphs- 1and 3).

The phylogenetic tree (Fig.2) of vancomycin resistance gene is categorized into 8 major clads on the basis of their evolutionary distances by Neighbor-Joining (NJ) method using percent identity. It is depicted in the NJ evolutionary tree that first clad has monoclad of VanC of *Enterococcus gallanarium* with a evolutionary distance of 1.27 (gi-AF162694) which is distantly related to VanA of *Enterococcs faecalis*. clad 2 is divided into two sub-clads with distance of 1.25 and 2.75 consisting VanZ of *Enterococcus faecalis* (gi-M97297.1) and *Lactococcus lactis* (gi-2467210) respectively that are closely related to each other.Further, clad 3 is sub divided into two

branches with one branch having VanS (gi-M97297.1) with evolutionary distance of 2.42 and VanZ from Taiwan(gi-312836941) with distance of 0.64.The other branch is depicted with VanR and VanY with 1.83 and 3.17 distance values respectively (gi-M97297.1). Clad 4 is represented by three subclads with evolutionary distance of 0.39,1.71, and 2.95 by HIV-1(gi-308743091), *Haemophilus influenzae* (gi-CP000671.1) and VanM (gi-220901852) respectively.

The internal clad 5 is sub grouped into A and B . Group A has 6 branched sub-clads with highly homologous VanA (gi-M97297.1) and S.aureus (AE017171.1) with distance of 0.41. Further, it is branched into sub-clads having *Bacillus circulans* (gi-6448487,distance-0.49), *P.thiaminolyticus* (gi-AY926880.2, distance-0.58), *P.apiarius* (gi-50082942,distance-0.84) *and Staphylococcus haemolyticus* (gi- 222159958, distance-0.33). Group B is sub divided into 5 clads with *Eggerthella lenta* (gi-AY655718.2) highly homologous with Ruminococcus sp.(gi-56123451) and clostridium sp.(gi-56123455) with evolutionary distances of 0.25 and VanB (gi-U00456.1)with 0.32 and *S.bovis*(gi-1262410) with 0.76 respectively. Clad 6 has monoclads VanD (gi-M97297.1) with 2.24 value of distance.

Later, clad 7 has subdivided into 4 clads comprising of VanX (gi-M97297.1), VanH(gi-M97297.1), *Salmonella enterica* (gi-M97297.1)., and *Danio rerio* gene(gi-197333707) with distance of 0.59,2.35, 0.42 and 2.90 respectively. Lastly, clad 8 is having two sub-clads with *Bacillus thurungiensis* and Asperigillus sp. with distance of 0.89 and 3.08.It is indicated in the phylogenetic tree (Fig.2) that VanA, VanB are more homologous with various organisms like Streptococcus sp. Staphyllococcus sp., Bacillus sp., Paenibacillus sp., Ruminococcus sp., Clostridium sp. compared to VanD. HIV-1, *Haemophilus influenzae* and Lactococcus sp. are homologous to VanS, VanY VanZ and VanM. Further, VanX, VanH are homologous to *Salmonella enterica* and *Danio rerio*(Zebra fish) further corroborating the pairwise comparison. Moreover, VanC Asparigillus sp. and *Bacillus thurungiensis* showed distant relationship with VanA and VanB.

The presence and significance of Van genes in various species is discussed as follows.

Streptococcus sp

Table-1 and Graph-1 show *Streptococcus gallolyticus, Streptococcus pneumonia, Streptococcus thermophilus, Streptococcus suis, and S. bovis* organisms' sequences that have identity of 75-95% with VanA, VanB, VanC, VanD and VanM genes of *Entercoccus sp.. Streptococcus pnemoniae, S. bovis, S.gallolyticus* cause acute sinusitis, meningitis, bacteremia, sepsis, endocarditis in humans while *S.thermophilus* is non pathogenic. Antibiotics like pencillin G or Centrixone are administrated to treat these diseases. However, these have shown to have acquired glycopeptide resistance in our study [29] .

Staphylococcus and Lactococcus sp

It is known that *S.aureus* has already acquired vancomycin resistance[10] and hence under present investigation it is identical with Van genes (Fig.2) . Further it is depicted that *S.aureus* is not similar to VanC type, indicating VanC has quite different capacity of resistance than VanA and VanB. Further, *S.haemolyticus* (VanA-100% and VanM-84% identity) has homologies with Van gene (Table-2, Graph-2). It is pathogenic as it causes endocarditis , and also methicillin resistant. This resistance may be due to Insertion Sequences (ISs) homologous to *S.aureus. S.haemolyiticus* showing resistance to Van gene may be a difficult to treat case of nosocomial infections [30].

In *Lactococcus sp*. VanY and VanM type genes are found (Table-2,Graph-2). *Lactococcus sp*. are used in food processing and dairy industry and also as probiotics (Lactococcus lactis,*Tetragenococcus halophilus).* While some are animal pathogens (*Lactococcus garviae*), some also opportunistic pathogens causing endocarditis and cholangitis in immunocompetent patients [31,32] and have been isolated from clinical samples of blood, skin lesions and urine [33].These are administered with extended spectrum beta-lactam antibiotics like tazobactum. However, in our study it is indicated that both *Lactococcus lactis* and *Lactococcus garviae* have

VanY and VanM sequence homologies. Perhaps, it indicates that there may be possible synergism towards resistance in presently administered antibiotics.

Paenibacillus sp

Paenibacillus strains have been reported to have VanA and VanB operons [34]. Further, the results (Table-3,Graph-3) obtained in our findings corroborate the report and indicate that all the three species of Paenibacillus viz *P.thiaminolyticus, P.apiaris and P.popillae* are having identity of 80-87% with the newly reported vanM gene. Perhaps the soil bacterium and pest control pathogens of this class that have Van comparable genes may be derived from common ancestor or may be integrated through conjugation [8]. Though *P.thiaminolyticus* is environmental bacteria it is reported to cause human disease in hemodialysis patients [35]. Therefore great care has to be taken by clinicians while prescribing vancomycin in haemodialysis patient as *Paenibacillus* sp. have acquired Van resistance gene.

Baccillus sp

B. cereus, B. Clausil , *B. Circulans, Baccillus thurungiensis, B. and Weihenstephanesis* have identity with VanA, VanD and VanM and VanY genes in the results (Table-3, Graph- 3). Usually these species are harmless, beneficial as pest control agents, food processing agents, and used as probiotics [36]. However, *B. cereus* causes vomiting and diarrhea due to food contamination and also skin infections [37]. *B. Clausil* causes gastrointestinal infections and is reported as methicillin resistant, [38]. Further, *B. circulans* has more number of Van resistance genes, which is marine pathogen [39]. Perhaps, the findings at hand provide clue that through the water bodies contaminated with human waste may synergistically disseminate the antibiotic resistance to many pathogens. Hence, there should be proper management of human waste.

Clostridium sp

The Clostridium sp. have shown greater similarity with most of the Van genes in our findings. The antibiotics which is effectively used to control clostridium is metronidazole. vancomycin is second line therapy if there is no response to metronidozole. But, the similarity results observed with all the Van genes in this sp. (Table-4, Graph-4) indicate that clostridium too has acquired resistance to vancomycin gene. The diseases and deaths due to Clostridium species (*C. difficle, and C. Sordellii)* infections are associated with wide antibiotics usage [40,41]. VanR, VanS ,VanA ,VanX, VanY and ,VanB, and VanM genes have identity (70-95%) with Ruminococcus sp. (Table-4,Graph 4). The Ruminococcus sp. are anaerobic, cellulytic bacteria found usually in the rumen of cattle, sheep, goats and human large intestine. Presently, these do not seem to pose any threat to human as these are non pathogenic.

Other Microorganisms

Van resistance genes from VanS, VanA, VanH, VanX, VanZ, VanB, and VanM are having similarity with *Eggerthella lenta*(Table-4,Graph-4) sequences. It is anaerobic organism present in human intestinal flora found to cause anaerobic spondylodiscitis [42] which is infection of intervertebral disc and adjoining the vertebral bones. Besides, it is also responsible for polymicrobial blood stream infection and bacteremia [43]. Eggerthella sp. are reported to be sensitive to penicillin [44]. But, in this study *Eggerthella lenta* has shown similarity up to 72-95% with VanA, VanB, and VanM genes that are responsible for vancomycin resistance. *E. lenta* also infectious in following cases of post gynecological surgery, chrrioamniotitis, appendicitis and cutaneous abscess. Therefore, clinicians should carefully advice the antibiotics wherein *E. lenta* may also pose threat to life of patient. Further, similarity of VanZ gene with pathogenic HIV-1 isolate from Belgium and USA envelope glycoprotein and *Schistosoma masoni* is depicted in our study (Table-1,Graph-4). It is noted that VanZ may confer resistance to teicoplanin antibiotics where the functions of VanZ are not clearly revealed [45]. Surprisingly, results under present investigation also indicated the presence of VanR, VanH,VanY type genes in (Table-4,Graph– 4) *Haemophilus influenzae* (influenza virus), *Salmonella enterica* (a gram negative bacteria), *Asperigillus sp.*(pathogenic fungi) and VanY type in *Trypanosoma Brunei. Plasmodium vivax and P.falciparum* are reported to account for 65% of malaria cases in Asia and south America. Interestingly, in this study *P.vivax* and *P.falciparum* show homologies to VanB (100% identity)

and VanY (93% identity, Table-5, Graph-5) respectively. This is probably related to modification in host-parasite interaction mechanism. As the pathogen is protozoan and transfer of resistance genes from bacteria cannot be expected [14]. However, *P.vivax and P.falciparum* have been mysterious and posing resistance to commonly used antimalarial drug chloroquinine, which is increasing problem around the world [46].

Higher Organisms

Higher organisms viz., *Anophelis gambiae* (malaria spreading mosquito), *Danio verio* (Zebra fish),*Mus musculas*[rodent), *Rattus norvegius* (rat), *Taeniopygia guttata* (zebra finch), *Gallus gallus* (domesticated fowl), *Monodelphis domestica* (marsupials) have presented higher homologies with VanB, VanR ,VanH, VanX ,VanY and VanZ genes up to 80-94% identity of sequences (Table-5, Graph-5). Presence of single gene from Van gene clusters in any organisms may not contribute for resistance to vancomycin. The presence of glycopeptide resistance gene sequences in all above diverse organisms is unclear and need to unmask behavior of organisms towards the important antibiotics. Perhaps, drug pressure is believed to be responsible for the emergence of drug resistance in pathogens.

In our results, the observation of similarity with Van resistance genes in unrelated [non Enterococci sp.) organisms and as well as in the organisms where glycopeptides are not administered, may be attributed for having common ancestral origin with Van gene clusters, that might have evolved independently [8] (Fig.2). Further, it is also reported that biopesticide, *P.popillae* widely used for agricultural purposes already has Van gene and might have served as donor of Van gene and has impact on bacterial resistance [47,1] in human pathogens. Genetic variations are responsible for different glycopeptide resistance types, which are present on transferable elements in Enterococcus and Staphylococcus sp. This has lead to emergence of several types of Van resistance genes . Dissemination of glycopeptide resistance to other bacteria has been evident with example of *E.faecalis to S.aureus* [48] because of absence of barriers for heterospecific expression of genes [14]. Further the phylogenetic analysis of Van genes has also justified BLAST (Pairwise alignment) results.

It may be noted that some of the organisms have Van resistance gene even though vancomycin is not administered. It is observed that vancomycin resistance was inducible to *Enterococcus faecalis* when exposed to vancomycin [49]. Probably presence of Van genes may be attributed to this fact .In our investigation presence of Van like sequences in other organisms may indicate change in the nature of their cell wall. Studies made in *S.aureus* have provided evidence that glycopeptide resistant organisms have thicker, more irregular cell walls than the glycopeptides susceptible ones [50].Probably this would pose resistance to frequently used different antibiotics.

2.3 Conclusion

Our findings indicate presence of vancomycin resistance like DNA sequences in various organisms irrespective of species and which may be pose synergistic resistance to presently used antibiotics. Hence the need of molecular characterization and detailed bioinformatics investigation of glycopeptide resistance genes in pathogenic, commercially important organisms and animals is emphasized. A continuous surveillance can be warranted to check the emergence of glycopeptide resistance in various organisms. Probably this would facilitate to minimize indiscriminate use of many antibiotics all over the world and pave new ways to find alternative measures to combat the human diseases.

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3. REFERENCES

1. S.Mendez-Alvarez,Xiomara, Perez-Hernandez,F.Claverie-Martín, "Glycopeptide resistance in Enterococci" , Internatl Microbiol , Vol. 3,pp.71–80, 2000.

2. Reynolds. P.E, "Structure, Biochemistry and Mechanism of Action of glycopeptide antibiotics",Eur.J.clin.Microbiol.Infect.Dis., Vol. 8(11),pp.943-950,1989.

3. R.Leclercq, E.Derlot., J.Duval, P.Courvalin, "Plasmid-mediated resistance to Vancomycin and teicoplanin in *Enterococcus faecium".* N Engl J Med. Vol. 21,319(3),pp157–161, 1988.

4. A.H.Uttley, C.H.Collins, J .Naidoo, and R.C.George., "Vancomycin resistant enterococci", Lancet , Vol. 1, no. 8575-76, pp. 57-58. 1988**.**

5. D.F.Sahm, J.Kissinger, M.S.Gilmore, P.R.Murray, R. Mulder, J. Solliday, and B. Clarke, "In vitro susceptibility studies of Vancomycin resistant *Enterococcus faecalis"* , Antimicrob. Agents Chemother, Vol.33,pp.1588-1591, 1989.

6. N.Fujita, M. Yoshimura, T. Komori, K.Tanimoto, and Y. Ike, "First report of the isolation of high-level Vancomycin-resistant *Enterococcus faecium* form a patient in Japan",Antimicrob Agents Chemother., Vol.42(8),2150, 1998.

7. Y.Ozawa, K .Tanimoto ,T Nomura, M.Yoshinaga, Y.Arakawam, and Y. Ike, "Vancomycin-resistant Enterococci in humans and imported chickens in Japan". Appl. Environ. Microbiol., Vol.68, pp.6457-6461, 2002.

8. X.Xu , D.Lin., G.Yan., X.Ye, S.Wu., Y .Guo., D.Zhu, F.Hu, Y.Zhang, F.Wang., G.A.Jacoby and M.Wang, "VanM, a New Glycopeptide Resistance Gene Cluster Found in *Enterococcus faecium*", Antimicrobial Agents and Chemotherapy, pp.4643-4647, Vol.54, No. 11. 2010.

9. B.H. Zheng, Y.H.Tomita, S .Xiao,.Y.Wang. Y.Li and Y.Ike. "Molecular characterization of Vancomycin-resistant *Enterococcus faecium* isolates form mainland China," J.Clin. Microbiol , Vol.45, pp.2813-2818,2007

10. K.Hiramatsu, H.Hanaki, T.Ino, K .Yabuta, T.Oguri and F.C .Tenover, "Methicillin-resistant S*taphylococcus aureus* clinical strain with reduced Vancomycin susceptibility", J.Antimicrob. Agents chemother, Vol. 40,pp.135-136, 1997.

11. F.D.Lowry,"*Stophylococcus aureus* infections",New Engl J Med.,Vol. 339,pp.520-532, 1998.

12. H.K.Tiwari and M.R.Sen , "Emergence of Vancomycin resistant *Staphylococcus aureus(*VRSA) from tertiary care hospital from northern part of India",BMC Infectious diseases, Vol.6,156, 2006.

13. C.G.Unkal and B.B.Kaliwal, "Vancomycin resistant Staphylococcus aureus containing Van A gene isolated from clinical samples of community health care centres of North Karnataka,India".,In Proceedings of ISBT held at Shillong, India,pp.464-469, 28th- 30, Dec 2008.

14. P.Courvalin,"Vancomycin resistance in gram-positive cocci", Clin Infect.,Dis Vol. 42(suppl 1),pp.S25-S34, 2006.

15. R.Quintiliani, Jr., S. Evers, and P. Courvalin, "The Van B confers various levels ofself-transferable resistance to Vancomycin in enterococci".J.Infect.Dis., Vol. 167,pp.1220-1223, 1993.

16. R.S Leclercq, J.Dutka-Malen , Duval and P Courvalin., "Vancomycin resistance gene VanC is specific to *Enterococcus gallinarum",*Antimicrob. Agents Chemother. , Vol.36,pp 2005-2008, 1992.

17. P.E.Reynolds and P. Courvalin , "Vancomycin resistance by synthesis of precursor terminating in D-alanyl D-alanine". Antimicrobial Agents and chemother., Vol.49,pp.21-5,2005.

18. F.Depardieu, P.E.Reynolds and P.Courvalin, "VanD type Vancomycin resistance *Enterococcus faecium* 10/96A.A", Antimicrob. Agents chemother, Vol.47,pp.7-18 ,2003

19. B.Perichon, P.Reynolds and P.Courvalin, "VanD type glycopeptide resistant *Enterococcus faecium* B M 4339", Antimicrob. Agents chemother, Vol. 41,pp.2016-2118, 1997.

20. M.Arthur and P. Courvalin, "Genetics and Mechanisms of glycopeptides resistance in enterococci", Antimicrob Agents chemother, Vol.37,pp.1563-1571,1993.

21. T.D. Bugg , H.S. Dutka- malen , M. Arthur, P.Courvalin, and C.T. Walsh, "Identification of Vancomycin resistance protein vanA as a D-alanine ligase of altered substrate specificity", Biochemistry , Vol.30, pp. 2017-2021, 1991.

22. M.Arthur, C.Mlinas, S. Dutka-Malen and P. Courvalin, "Structural relationship between the Vancomycin resistance protein VanH and 2-hydroxycarboxylic acid dehydrogenasesGene" , Vol.103 ,pp.133-134, 1991.

23. L.D.Gutmann, S.Billot-Klein, I.Al-Obeid,S.Klare,E.Francoual,Collatz and J van Heijenoort, "Inducible carboxypeptidase activity in Vancomycin–resistant enterococci",Antimicrob. Agents Chemother, Vol.36,pp.77-80 ,1992 .

24. L. L.Carias, S D Rudin, C.J. Donskey and L.B. Rice, "Genetic linkage and cotransfer of a novel Van B-containing Transposon (Tn 5382) and alow-affinity penicillin-binding protein gene in a clinical Vancomycin resistant *Enterococcus faecium* is late" ,J. Bacteriol , Vol.180,pp.4426-4434,1998.

25. K.H.Dahl, G.S. Simonsen, O.Olsvik and A.Sundsfjord, "Heterogeneity in the vanB gene cluster of genomic ally diverse clinical strains of Vancomycin-resistant enterococci", Antimicrob Agents Chemother, Vol.43,pp.1105-1110, 1999.

26. S.F. Altschul, W. Gish, W. Miller, E.W.Myers and D.J. Lipman, "Basic local alignment search tool". J. Mol. Biol. Vol.215,pp.403-410, 1990.

27. http://www.ncbi.nlm.nih.gov/blast(December,2010)

28. http://www.ebi.ac.uk/clustalw (February,2011)

29. D.L. Headings, A.Herrera, E. Mazzi, and M. A. Bergman. "Fulminant neonatal septicemia caused by *Streptococcus bovis".* J. Pediatr. , Vol.92,pp.282-283, 1978.

30. M.Falcone, F.Campanile ,M.Giannella ,S.Borbone,S.Stefani ,M.Venditti, "*Staphylococcus haemolyticus* endocarditis: clinical and microbiologic analysis of 4 cases", Diagn.Microbiol. Infect .Dis. ,Vol.57(3),325-31, 2007.

31. J.M.Davies, D. Burkitt and A.Watson, "Ascending cholangitis presenting with *Lactococcus lactis cremoris* bacteraemia: a case report",*Journal of Medical Case Reports*, 3:3doi:10.1186/1752-1947-3-3,2009.

32. J.J.Fefer, K.R.Ratzan, S.E.Sharp and E.Saiz, *"Lactococcus garvieae* endocarditis: report of a case and review of the literature",Diagnostic Microbiology and Infectious Disease, Vol.32, 2, pp. 127-130 ,1998.

33. R.Facklam and J.A.Elliott, "Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci", *Clin Microbiol Rev.* ,Vol.8(4),pp.479–495,1995.

34. G.Luca, B.Perichon,J. V.Heijenoort, D .Blanot and P.Courvalin, "Glycopeptide Resistance *vanA* Operons in *Paenibacillus* Strains Isolated from Soil", Antimicrobial Agents and chemotherapy, 49 (10),pp. 4227-4233, 2005.

35. J.Ouyang, Z.Pei,L.Lutwick, S.Dalal, L.Yang ,N.Cassai, K.Sandhu ,B.Hanna, R.L.Wieczorek, M.Bluth and M.R.Pincus, "*Paenibacillus thiaminolyticus:* A New Cause of Human Infection, Inducing Bacteremia in a Patient on hemodialysis", Annals of Clinical & Laboratory Science, Vol.38,pp.393-400,2008.

36. K .V. Tilak and B.R.Srinivasa Reddy , "*Bacillus cereus* and B. *circulans* –novel inoculants for crops", Current Scince, Vol.90 (5),pp.642-644, 2006.

37. A. Kotiranta, K.Lounatmaa , M.Haapasalo , "Epidemiology and pathogenesis of *Baccillus cereus* infections" , Microbes Infect Vol. 2 (2),pp.189-198,2000.

38. http://www.genome.jp/kegg/ (December,2010)

39. M.Thangaraj , P.Vishruth , T.Ramesh and A.P.Lipton, "RAPD Fingerprinting and Demonstration of Genetic Variation in Three Pathogens Isolated form Mangrove Environment" ,Asian Journal of Biotechnology, Vol.3(3),pp.269-274, 2011.

40. E.Meites,S.Zane,C.Gould, "Fatal Clostridium sordellii infections after medical abortions",N Engl J Med. , Vol.30,363(14),pp.1382-3,2010.

41. B.E.Murray.,"Diversity among multidrug-resistant entrococci", Emerg.InfectDis, Vol.4,pp.37-47,1998.

42. C.W.Bok and Y.S.Ng, "*Eggerthella lenta* as a cause of anaerobic Spondylodiscitis, Singapore", Med .J , Vol.50(12), e393-e396, 2009.

43. K.Liderot, M. Larsson, S.Borang and V.Ozenci, "Polymicrobial Bloodstream Infection with *Eggerthella lenta* and Desulfovibrio desulfuricans",Journal of Clinical Microbiology, Vol. 48,pp.3810-3812, 2010.

44. W.G.Wade, J.Downes, D.Dymock, S.J.Hiom, A.J.Weightman, F.E.Dewhirst, B.J. Paster, N.Tzellas, B.Coleman, "The family *Coriobacteriaceae*: reclassification of *Eubacterium exiguum*(Poco et al., 1996) and *Peptostreptococcus heliotrinreducens* (Lanigan 1976) as *Slackia exigua* gen. nov., comb. nov. and *Slackia heliotrinireducens* gen. nov., comb. nov., and *Eubacterium lentum* (Prevot 1938) as *Eggerthella lenta* gen. nov., comb. Nov" . *Int J Syst Bacteriol*, Vol. 49,595-600,1999.

45. M.Arthur, C.Molinas, F.Depardieu and P.Courvalin,"Characterization of Tn/546,aTn3-related Transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycon precursors *Enterococcus faecium* BM4147",J. Bacteriol., Vol. 175,pp.117-127, 1993.

46. K.S.Lee,T.H.Kim,E.S.Kim, H.S.Lim, J.S.Yeom, G.Jun and J.W.Park, "Chloroquine-resistant *Plasmodium vivax* in the Republic of Korea", Am. J. Trop. Med.Hyg.,80(2),pp.215-217,2009.

47. R.K.Patel,.K.Piper, F.R Cockerill III, J.M Steckelberg and A.A.Yousten., "The biopesticide *Paenibacillus popillae* has a Vancomycin resistance gene cluster homologous to the enterococcal VanA Vancomycin resistance gene cluster", Antimicrob. Agents chemother, Vol. 44,pp.705-709,2000.

48. W.C. Noble, Z.Virani, R.G .Cree, "Co-transfer of Vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus"*. ,FEMS Microbiol Lett., 1, Vol. 72(2),pp.195–198,1992 .

49. N.Woodford , A.P.Jhonson,D.Morrison and D.C.Spellar, "Current perspectives on Glycopeptide Resistance", Clinical Microbiology Reviews, Vol. 8,pp.585-615,1995.

50. R.S.Daum,S.Gupta,R.Sabbagh,W.M.Milewski, "Characterization of S*taphylococcus aureus* isolates with decreased susceptibility to vancomycin and teicoplanin: isolation and purification of a constitutively produced protein associated with decreased susceptibility", J Infect Dis., Vol.166(5),pp.1066–1072,1992.

TABLE 1: Percent identity of Vancomycin resistant gene sequences in Enterococcus and Streptococcal sp.

Sr. No	Organisms	Percent identity(%)										
	Enterococcus and Streptococcal sp.	**Van R**	**Van S**	**Van H**	**Van A**	**Van X**	**Van Y**	**Van Z**	**Van B**	**Van C**	**Van D**	**Van M**
1.	*Enterococcus faecium (Van A)*	100	100	100	100	100	99	100	73	--	70	81
2.	*Enterococcus faecium (Van B)*	75	99	74	75	72	--	--	100	--	69	73
3.	*Enterococcus faecium v583*	94	96	71	74	73	94	--	99	--	--	72
4.	*Enterococcus faecium (Van D)*	72	69	71	75	71	--	--	68	--	100	--
5.	*Enterococcus gallinarum (Van C)*	--	--	67	99	--	--	--	69	100	78	--
6.	*Streptococcus gallolyticus*	89	100	--	75	--	--	92	95	--	--	--
7.	*Streptococcus pneumoniae*	--	--	--	--	--	--	--	--	90	--	--
8.	*Streptococcus thermophilus*	--	--	--	--	--	--	--	--	85	--	100
9.	*Streptococcus suis*	--	--	--	--	--	--	--	--	--	--	99
10.	*Streptococcus bovis*	--	--	--	74	--	--	--	96	--	--	--

Note:VanR,VanS, VanH,VanA, VanX,VanY, VanZ are components of VanA gene

TABLE 2: Percent identity of Vancomycin resistant gene sequences in Staphylococcus and Lactococcus sp.

Sr.No	Organisms	Percent identity (%)										
	Staphylococcus and Lactococcus sp.	**Van R**	**Van S**	**Van H**	**Van A**	**Van X**	**Van Y**	**Van Z**	**Van B**	**Van C**	**Van D**	**Van M**
1.	*Staphylococcus aureus*	100	100	100	100	100	99	100	75	--	70	81
2.	*Staphylococcus haemolyticus*	--	--	--	100	--	--	--	--	--	--	84
3.	*Lactococcus lactis*	--	--	--	--	--	71	--	--	--	--	100
4.	*Lactococcus garvieae*	--	--	--	--	--	--	--	--	--	--	99
5.	*Tetragenococcus halophilus*	--	--	--	--	--	--	--	--	--	--	100

Note:VanR,VanS, VanH,VanA, VanX,VanY, VanZ are components of VanA gene

TABLE 3: Percent identity of Vancomycin resistant gene sequences in Paenibacillus and Bacillus species.

Sr. No	Organisms	Percent identity(%)										
	Paenibacillus and Bacillus sp.	**Van R**	**Van S**	**Van H**	**Van A**	**Van X**	**Van Y**	**Van Z**	**Van B**	**Van C**	**Van D**	**Van M**
1.	*Lactobacillus salivarius*	--	--	--	--	--	--	--	--	--	--	100
2.	*Paenibacillus thiamino-lyticus*	99	91	91	91	93	84	--	74	--	--	83
3.	*Paenibacillus popillae*	--	--	74	77	80	73	--	70	--	--	79
4.	*Paenibacillus apiarius*	94	90	79	91	84	81	--	--	--	--	87
5.	*Bacillus cereus*	88	--	--	97	--	73	--	--	--	--	--
6.	*Bacillus clausil*	96	--	--	--	--	--	--	--	--	--	--
7.	*Bacillus circulans*	91	--	91	93	--	94	91	--	--	70	82
8.	*Bacillus thuringiensis*	--	86	--	97	--	70	--	--	--	--	93
9.	*Bacillus weihenste-phanensis*	--	--	--	--	--	93	--	--	--	--	93

Note:VanR,VanS, VanH,VanA, VanX,VanY, VanZ are components of VanA gene

TABLE 4: Percent identity of Vancomycin resistant gene sequences in Clostridium sp. and other microorganisms

Sr. No	Organisms	Percent identity(%)										
	Clostridium sp. and other microorganisms	**Van R**	**Van S**	**Van H**	**Van A**	**Van X**	**Van Y**	**Van Z**	**Van B**	**Van C**	**Van D**	**Van M**
1.	*Clostridium sp.*	95	--	--	--	72	90	71	99	72	--	90
2.	*Ruminococcus sp.*	76	70	--	75	70	84	--	95	--	--	73
3.	*Eggerthella lenta*	--	72	71	75	72	--	85	95	--	--	74
4.	*HIV-1 isolate*	--	--	--	--	--	--	96	--	--	--	--
5.	*Schistosoma masoni*	--	--	--	--	--	--	92	--	--	--	--
6.	*Aspergillus sp.*	100	--		--	--	--	--	--	--	--	--
7.	*Haemophilous influenzae*	--	--	71	--	--	--	--	--	--	--	--
8.	*Salmonella enterica*	85	--	--	--	71	--	--	--	--	--	--
9.	*Trypanosoma brunei*	--	--	--	--	82	82	--	--	--	--	--
10.	*Plasmodium vivax*	--	--	--	--	--	--	--	100	--	--	--
11.	*Plasmodium falciparum*	--	--	--	--	--	93	--	--	--	--	--

Note:VanR,VanS, VanH,VanA, VanX,VanY, VanZ are components of VanA gene

TABLE 5: Percent identity of Vancomycin resistant gene sequences in higher organisms

Sr. No	Organisms	Percent identity(%)										
	Higher organisms	**Van R**	**Van S**	**Van H**	**Van A**	**Van X**	**Van Y**	**Van Z**	**Van B**	**Van C**	**Van D**	**Van M**
1.	*Anophelis gambiae*	93	--	--	--	--	--	--	--	--	--	--
2.	*Danio verio*	--	--	--	--	91	90	--	87	--	--	--
3.	*Mus musculus*	--	--	--	--	96	--	91	--	--	--	--
4.	*Rattus norvegicus*	--	--	--	--	--	84	82	--	--	--	--
5.	*Taeniopygia guttata*	87	--	96	--	--	--	--	--	--	--	--
6.	*Gallus gallus*	--	--	--	--	92	92	--	--	--	--	--
7.	*Monodelphis domestica*	--	--	87	--	--	93	--	--	--	--	--

Note:VanR,VanS, VanH,VanA, VanX,VanY, VanZ are components of VanA gene

FIGURE 1: Flow chart for screening of Vancomycin resistance genes in various organisms

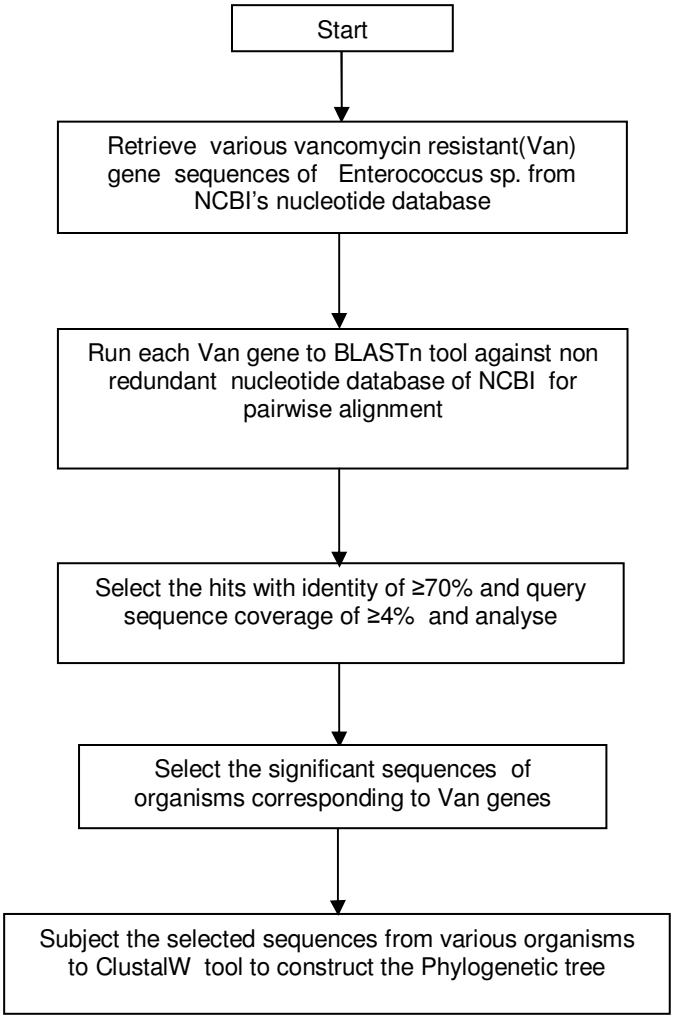
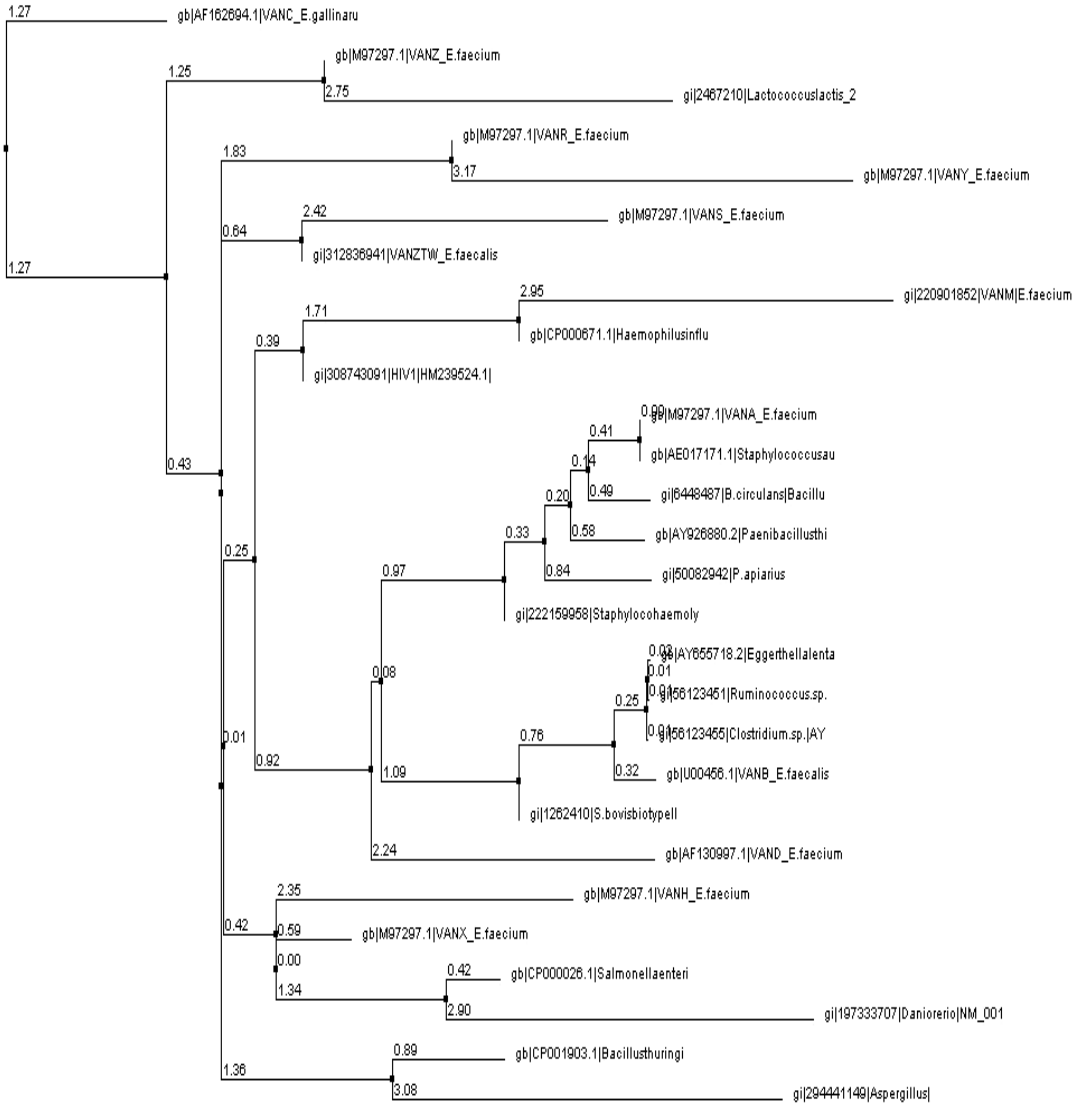
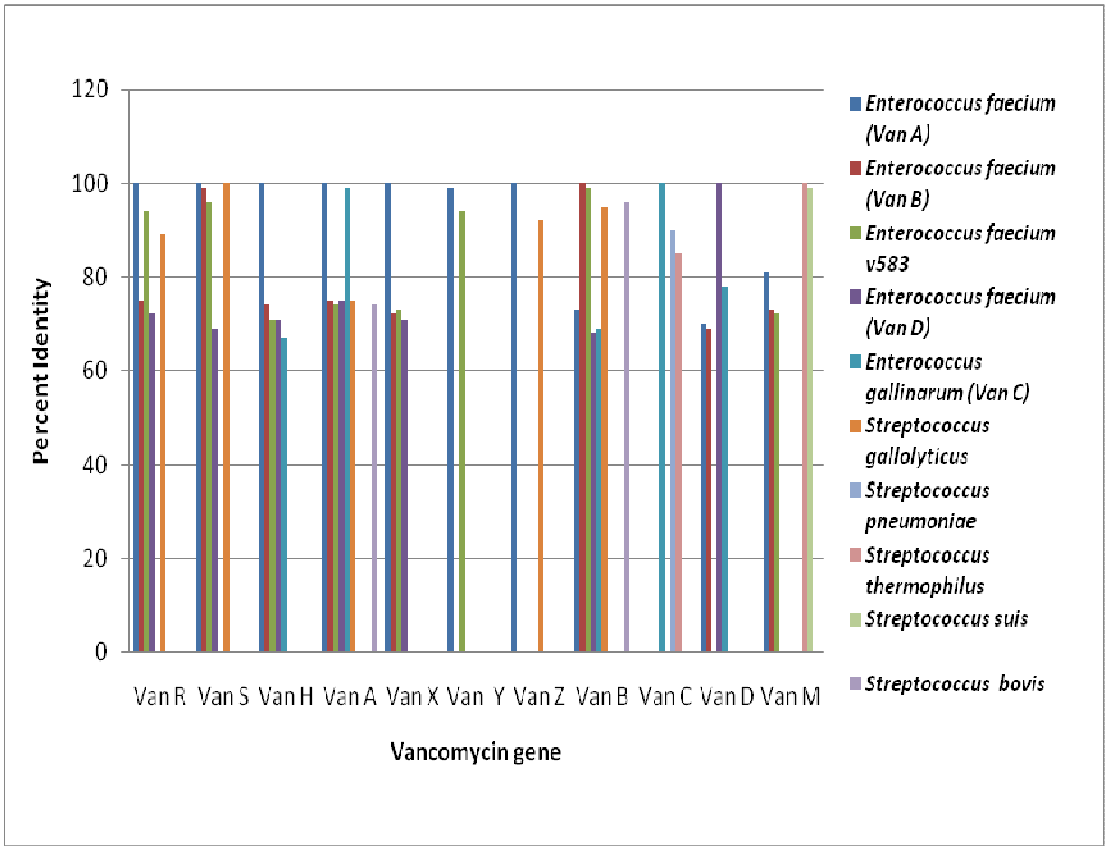


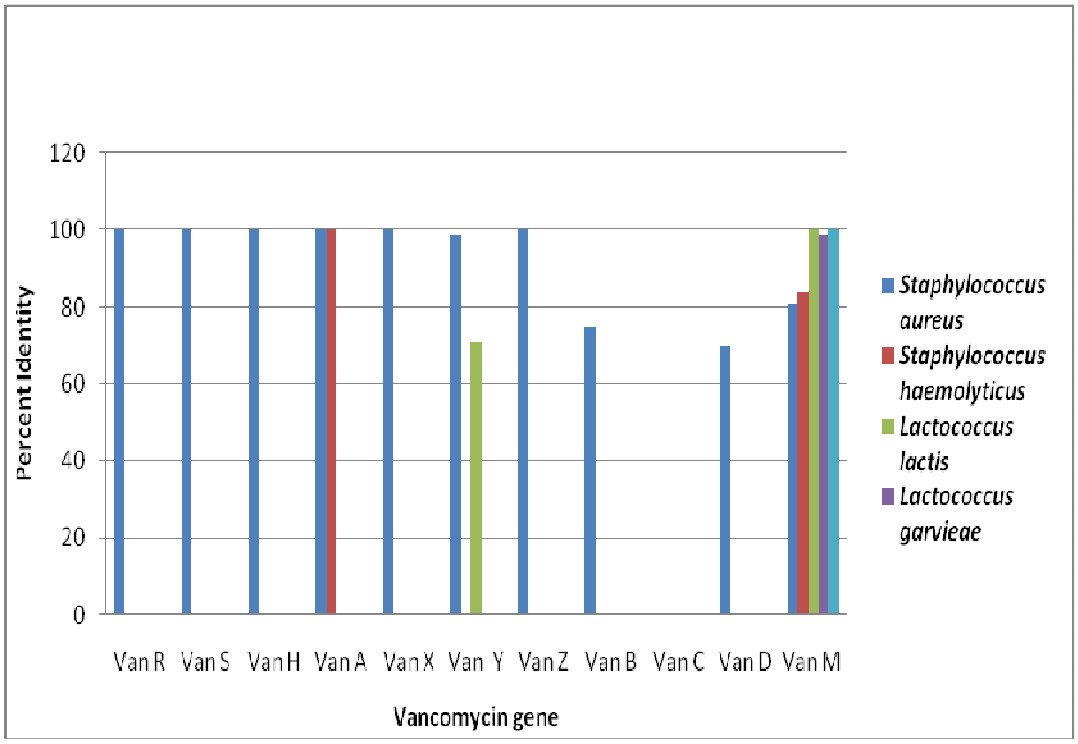
FIGURE 2: Distance based Phylogenetic tree (Neighbor- Joining) using percent identity



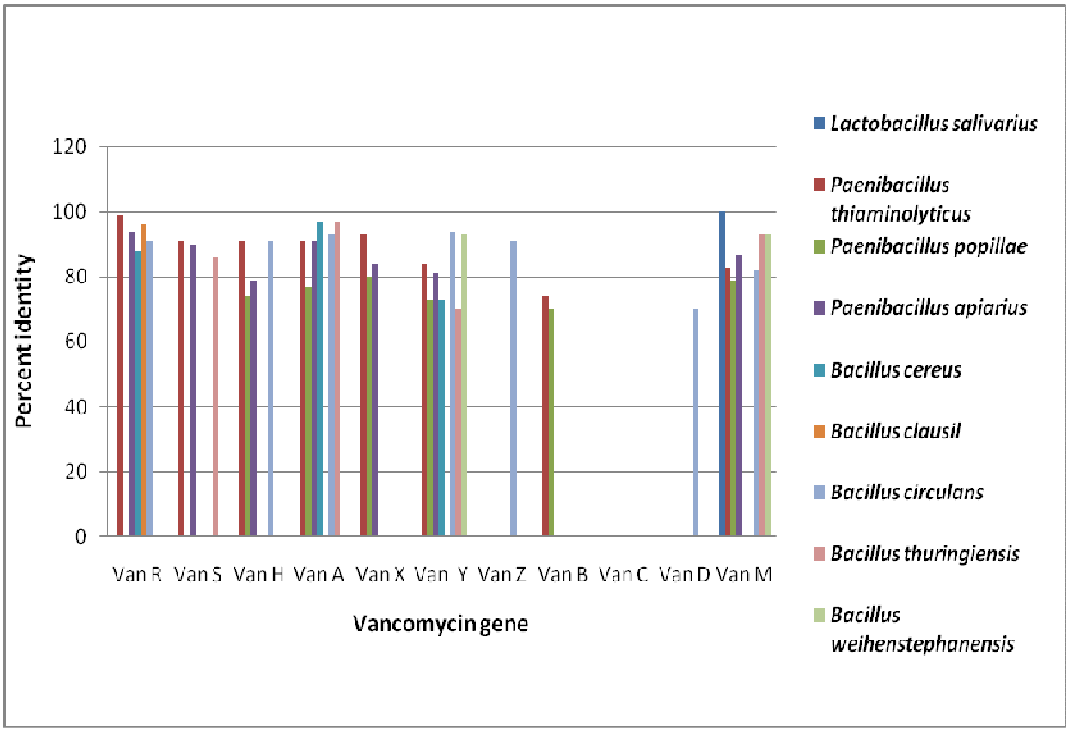
GRAPH 1: Presence of Vancomycin genes in Enterococcus and Streptococcus species



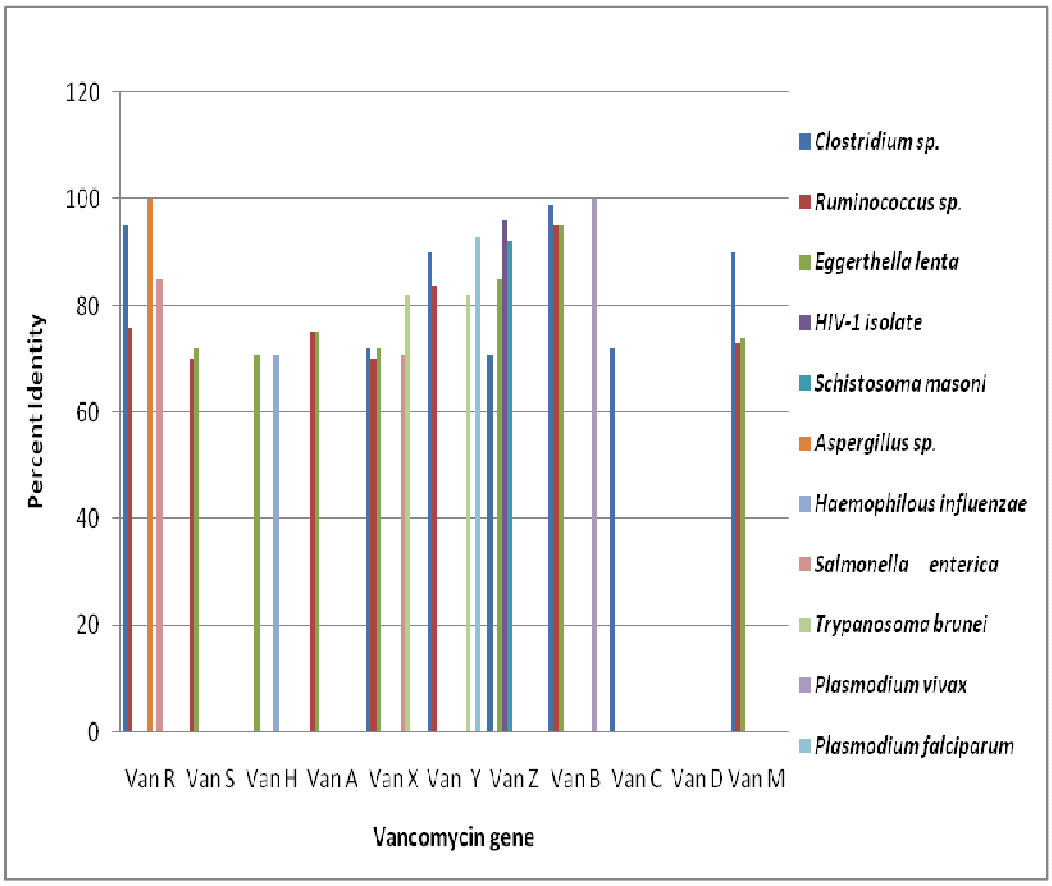
GRAPH 2: Presence of vancomycin genes in Staphylococcus and Lactococcus species



GRAPH 3: Presence of Vancomycin genes in Paenibacillus and Bacillus species



GRAPH 4: Presenceof vancomycin like genes in other organisms



GRAPH 5: Presence of vancomycin like genes in higher organisms