Molecular characterization of vancomycin resistant Enterococcus faecium clinical isolate in Makkah, Saudi Arabia

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ABSTRACT

Aims: The aims of this study is to characterize the mechanism of vancomycin resistance in clinical Enterococcus faecium isolate recovered from ICU patient in Al-Nour Specialized Hospital in Makkah. Methods: In this study, resistant vancomycin resistant isolate was studied using whole genome sequencing. The genome was sequenced using Illumina MiSeq and sequence data was used to predict both the antibiotics resistant genes and the sequence type. In addition, the sequence data was used to screen for plasmids and virulence genes. Results: This resistant isolate were found to be belonging to sequence type 80 and harboring all known VanA genes, which include VanA, VanX, VanH, VanR, VanS, VanY and VanZ genes. Conclusion: Whole genome sequence of Vancomycin resistant E. faecium clinical strain revealed that it belonged to ST-80 which is a worldwide distributed sequence type. The vancomycin resistance was found to be due to the presence of VanA gene cluster. Three plasmids were predicted, two of them reported from E. faecium strains in many countries. Relation between transposon Tn-1546 and E. faecium plasmids also have been reported.

Keywords: Enterococcus faecium, Vancomycin resistant, Whole genome sequencing

INTRODUCTION

Antimicrobial resistance is a rapidly evolving problem associated with high cost and high mortality rates [1, 2]. Although, gastrointestinal tract of human consider to be the natural habitat of Enterococci, now it has become evident that environmental habitats are significant source of infection [3]. Like other opportunistic bacteria, enterococci causes infection when it finds a chance to reach other body sites. Enterococci can cause infections in urinary tract and cardiovascular system. In addition, enterococci can infect burns and wounds, abdomen, biliary tract, catheters, and other implanted medical devices [2].
Genus Enterococcus naturally resistant to many antibiotics such as cephalosporin, co-trimoxazole and aminoglycoside [4]. Several properties contributed to increase infection of multi-drug resistant enterococci including exposure to broad-spectrum antibiotics, ability to acquire new genetic characteristics and possibility to live in hospital settings. Increased antibiotic consumption plays a major role in acquiring antibiotic resistance to Enterococci [5]. Antibiotics used for infection prevention and growth promotion in food industry also play a role in resistance [6]. VRE are linked with high incidence of mortality in hospitals especially in very ill patients in intensive care units [7] and of resistant isolates recovered from intensive care units and surgical wards [8, 9]. Few studies have reported VRE from Saudi Arabia. In one study, the frequency of VRE in the gut microflora has been reported using conventional cultural methods and antimicrobial susceptibility testing. In a latter study, the VRE were identified in only six patients out of 4276 patients [10]. The second study characterized 34 vancomycin-resistant VanA E. faecium isolates obtained from two hospitals in Saudi Arabia, MLST of 33 isolates as determined by PFGE revealed that these isolates belonged to clonal complex CC17 [11].

MATERIALS AND METHODS

This study was carried out in Makkah Holy City, Saudi Arabia to study Enterococcus faecium clinical isolate from Al Nour Specialized Hospital.

DNA Extraction and Genome Sequencing

Bacterial cells were suspended in Tris-EDTA buffer and 0.1 mm glass beads were added to the harvested cells and subjected to 2 rounds of beads-beating in Mini Beadbeater 16 (BiospecInc, USA) followed by cool in ice. DNA was then purified using phenol-chloroform. Extracted DNA was quantified using Qubit Fluorometer (Invitrogen, USA). DNA libraries for whole genome sequence were prepared to use Illumina NexteraXT Library Preparation Kit and sample were barcoded using NexteraXT Index Kit (Illumina Inc., USA). DNA sequence libraries were prepared using 1 ng input genomic DNA, and validated and quantified using Agilent Bio analyzer 2100 high sensitivity DNA Chip (Agilent Inc., USA). Vancomycin Resistant Enterococci (VRE) genomes were sequenced in Illumina MiSeq using pair ends protocol and version-3 600 cycles kit.

The quality of the pair ends sequence reads were checked by FastQC before sequence assembly (BaseSpace Labs, Illumine Inc., USA). De novo assembly of (VRE) genomes was done using DNASTAR SeqManNGen 13.0.0 (DNASTAR, Madison, USA) using default settings, which included trimming of low quality sequences ends.

Assembled genome was used for species identification and genome characterization. Species identification, Multi Locus Sequencing Typing, identification of plasmid, virulence factors and antibiotics resistant genes were predicted using Bacterial Analysis Pipeline from GoSeqIt (GoSeqIt, Denmark).

RESULTS

Isolated bacteria on MacConkey agar was identified based on cultural characteristics as Enterococci. Antimicrobial susceptibility testing showed that isolate was resistant to vancomycin. DNA was sequenced and sequencing reads were assembled (de novo assembly) and resulted in 600,797 assembled sequences forming 818 contigs. Average quality of assembled sequences was 35 and average coverage was 41X. Based on the 16SrRNA sequences, the isolate was found to be E. faecium determined by the Bacterial Analysis Pipeline from SeqIt. Multi-Locus Sequence Typing using Center of Genomic Epidemiology showed that E. faecium strain is belonging to ST-80 like (Table 1). Raw reads mapping using DNASTAR bioinformatics software revealed the presence of VanA, VanX, VanH, VanS, VanY and VanZ vancomycin resistance genes (Table 2). Three plasmids were identified using the Bacterial Analysis Pipeline from GoSeqIt (Table 3).

DISCUSSION

Using whole genome sequencing, we are able to document the presence of vancomycin resistant E. faecium belonging to ST-80 in one of the major hospitals in Makkah, Saudi Arabia. Previous studies from Saudi Arabia reported emergence of VRE nosocomial infections from different hospitals. However, in other studies only PCR and PFGE were used to investigate vancomycin resistance mechanism and to trace the strains epidemiically [11–15]. ST-80 is included in Clonal Complex 17 (CC17) lineage as found in clinical, environmental and animal samples from Tunisia, UK, Denmark, Canada, Portugal, Sweden and Korea [16–22]. Based on the epidemiological data provided by PubMLST, ST-80 isolated from blood sample of hospitalized patient in Israel in 1997 then appear in UK, South Korea (sample type is unknown). From 1999 until 2004 this sequence type appeared in Germany and Hungary (blood samples from hospitalized patients). In 2005, ST-80 was reported from skin and abdominal drainage of hospitalized patients in Italy, from blood samples of hospitalized patients in Sweden, Denmark and Canada. In 2014, this sequence type was reported from Russia (blood samples from hospitalized patients).

In 2015 and 2016, this sequence type was reported in Algeria from infected surgical wounds and peritoneal pus of hospitalized patients. Most of the isolates belonging to this sequence type were vancomycin resistant [23]. Our study showed presence of VanA, VanX, VanH, VanR,
VanS, VanY and VanZ genes which known to confer vancomycin resistance. Our findings are consistent with other studies that reported VRE infections caused by *E. faecium* are carrying VanA [24–30]. We are to document the presence of transposon Tn1546, which known to carry VanA, VanX, VanH, VanR, VanS, VanY and VanZ genes. Transposon Tn1546 have different insertion sequences. Insertion element IS1216V is one of the most frequently detected insertion sequences within the transposon Tn1546, which was reported in VRE isolates from all over the world, in transposons containing vanA from different sources [28–31]. Studies from Saudi Arabia also approved that transposon Tn1546 containing IS1216V has been associated with CC17 lineage [11, 15]. This insertion element has been detected at different positions and orientations in association with deletion of VanY and duplication of VanZ [32–35]. our findings indicate presence of VanY and VanZ in existence of IS1216V, our findings are similar to Chao et al. [22]. Some studies found that deletion of VanY gene associated with presence of Table 1: Multi-Locus Sequence Typing of vancomycin resistant *Enterococcus faecium* as determined by MLST server, Center of Genomic Epidemiology (this study).

| Strain ID | Sequence Type Genes Alleles | Sequence Type |
|----------|-----------------------------|---------------|
| VRE2     | Adk Atpa ddl Gdh gyd psts purk | ST-80 like    |

Table 2: Vancomycin Resistance genes prediction in vancomycin resistant *Enterococcus faecium* using DNASTAR SeqManNGen 13.0.0 (this study).

| Template title and accession number | Template Length | Total mapped sequences | Template Coverage% | Median Coverage | Total Sequences count |
|------------------------------------|-----------------|------------------------|--------------------|-----------------|----------------------|
| VanS-A_2_M97297                    | 1155            | 594                    | 100                | 101             | 915794               |
| VanH-A_1_FJ866609                  | 969             | 585                    | 100                | 118             | 915794               |
| VanA_2_M97297                      | 1032            | 549                    | 100                | 103             | 915794               |
| VanA_1_FJ866609                    | 1032            | 533                    | 100                | 104             | 915794               |
| VanX-A_1_FJ866609                  | 609             | 444                    | 100                | 133             | 915794               |
| VanS-Pt2_4_AY926880                | 1161            | 441                    | 100                | 64              | 915794               |
| VanY-A_2_M97297                    | 912             | 357                    | 100                | 78              | 915794               |
| VanY-A_1_FJ866609                  | 891             | 350                    | 100                | 77              | 915794               |
| VanX-Pt2_4_AY926880                | 609             | 337                    | 100                | 93              | 915794               |
| VanX-Pt_3_DQ018710                 | 609             | 336                    | 100                | 92              | 915794               |
| VanR-A_1_FJ866609                  | 696             | 327                    | 100                | 87              | 915794               |
| VanR-Pt2_4_AY926880                | 696             | 321                    | 100                | 82              | 915794               |
| VanH-Pt2_4_AY926880                | 969             | 298                    | 100                | 61              | 915794               |
| VanH-Pt_3_DQ018710                 | 969             | 275                    | 100                | 53              | 915794               |
| VanH(A(bc)_7_Y15704                 | 1032            | 260                    | 100                | 50              | 915794               |
| VanX-M_1_FJ349556                  | 609             | 206                    | 100                | 68              | 915794               |
| VanS-Pt_5_DQ018711                 | 1167            | 195                    | 100                | 36              | 915794               |
| VanR-Pt_5_DQ018711                 | 696             | 145                    | 100                | 33              | 915794               |
| VanR-Pt_3_DQ018710                 | 696             | 144                    | 100                | 33              | 915794               |
| VanZ-A_1_FJ866609                  | 486             | 122                    | 100                | 35              | 915794               |

Table 3: Plasmids identified in vancomycin resistant *Enterococcus faecium* isolate using Bacterial Analysis Pipeline from GoSeqIt (this study).

| Isolate | Plasmid | % Identity | Accession no. |
|---------|---------|------------|---------------|
| VRE2    | Rep11   | 100        | AB178871      |
|         | Rep18   | 99.36      | AF408195      |
|         | Repus15 | 99.69      | NZAAK0100000287 |
of insertion sequence IS-1216V, however, our findings showed that VanY gene has been found together with the existence of IS-1216V [36]. Plasmids as carrier for VanA determinants played major role in spreading glycopeptides resistance [35]. Our results show that \textit{E. faecium} contains plasmid replicons of different families, rep11 (repA-pB82) and rep18 (repA-pEF418) plasmids, recovered also from Portugal, Canada, Denmark, Germany, Italy, Spain, Sweden, Norway, Netherlands and Poland [32, 37–40]. Plasmid rep11 (repA-pB82) is associated with human colonizing isolates. The high abundance of this plasmid family in \textit{E. faecium} is important to the development of these important clinical lineages of \textit{E. faecium} [38]. Plasmid rep18 (repA-pEF418) reported on this study was found to be related to Tn1546 as mentioned above [32].

**CONCLUSION**

Whole genome sequencing of Vancomycin resistant \textit{E. faecium} clinical strain revealed that it’s belonged to ST-80 which is a worldwide distributed sequence type. The vancomycin resistance was found to be due to the presence of VanA gene cluster. Three plasmids were predicted, two of them reported on \textit{E. faecium} strains in many countries. Relation between transposon Tn-1546 and \textit{E. faecium} plasmids also have been reported.

**REFERENCES**

1. Kaveh M, Bazargani A, Ramzi M, Sedigh Ebrahim-Saraie H, Heidari H. Colonization rate and risk factors of vancomycin-resistant Enterococci among patients received hematopoietic stem cell transplantation in Shiraz, Southern Iran. Int J Organ Transplant Med 2016;7(4):197–205.
2. Michael KE, No D, Roberts MC. vanA-positive multidrug-resistant Enterococcus spp. isolated from surfaces of a US hospital laundry facility. J Hosp Infect 2017 Feb;95(2):218–23.
3. Vazquez-Guillamet C, Kollef MH. Treatment of Gram-positive infections in critically ill patients. BMC Infect Dis 2014 Nov 28;14:92.
4. Rice L, Bonomo R. Mechanisms of resistance to antibacterial agents. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. Manual of Clinical Microbiology. Washington, DC: ASM Press; 2011. p. 350–64.
5. Goossens H, Ferech M, Vander Stichele R, Elseviers M; ESAC project group. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. Lancet 2005 Feb 12–18;365(9459):579–87.
6. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol 2014 Jul;12(7):465–78.
7. Alotaibi FE, Bukhari EE. Emergence of Vancomycin-resistant Enterococci at a teaching hospital, Saudi Arabia. Chin Med J (Engl) 2017 Feb 5;130(3):340–6.
8. Shibli AM, Memish ZA, Kambal AM, et al. National surveillance of antimicrobial resistance among Gram-positive bacteria in Saudi Arabia. J Chemother 2014 Feb;26(1):13–8.
9. Leavis HL, Bonten MJ, Willems RJ. Identification of high-risk enterococcal clonal complexes: Global dispersion and antibiotic resistance. Curr Opin Microbiol 2006 Oct;9(5):454–60.
10. Shorman M, Al-Tawfiq JA. Risk factors associated with vancomycin-resistant enterococci in intensive care unit settings in saudi arabia. Interdiscip Perspect Infect Dis 2013;2013:369674.
11. Khan MA, van der Wal M, Farrell DJ, et al. Analysis of VanA vancomycin-resistant Enterococcus faecium isolates from Saudi Arabian hospitals reveals the presence of clonal cluster 17 and two new Tn1546 lineage types. J Antimicrob Chemother 2008 Aug;62(2):279–83.
12. Tayfour MA, Al-Ghamdi SM, Al-Ghamdi AS. Surgical wound infections in King Fahad Hospital at Al-Baha. Saudi Med J 2005 Aug;26(8):1305–7.
13. Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J Med Microbiol 2012 Jan-Mar;30(1):44–51.
14. Al-Otaibi FE, Kambal AM, Baabbad RA. Enterococcal bacteraemia in a teaching hospital in the Central region of Saudi Arabia. Saudi Med J 2004 Jan;25(1):21–5.
15. Khan MA, Shorman M, Al-Tawfiq JA, Hays JP. New type F lineage-related Tn1546 and a vanA/vanB type vancomycin-resistant Enterococcus faecium isolated from patients in Dammam, Saudi Arabia during 2006–2007. Epidemiol Infect 2013 May;141(5):1109–14.
16. Elhani D, Klibi N, Dziri R, et al. vanA-containing E. faecium isolates of clonal complex CC17 in clinical and environmental samples in a Tunisian hospital. Diagn Microbiol Infect Dis 2014 May;79(1):60–3.
17. Brodrick HJ, Raven KE, Harrison EM, et al. Whole-genome sequencing reveals transmission of vancomycin-resistant Enterococcus faecium in a healthcare network. Genome Med 2016 Jan 12;8(1):4.
18. Pinholt M, Lerner-Svensson H, Littauer P, et al. Multiple hospital outbreaks of vanA Enterococcus faecium in Denmark, 2012–13, investigated by WGS, MLST and PFGE. J Antimicrob Chemother 2015 Sep;70(9):2474–82.
19. McCracken M, Wong A, Mitchell R, et al. Molecular epidemiology of vancomycin-resistant enterococcal bacteremia: Results from the Canadian Nosocomial Infection Surveillance Program, 1999-2009. J Antimicrob Chemother 2015 Jul;68(7):1505–9.
20. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L. Dispersion of multidrug-resistant Enterococcus faecium isolates belonging to major clonal complexes in different Portuguese settings. Appl Environ Microbiol 2009 Jul;75(14):4904–8.
21. Billström H, Top J, Edlund C, Lund B. Frequent occurrence of multidrug-resistant CC17 Enterococcus faecium among clinical isolates in Sweden. J Appl Microbiol 2010 May;108(5):1810–6.
22. Cha JO, Yoo JI, Kim HK, et al. Diversity of Tn1546 in vanA-positive Enterococcus faecium clinical
isolates with VanA, VanB, and VanD phenotypes and susceptibility to vancomycin. J Appl Microbiol 2013 Oct;115(4):796–79.

23. PubMLST. Enterococcus faecium isolates database. [Available at: https://pubmlst.org/cgi-bin/bigsdb/bigsdb.pl?id=designation_value=ST&page=query&db=pubmlst_efaecium_isolates&submit=1&designation_operator=equal&designation_field1=s_1_ST]

24. Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in Enterococcus faecium BM4147. J Bacteriol 1993 Jan;175(1):26–27.

25. Arthur M, Depardieu F, Reynolds P, Courvalin P. Quantitative analysis of the metabolism of soluble cytoplasmic peptidoglycan precursors of glycopeptide-resistant enterococci. Mol Microbiol 1996 Jul;21(1):33–44.

26. Ko KS, Baek JY, Lee JY, et al. Molecular characterization of vancomycin-resistant Enterococcus faecium isolates from Korea. J Clin Microbiol 2005 May;43(5):2303–6.

27. Naas T, Fortineau N, Snanoudj R, Spicq C, Durrbach A, Nordmann P. First nosocomial outbreak of vancomycin-resistant Enterococcus faecium expressing a VanD-like phenotype associated with a vanA genotype. J Clin Microbiol 2005 Aug;43(8):3642–9.

28. Cha JO, Jung YH, Lee HR, Yoo JI, Lee YS. Comparison of genetic epidemiology of vancomycin-resistant Enterococcus faecium isolates from humans and poultry. J Med Microbiol 2012 Aug;61(Pt 8):1121–8.

29. Willems RJ, Top J, van den Braak N, et al. Molecular diversity and evolutionary relationships of Tn1546-like elements in enterococci from humans and animals. Antimicrob Agents Chemother 1999 Mar;43(3):483–91.

30. Handwerger S, Skoble J, Discotto LF, Pucci MJ. Heterogeneity of the vanA gene cluster in clinical isolates of enterococci from the northeastern United States. Antimicrob Agents Chemother 1995 Feb;39(2):362–8.

31. Camargo IL, Zanella RC, Brandileone MC, et al. Occurrence of insertion sequences within the genomes and Tn1546-like elements of glycopeptide-resistant enterococci isolated in Brazil, and identification of a novel element, ISFa5. Int J Med Microbiol 2005 Mar;294(8):513–9.

32. Novais C, Freitas AR, Sousa JC, Baquero F, Coque TM, Peixe LV, et al. Diversity of Tn1546 nd its role in the dissemination of vancomycin-resistant enterococci in Portugal. Antimicrob Agents Chemother 2008 Mar;52(3):801–8.

33. Szakacs TA, Kalan L, McConnell MJ, et al. Outbreak of vancomycin-susceptible Enterococcus faecium containing the wild-type vanA gene. J Clin Microbiol 2014 May;52(5):1682–6.

34. Gu L, Cao B, Liu Y, et al. A new Tn1546 type of VanB phenotype-vanA genotype vancomycin-resistant Enterococcus faecium isolates in mainland China. Diagn Microbiol Infect Dis 2009 Jan;63(1):70–5.
Consent Statement
Written informed consent was obtained from the patient for publication of this study.

Conflict of Interest
Authors declare no conflict of interest.

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