*Journal of Experimental Research*

*December 2017, Vol 5 No 2*

***Email: editorinchief.erjournal@gmail.com editorialsecretary.erjournal@gmail.com***

*Received: April, 2016*
*Accepted for Publication: July, 2017*

# DETECTION OF VAN C IN SOME *ENTEROCOCCUS* SPP. ISOLATED FROM A TERTIARY HOSPITAL IN ABUJA, NIGERIA.

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## Abstract

This study investigated the presence of van C, a vancomycin resistant gene in some *Enterococcus* spp. isolated from clinical samples from National Hospital, Abuja (NHA), a tertiary hospital in Abuja, Nigeria. The samples collected for the research included stool, urine, wound and environmental swabs which were cultured on bile esculin azide agar and the isolates were identified with microgen test kit. The susceptibility testing was done with vancomycin disks. Isolates that were resistant to vancomycin by Kirby-Bauer disk diffusion method were selected for minimum inhibition concentration using E-test strips. Their DNA was extracted to determine the presence of vanC genes in 17 of the isolates having MIC of ≥ 4µg/ml and 8µg/ml. The van genes present were amplified by polymerase chain reaction.

**Key Word**: *Enterococcus* spp., van genes, susceptibility, resistance, vancomycin.

## INTRODUCTION

*Enterococcus* spp are facultative anaerobic Gram-postives coccal bacteria that appear in singles, pairs and short chains (Gilmore, 2002). They are commensals in the gastrointestinal tract (GIT) of humans (Miller et al.2014). *Enterococcus* spp. are important causative agents of both nosocomial and community acquired infection and of recent are increasingly resistant to multiple antibiotics (Teixeira and Facklam, 2003). Vancomycin resistance in enterococci have emerged as a threat in medical community which has led to increased hospital length of stay of patients, increase in medical bills and mortality rate due to multi resistant nature of VRE (Edmond, 1996). This antibiotic resistance has been attributed to the overuse and misuse of medications (Ventola, 2015).

Among the medically important genes responsible for vancomycin resistance in enterococci include vanA and vanB genes found in *Enterococcus faecalis* and *Enterococcus faecium* which are acquired resistance genes and can be transferred to other *Enterococcus* spp through plasmids and transposons (Woodford, 1998). The vanC genes are intrinsic and are mainly found in *E.gallinarum* and *E.casselliflavus* and are non-transferable to other species (Couvalin, 2006). Our aim was to detect the presence of this vanC genes in other enterococcal species isolated in this study. The presence of vanC genes in *E.faecalis, E.faecium* and *E.mundtii* in this study shows that vanC genes alone cannot be used to determine or identify *Enterococcus* species.

## MATERIAL AND METHOD

One hundred and fifty samples were collected from the National hospital, Abuja (NHA) which is a tertiary care hospital after ethical approval was obtained from the management. The 150 samples included 120 clinical and 30 environmental samples. The clinical samples collected included 30 stool, 70 urine, 20 wound swabs. From the 120 clinical samples cultured, 39 strains were isolated while nil was isolated from the environment. The procedure included inoculation of the stool, urine and swabs onto bile esculin azide agar,

incubation for 24 hours at 37c°, observation of the morphological characteristic such as formation of dark brown colonies which is assumed presumptive identification of *Enterococcus* spp. (Cheesbrough, 2005), further characterization of the isolates by growing at 45c°, growth in 6.5% salt (NaCl) broth, growth on 40% bile agar, catalase test (Manero and Blanch, 1999) before being subjected to further confirmatory test with microgen test kit. Antibiotics susceptibility of the isolates were conducted using Kirby-Bauer disk diffusion method using vancomycin disk (30µg). Out of the 39 strains isolated, 24 strains were resistant by Kirby Bauer disk diffusion method while 10 strains were further confirmed to be intermediately susceptible (MIC≥8µg/ml) using E-test MIC strips. These 10 resistant strains with 7 strains having MIC of ≥4µg/ml were subjected to DNA extraction, PCR amplication of their van genes.

## RESULT:

Table 1 shows the no of samples cultured and the no of strains isolated. Urine with 70 samples yielded 11(15.7%) strains, stool with 30 samples yielded 25 (83.3%) strains, wound swab with 20 samples yielded 2(15%)of the strains while 30 environmental samples taken yielded no isolates. The 150 samples yielded 39(26.0%) enterococcal strains (<0.001) with p-value showing significant difference between the samples and isolates.

**Table 1: Isolates and their sources from NHA**

| Source | No of samples | No of *Enterococcus* (%) | Chi-square | p-value |
|---|---|---|---|---|
| Urine | 70 | 11(15.7) | 67.310 | <0.001** |
| Stool | 30 | 25(83.3) | | |
| Wound | 20 | 3(15.0) | | |
| Environmental | 30 | 0(0.0) | | |
| Sub-total | 150 | 39(26.0) | | |

Table 2 shows the species isolated from the various samples. Five species made up the 39 strains isolated. The various samples yielded 19(48.7%) *Enterococcus faecalis*, 11(28.2%) *Enterococcus faecium*, 7(17.9%) *Enterococcus mundtii*, 1(2.6%) *Enterococcus gallinarum*, 1(2.6%) *Enterococcus dispar*.

**Table 2: Enterococcus species and their sources from NHA**

| Source | No +ve for *Enterococcus* | *E.faecalis* (%) | *E.faecium* (%) | *E.gallinarum* (%) | *E.mundtii* (%) | *E.dispar* (%) |
|---|---|---|---|---|---|---|
| Urine | 11 | 11(100.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Stool | 25 | 5(20.0) | 11(44.0) | 1(4.0) | 7(28.0) | 1(4.0) |
| Wound | 3 | 3(100.0) | 0(0.0) | 0(0.0) | 0(0.0) | 0(0.0) |
| Total | 39 | 19(48.7) | 11(28.2) | 1(2.6) | 7(17.9) | 1(2.6) |

Figure 1 shows the susceptibility to vancomycin by Kirby Bauer disk diffusion method of the various Enterococcus species isolated from NHA. E. faecalis had 7(36.8%) resistant and 12(63.2%) susceptible strains; *E. faecium* had 8(72.7%) resistant and 3(27.3%) susceptible strains, *E. gallinarum* had 1(100%) resistant strain; *E. mundtii* had 7(100%) all resistant strains while *E. dispar* had 1(100%) resistant strain. None of the isolates had intermediate susceptibility. Out of the 39 enterococcal isolates, 24 of the species were resistant to the vancomycin disk (oxoid). E-test strips confirmed 10 of the isolates to have intermediate susceptibility with MIC of 8µg/ml using CLSI 2014 interpretive standard.

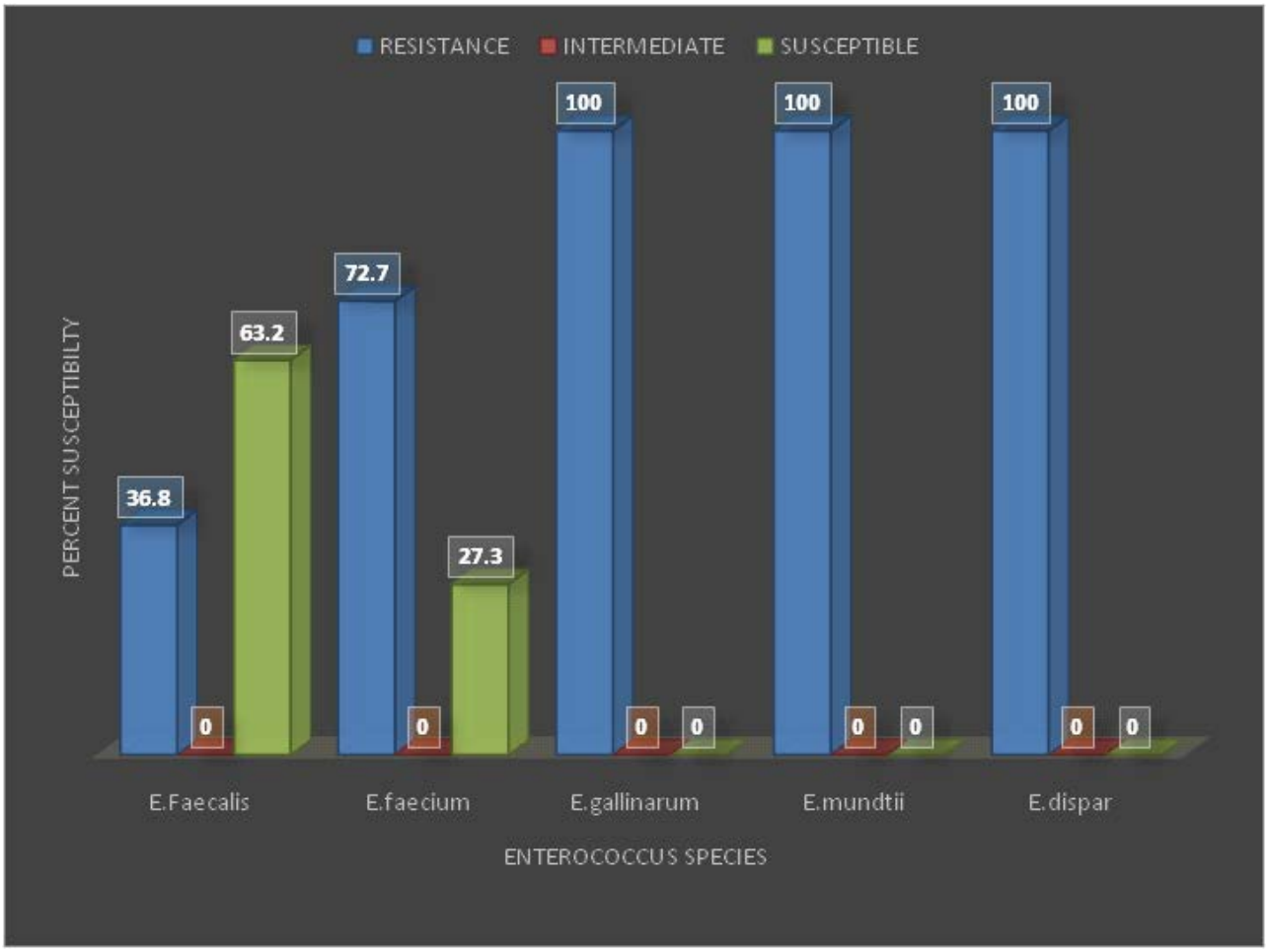


Figure 1: vancomycin susceptibility of the isolates.

Table 3 shows the MIC interpretive standard for vancomycin according to CLSI, 2014. Out of the 7 resistant *E. faecalis* isolated, 4 were susceptible while 3 were confirmed to have intermediate susceptibility; 5 were susceptible while 3 were confirmed intermediate out of 8 *E. faecium*; 1 intermediately susceptible *E. gallinarum* were also obtained. For *E. mundtii*, 5 were susceptible while 2 had intermediate susceptibility out of 7 resistant strains. One *E. dispar* was confirmed to have intermediate susceptibility.

**Table: 3: Etest MIC of Vancomycin susceptibility**

| E.faecalis (7) | | | E.faecium (8) | | | E.gallinarum(1) | | | E.mundtii(7) | | | E.dispar(1) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| <4 | 8-16 | >32 | <4 | 8-16 | >32 | <4 | 8-16 | >32 | <4 | 8-16 | >32 | <4 | 8-16 | >32 |
| 4 | 3 | 0 | 5 | 3 | 0 | 0 | 1 | 0 | 5 | 2 | 0 | 0 | 1 | 0 |

KEY: S: Susceptible, I: Intermediate, R: Resistance.

Figure 2 shows the PCR result of the amplified vanC genes from some of the resistant Enterococcus spp. Five *Enterococccus* species harboured vanC genes which included 1 *E .faecalis*, 2 *E. mundtii*, 1 *E. faecium* and 1 *E. gallinarum*.

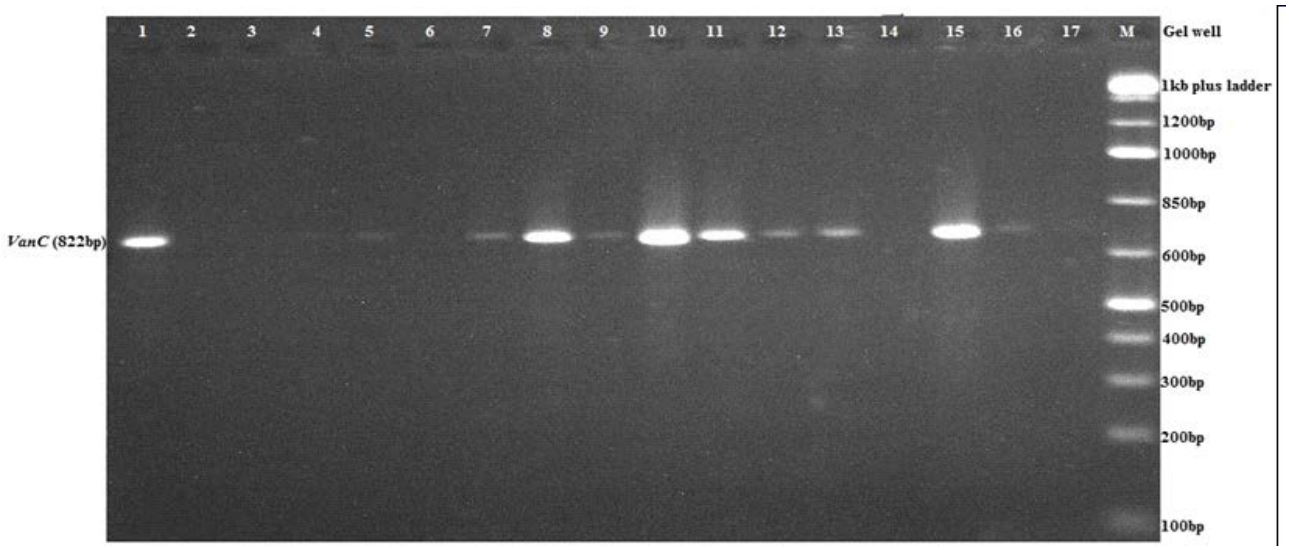


Figure: 2 shows that 29.0% (5) out of the 17 extracted DNA samples harbored vanC gene.
Key: 1: NH17(*E.feacalis*), 8:NH25(*E.mundtii*),10:NH9(*E.mundtii*), 11:NH33(*E.faecium*), 15:NH2(*E.galliarum*)

## DISCUSSION

*Enterococcus spp.* cause both nosocomial and community acquired infections. Antimicrobial resistance is on the increase in most Nigerian hospitals with its challenge for the medical community. Treatment failure due to bacterial infections especially those cause by *Enterococcus* spp. is on the increase. Infections such as bacteremia is caused by *Enterococcus* spp. and it is regarded as the third most causative agent (Fatholahzadeh et al. 2006), second most causative agent for urinary tract infections ( Mascini and Bonten, 2005) and endocarditis. These infections are increasing as a result of lack of information on vancomycin resistance among the commonly isolated enterococci.

Five phenotypes of vancomycin resistance such as VanA, VanB, VanC, VanD, and VanE are recognized but VanA and VanB are mediated by newly acquired gene clusters not previously found in enterococci. These vanA and vanB phenotypes were described primarily in *E. faecalis* and *E. faecium*. Strains with vanC gene possess low level resistance to vancomycin (MICs ≥ 4 to 32 μg≤ml) but susceptible to teicoplanin *(Cetinkaya et al. 2000).* The vanC phenotype is chromosomally encoded by the $vanC_1$ and $vanC_{2/3}$ genes, which are intrinsic to *Enterococcus gallinarum* and *Enterococcus casseliflavus*, respectively, and therefore can be used for species identification (Moura et al. 2013)

In this study, we investigated the intrinsic resistant genes among the five *Enterococcus* species isolated from National Hospital Abuja, Nigeria. Out of the 150 clinical and environmental samples collected, 39 *Enterococcus* spp. were isolated which included 19 E. faecalis, 11 E.faecium, 7 *E.mundtii*, 1 each of *E. gallinarum* and *E. dispar*. Out of these 39 isolates, 24 were resistant to vancomycin disk by Kirby-Bauer disk diffusion method but further depreciated to 10 resistant strains by E-test MIC using CLSI interpretive standard (CLSI, 2014). Among 17 strains tested for the presence of vanC genes, 5 strains were discovered to harbor vanC genes. The *Enterococcus* spp. with the vanC genes included 1 *E. faecalis* (NH7), 2 *E. mundtii* (NH25 and NH9), 1 *E. faecium* (NH33) and 1 *E. gallinarum* (NH2). The vanC genes have been known to be intrinsically present in *E. gallinarum*, *E. casseliflavus* and *E. flavescens* but our finding revealed the presence of the genes in *E. faecalis*, *E. faeccium* and *E. mundtii* showing that there could be transferability of the genes among the *Enterococcus* spp. Our findings corroborated with the work of Schwaiger et al. (2012) who detected $vanC_1$ gene in a vancomycin-susceptible strain of *E. faecalis and Sun et al. (2014) who detectected* $vanC_1$ *gene in E .faecium strain from blood culture* unlike Praharaj et al. (2013) who noted that vanC genes

are *constitutive in E. casseliflavus and E. gallinarum and are not transferable to other enterococci. E. faecalis, E. faecium, E. mundtii* may have acquired vanC genes by horizontal transfer from *E. gallinarum* and *E. casseliflavus* which are part of the intestinal normal flora. The exchange of this vanC genes between enterococcal species is important because the genes are often used to identify species. The gene transfer also shows that the chromosomal location of the genes in intrinsically resistant strains does not protect against transfer to other species (Moura et al. 2013).

## CONCLUSION

The presence of vanC genes in *E. faecalis*, *E. faecium* and *E. mundtii* isolated in this study shows that there may be horizontal transfer of vanC genes from *E. gallinarum* and *E. casseliflavus* which are natural carriers to other *Enterococcus* spp. Caution should be taken in using vanC genes as enterococcal species-specific markers.

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