Assessment of Surface Contamination by Vancomycin-Resistant *Enterococcus* Species in Selected Hospitals in the Akoko Region of Ondo State, Nigeria

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ABSTRACT

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| **Aims:** Vancomycin-resistant *Enterococcus* (VRE) species are among the most significant nosocomial pathogens, known for their ability to survive on environmental surfaces for extended periods and contribute to hospital-acquired infections (HAIs). This study evaluated the prevalence and persistence of VRE on high touch surfaces in selected hospitals in Akoko area of Ondo State, southwest Nigeria.  **Methodology:** High touch surfaces including doorknobs, bedframes, patient bedside tables and hand washing basin were sampled with moistened swab sticks. The samples were enriched in Tryptic soy broth before being inoculated on Bile esculin azide (BEA) agar for the isolation of *Enterococcus* species. The identity of colonies characteristic of *Enterococcus* species on BEA agar were confirmed based on their cellular morphology and biochemical reactions. The susceptibility of the *Enterococcus* species isolates to vancomycin and selected antibiotics was evaluated based on CLSI guidelines.  **Results:** *Enterococcus* species were isolated from 14 (15.5%) of the samples collected from the three hospitals studied. Most of isolates were resistant to one or more antibiotics tested with three (21.43 %) of the isolates resistant to Vancomycin. **Conclusion:** Result from this study demonstrated the potential for hospital surfaces to act as reservoirs of VRE infection in medical facilities studied. |

*Keywords: Enterococcus species, Healthcare associate infections, Vancomycin resistance, HAI, VRE*

1. INTRODUCTION

*Enterococcus* species are Gram-positive facultative anaerobic cocci commonly found as commensals in the gastrointestinal tract and less frequently as part of the normal floral of the female genital tract (Krawczyk et al., 2021). *Enterococcus* species are also frequently found as commensals in environmental reservoirs, such as soil and water sources (Ramos et al., 2020). Despite existing commonly as commensal bacteria, *Enterococcus* species such as *E. faecalis and E. faecium* can cause opportunistic infections in susceptible hosts, especially in individuals with weakened immune systems and underlying conditions like cancer, diabetes, and chronic kidney disease (Fiore et al., 2019; Ramos et al., 2020). In susceptible hosts, *Enterococcus* species may cause mild to severe infections, including urinary tract infections (UTIs), bacteraemia, endocarditis, intra-abdominal abscesses, and, less commonly, meningitis and pneumonia (Fahim et al., 2025)*.*

Although *Enterococcus* species are opportunistic pathogens but the emergence of strains resistant to multiple antibiotics poses a significant challenge to the management of enterococcal infections. Specifically, resistance to vancomycin, the antibiotic commonly used in the treatment of infections caused by multidrug-resistant Gram-positive bacteria, is a significant public health challenge. The World Health Organisation (WHO) categorised vancomycin-resistant *Enterococcus* (VRE) as a high-priority pathogen which requires urgent intervention (Tacconelli et al., 2018). Vancomycin-resistant *Enterococcus* infection has emerged as an important healthcare-associated infection (HAI) globally (Brinkwirth et al., 2021). A significant number of HAIs globally are caused by *Enterococcus* species, with increasingly more cases being caused by vancomycin resistant strains. For instance, Vancomycin-resistant *Enterococcus* is now a common cause of HAI in the United States., and an estimated 10.9% of HAI infections in Europe were due to *Enterococcus* infection (Brinkwirth et al., 2021; Joshi et al., 2021). VRE infection also constitutes a substantial healthcare associated infection threat in Africa (Fraser et al., 2021).

The inherent ability of *Enterococcus* species to persist on environmental surfaces is an important factor enhancing the prevalence of healthcare-associated VRE infections (Agarwal et al., 2019; Fiore et al., 2019). Thus, the continued surveillance of the distribution of VRE in hospital environment will provide important information required to develop effective control strategies. This is particularly important in low- and middle-income countries like Nigeria with disproportionally high burden of infectious diseases compared to developed countries. This study evaluated and estimated the distribution of VRE in hospital surfaces in selected hospitals in Akoko area of Ondo state Nigeria to provide insight into the risk of healthcare associated VRE infection in the study region.

2. material and methods

2.1 Sample collection:

*Enterococcus* species were isolated from surfaces in clinical area of selected hospitals in Ondo State Nigeria. A total of three hospitals were sampled. These included two publicly owned acute hospitals (site A and B) and a privately owned acute hospital (site C). Surfaces of bedframes, beddings, patient bedside tables, doorknobs, hand washing basin were sampled using sterile swab stick moistened with sterile distilled water. The samples were transported on ice to the laboratory for isolation of *Enterococcus* species. On each site visit, 10 surfaces were sampled in each medical facility with each surface sampled on three different occasions to obtain a total of 30 samples from each studied facility and a total of 90 samples from all the studied hospitals.

2.2 Isolation of *Enterococcus* species:

The cotton bud on the swab stick used was aseptically transferred into 10mL of sterile distilled water in sterile 20 mL glass bottles. The bottles were vigorously vortexed for 1 minute after which 1 mL was transferred into 9 mL of sterile Tryptic soy broth (TSB). The inoculated TSB was incubated at 37oC for 18 hours. For the isolation of *Enterococcus* species, 100 µL from the inoculated TSB was spread on Bile esculin azide (BEA) agar after which the inoculated agar was incubated at 37oC. After 18 hours of incubation, plates were observed for brown colonies which is indicative of enterococci growth on BEA agar. The colonies were subsequently sub-cultured by streaking on freshly prepared BEA agar plates and incubated for 18 hours at 37oC to obtain pure colonies.

2.3 Identification of *Enterococcus* species:

The colonies of presumptive *Enterococcus* species isolated were subjected to Gram staining, after which the catalase and oxidase reactions of all Gram-positive isolates showing characteristics cellular morphologies of *Enterococcus* species were initially done. Catalase and oxidase negative isolates were subjected to further biochemical tests including Citrate, Indole and Methyl red tests as well as motility test to confirm the identity of the isolates.

2.4 Antibiotics susceptibility profile of isolates:

The susceptibility of colonies identified as *Enterococcus* species to antibiotics was determined using the agar diffusion method. Disc of selected antibiotics, including Amoxicillin-clavulanate (20/10 µg), Vancomycin (30µg/L), Levofloxacin (5μg), Ciprofloxacin (5μg) and Erythromycin (15μg), disk were placed on Muller Hinton agar seeded with fresh culture of the Enterococcal isolates standardised to the 0.5 McFarland standard. The susceptibility of the isolates to vancomycin was evaluated based on the zone of inhibition after 18 hours incubation at 37oC according to CLSI (2021)

3. results and discussion

This study evaluated the antibiotics susceptibility profile of strains of *Enterococcus* species isolated from surfaces in selected medical facilities in Akoko are of Ondo state, southwest Nigeria. Out of a total of 90 samples collected from the surfaces for the isolation of *Enterococcus* species, as shown in Table 1, 14 (15.5%) were positive for *Enterococcus* species. This demonstrated the ability of *Enterococcus* species to colonise and persist on the sampled surfaces in the clinical facilities studied.

The colonised surface can thus potentially serve as reservoir for *Enterococcus* species from where they can be disseminated in the facility. This observation also demonstrated that routine cleaning and disinfection in the studied facility are not adequate to achieve a total removal of viable *Enterococcus* species from contaminated surfaces. This can be due to the ability of *Enterococcus* species to form good biofilms. For instance, *E. faecalis* and *E. faecium,* the most common *Enterococcus* HAI pathogens are known to be good biofilm formers (Ruhal et al., 2024).

The highest number of Enterococcus isolates were recovered from samples collected from sample site B which is a publicly owned medical facility while the lowest number of isolates were recovered from samples collected from site C. The difference in the surface contamination rate in the different facility sampled can be attributed to a range of factors. For instance, the difference in the stringency of the surface disinfection procedures in the different facilities as well as environmental surface contamination potential due to difference in colonised patients who could shed the bacteria into the environment.

The antibiotics susceptibility profiles of the *Enterococcus* isolates are shown in Table 2. Most of the isolates exhibit resistance to one or more antibiotics tested with three of the isolates resistant to Vancomycin. Resistant to Erythromycin was the highest (42.86 %, n=6) while the least number of isolates was resistant to Levofloxacin (14.29%, n=2). The resistance of some of the isolates to multiple antibiotics emphasises the role of the hospital surfaces as reservoirs of multiple-drug resistant HAI pathogens like *Enterococcus* species. The hospital surfaces is one of the most common sources of multidrug resistant HAI pathogen (Cobrado et al., 2017; Hanczvikkel and Tóth, 2018). Six of the isolates (42.86%) were resistant to three or more antibiotics belonging to different classes of antibiotics thus demonstrating multidrug resistant phenotypic attributes.

**Table 1: Biochemical Characteristics of Bacteria Isolated from samples**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sites | **Isolates** | **Catalase** | **Oxidase** | **Motility** | **Citrate** | **Indole** | **Methyl Red** | **Presumptive *Enterococcus*** |
| **A** | A3 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| A7 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| A12 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| A18 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| **B** | B5 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B6 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B9 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B13 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B15 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B22 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B24 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| B29 | +ve | +ve | nd | nd | nd | nd | *-* |
| **C** | C7 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| C10 | -ve | -ve | -ve | -ve | -ve | -ve | + |
| C24 | -ve | -ve | -ve | -ve | -ve | -ve | + |

nd: test not done

**Table 2: Antibiotics susceptibility profile of *Enterococcus* species isolated from Surfaces in Studied Hospital**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Isolates* | AUG | VAN | LFX | CIP | ERY |
| *A3* | R | S | S | R | R |
| *A7* | R | S | I | I | I |
| *A12* | S | S | S | S | I |
| *A18* | S | R | S | S | R |
| *B5* | R | S | S | S | I |
| *B6* | S | S | I | S | S |
| *B9* | S | R | R | I | I |
| *B13* | R | R | S | S | R |
| *B15* | S | S | S | I | S |
| *B22* | S | S | S | I | R |
| *B24* | R | S | S | R | I |
| *C7* | S | I | R | R | R |
| *C10* | S | S | S | S | R |
| *C24* | S | S | I | S | S |

Amoxicillin clavulanate (AUG), Levofloxacin (LFX), Ciprofloxacin (CIP),

Erythromycin (ERY), Vancomycin (VAN**)**

4. Conclusion

The persistence of resistant *Enterococcus* species on hospital surfaces as shown in this study poses a significant threat to patient safety. The implementation of effective infection prevention strategy must include effort to eradicate the environmental reservoirs of opportunistic pathogens such as *Enterococcus* species through stringent surface decontamination practices and active surveillance. The adoption of antibiotics stewardship and adapting emerging disinfection technologies are useful strategies that can be embraced to combat this persistent threat. This will entail a multidisciplinary approach to control the spread of antibiotics resistant pathogens such as VRE in hospital environment to improve patients’ outcomes.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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