Prevalence of Vancomycin-resistant *Staphylococcus aureus* and Associated Risk Factors in well water used for domestic purposes in Ile-Ife, Southwestern Nigeria

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Introduction

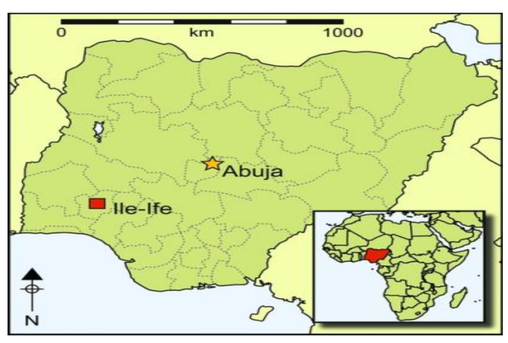
- Vancomycin resistant *Staphylococcus aureus* (VRSA) is among the bacteria designated as a high-priority pathogen by the World Health Organization.
- The prevalence of VRSA in Nigeria varies by location and sample type, with recent studies showing rates from around 1.4% in clinical settings to over 10% in specific populations like abattoir workers, highlighting its presence in both healthcare-associated and community environments
- Building upon our previous research identifying domestic wells as reservoirs for pathogenic and resistant *E. coli*, this study determined the prevalence of VRSA in these wells, investigated the mechanisms of VRSA resistance within well water, identified potential reservoirs for VRSA in the community and analyzed factors influencing VRSA presence in well water. This information will guide the development of effective interventions to prevent the spread of VRSA through drinking water sources in the study area.

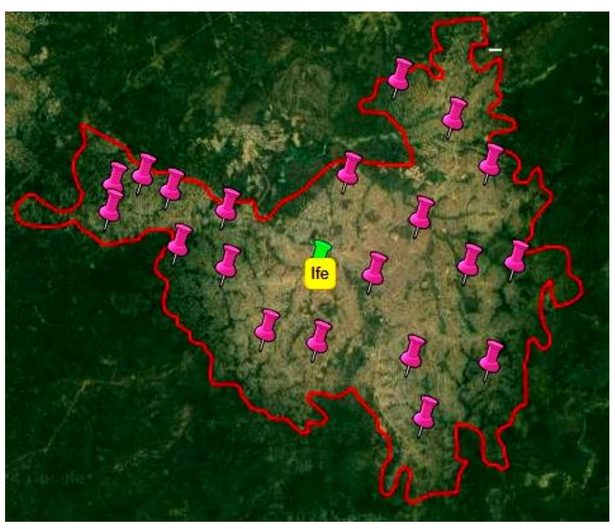
Methods

- We assessed 350 wells for VRSA, and questionnaires were administered to obtain information about well characteristics and owners' bio-demographic data.
- Isolates were identified using biochemical and molecular methods.
- The isolates were tested against nine antibiotics using the Kirby-Bauer disc diffusion method and vancomycin minimum inhibitory concentration (MIC) was determined by the agar dilution method.
- Biofilm formation test was done using tissue culture plate method.
- The resistance and virulence genes of VRSA were detected by Polymerase chain reaction.
- Data analysis was done with R statistical software.

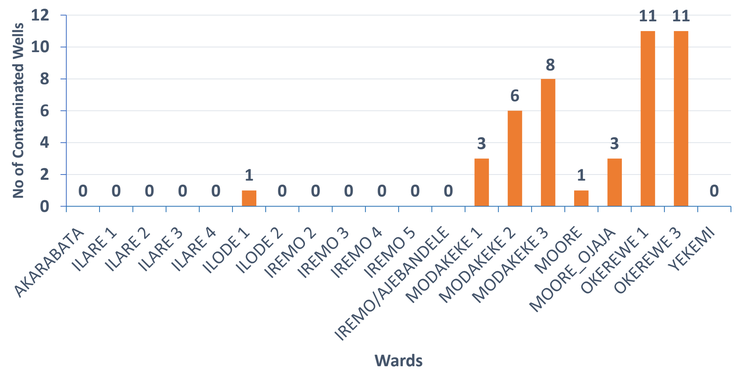
Results

The sampling locations are shown on the map. Thirty-nine samples were obtained from Moore-Ojaja ward, 23 samples from Ilare ward 4, 25 samples from Ilode ward 2, 21 samples from Iremo ward 2, 18 samples from Ilode ward 1, and 17 samples from Modakeke ward 1

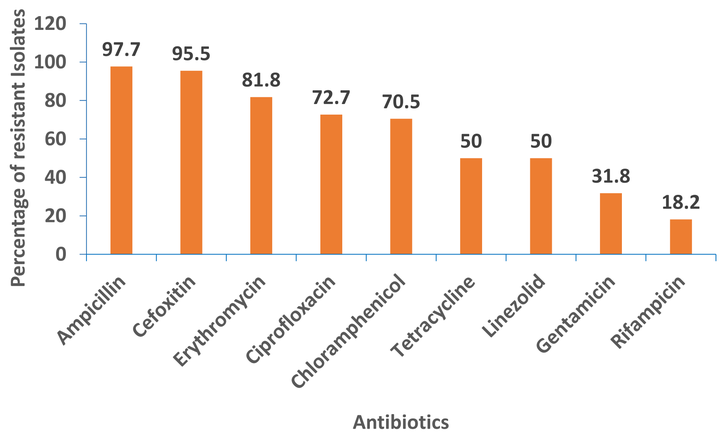


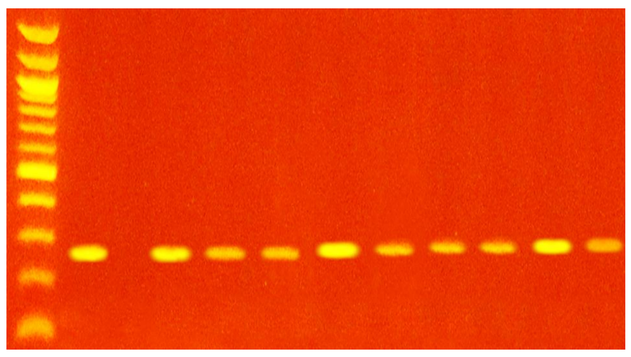


Forty-four (12.6%) wells were contaminated by VRSA, with counts ranging from $1X10^2$ to $3X10^4$ CFU/100mL.

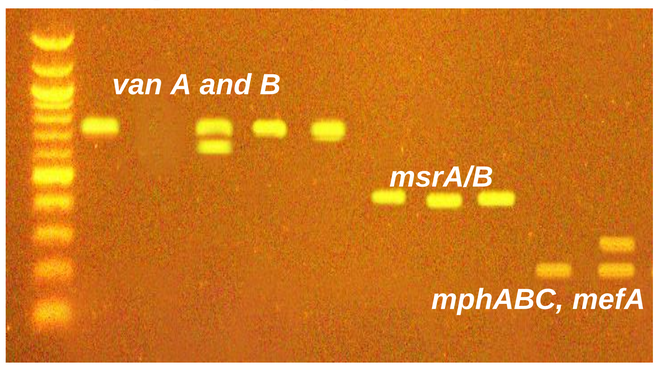


- Biofilm assessment of the 44 VRSA isolates showed 22.7% were biofilm producers; 13.6% strong and 9.1% moderate.
- Environmental and structural risk factor analysis identified animal related pollution as the strongest predictor of VRSA contamination ($p = 0.016$).
- The highest resistance rates were observed for ampicillin (97.7%) and cefoxitin (95.5%), lower resistance was observed to gentamicin (31.8%) and rifampicin (18.2%). The MIC values of the isolates ranged from 32 to 4096 ug/mL, and all exhibited multidrug resistance. The Multiple Antibiotic Resistance (MAR) indices varied between 0.1 and 1.





A representative gel image of amplified *STPY gene* of *Staphylococcus aureus*



A representative gel image of amplified *vanA, vanB, msrA*/B, *mefA*, *mph (ABC)* of *VRSA*.

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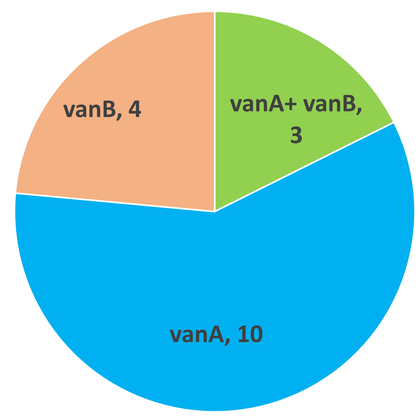
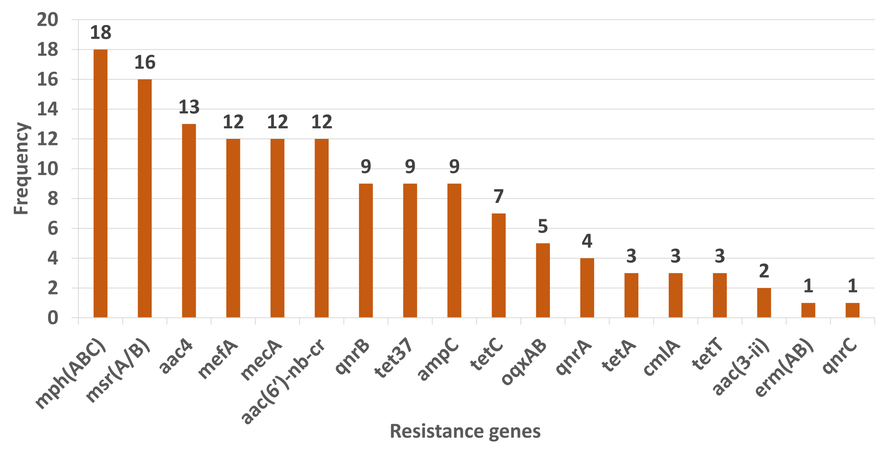
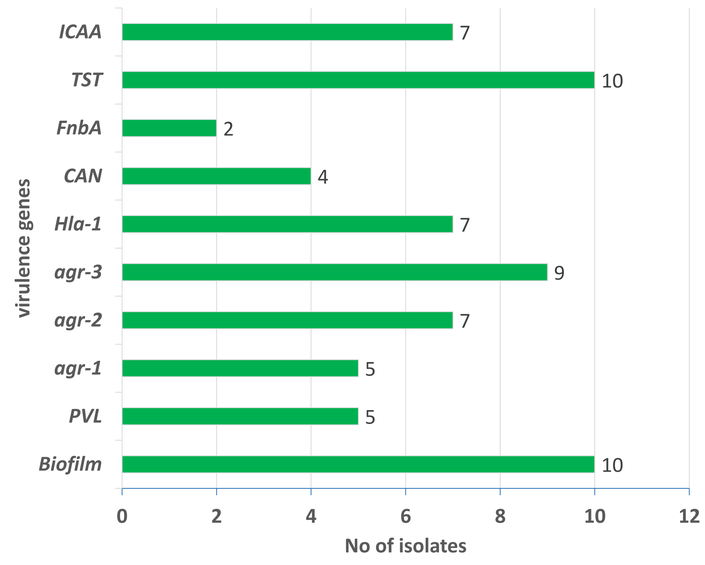
- Twenty isolates haboured only *the vanA* gene, four harboured only *vanB* and three haboured both *the vanA* and *vanB* genes. Resistance gene analysis showed a predominance of macrolide resistance, with mph(ABC) (n = 18) and msr(A/B) (n = 16) being most frequent. β-lactam resistance genes (mecA and ampC) were commonly detected. Aminoglycoside and tetracycline resistance genes occurred at moderate frequencies, while quinolone resistance genes were less common. Overall, multiple resistance determinants were observed, indicating widespread multidrug resistance among the isolates. Virulence gene prevalence- biofilm genes (27.2%), tst (22.7%), agr-3 (20.5%), agr-2 (15.9%), hla-1 (15.9%), ICAA (15.9%), and PVL (11.4%). Among biofilm-positive isolates, agr-1, agr-2, and tst were most frequent (30% each), while agr-3 and CAN were absent.







Conclusion

This study indicates that hand-dug wells may serve as a potential source of VRSA in this environment. The identification of factors such as improper well construction, proximity to pollution sources, and habitation patterns that contribute to contamination underscores the necessity for regular sanitation and inspection of wells for cracks to prevent seepage, runoff, and potential outbreaks of waterborne diseases. It is essential to educate well owners and consumers on the importance of boiling untreated water before use. Regulatory agencies responsible for well construction and water quality must implement measures to ensure proper sitting, construction, and maintenance of wells to prevent contamination.

Referencess

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