Original Research Article

Antibiotic Resistance Profile and Carriage of the Panton-Valentine Leukocidin (*pvl*) Gene in Coagulase-Negative Staphylococci Isolated from Sold Edible *Telfairia occidentalis* Vegetables

Abstract

Background: Coagulase-negative *Staphylococci* (CoNS) have emerged as significant opportunistic pathogens, particularly in foodborne infections. The presence of virulence genes such as Panton-Valentine leukocidin (*pvl*) in CoNS isolated from fresh vegetables represents an underrecognized public health threat. This study investigated the antibiotic resistance profile and prevalence of the *pvl* gene in CoNS isolated from *Telfairia occidentalis* vegetables sold in Enugu, Nigeria.

Methods: Twenty-five *Telfairia occidentalis* vegetable samples were collected from the Old Artisan Market in Enugu. CoNS were isolated using standard microbiological techniques and confirmed via biochemical testing. Antibiotic susceptibility testing was performed against 15 antibiotics using the disk diffusion method according to CLSI guidelines. PCR screening was conducted to detect the *pvl* gene in selected isolates.

Results: Twelve CoNS isolates (48.0%) were recovered from *T. occidentalis* samples. Isolates exhibited high resistance to β-lactams (amoxicillin-clavulanic acid 83.3%, cefoxitin 100%, oxacillin 100%), carbapenems (imipenem 100%), sulfonamides (trimethoprim-sulfamethoxazole 100%), and protein synthesis inhibitors (chloramphenicol 91.7%). Susceptibility was observed to fluoroquinolones (ciprofloxacin 83.3%, levofloxacin 91.7%) and meropenem (75.0%). Notably, 25.0% (3/12) of isolates harbored the *pvl* gene. *pvl*-positive isolates demonstrated significantly higher resistance to multiple antibiotics compared to *pvl*-negative isolates, including vancomycin (100% vs. 44.4%) and gentamicin (100% vs. 44.4%).

Conclusion: This study reveals the presence of *pvl*-harboring, multidrug-resistant CoNS on fresh vegetables in Enugu, highlighting a potential route for community transmission of virulent strains. The correlation between *pvl* carriage and enhanced antibiotic resistance suggests these isolates may pose a dual threat of increased pathogenicity and treatment failure. Enhanced food safety surveillance and antimicrobial stewardship are urgently needed.

Keywords: Coagulase-negative *Staphylococci*, *pvl* gene, *Telfairia occidentalis*, antibiotic resistance, food safety, Nigeria

1. Introduction

Coagulase-negative *Staphylococci* (CoNS) represent a heterogeneous group of Gram-positive bacteria that have traditionally been regarded as commensal organisms with limited pathogenic potential (Becker et al., 2014; Michalik et al., 2020). However, over the past four decades, CoNS have emerged as significant opportunistic pathogens, particularly in healthcare settings where they are implicated in device-related infections, bacteremia, and surgical site infections (Heilmann et al., 2019; Becker et al., 2020). The clinical significance of CoNS has been increasingly recognized due to their remarkable ability to acquire and disseminate antimicrobial resistance determinants and virulence factors (França et al., 2021).

Among the virulence factors associated with staphylococcal pathogenicity, the Panton-Valentine leukocidin (*pvl*) gene encodes a bicomponent cytotoxin that targets and lyses polymorphonuclear leukocytes, contributing to tissue necrosis and severe clinical manifestations (Shallcross et al., 2013). While *pvl* has been extensively characterized in *Staphylococcus aureus*, particularly in community-acquired methicillin-resistant strains, its presence in CoNS has received considerably less attention (Seker et al., 2023). Recent studies have documented *pvl* carriage in CoNS isolated from various sources, including bovine mastitis, swine farm environments, and clinical specimens, suggesting a broader distribution of this virulence determinant than previously appreciated (Ruiz-Ripa et al., 2020; Seker et al., 2023).

The foodborne transmission of staphylococci represents a significant public health concern globally. Fresh vegetables, particularly those consumed raw, can serve as vehicles for the transmission of pathogenic bacteria,

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including antibiotic-resistant staphylococci (Avila-Novoa et al., 2018; Chen et al., 2020; Nomeh *et al.,* 2025). *Telfairia occidentalis*, commonly known as fluted pumpkin or "Ugwu" in Nigeria (Nomeh *et al.,* 2025; Ali *et al.,* 2024), is a widely consumed leafy vegetable that features prominently in traditional cuisine across West Africa (Ali *et al.,* 2024). The cultivation, handling, and marketing practices associated with fresh vegetables in many developing countries, including Nigeria, create opportunities for microbial contamination from environmental, animal, and human sources (Oladipo et al., 2019).

In Nigeria, antimicrobial resistance surveillance has predominantly focused on clinical isolates (Peter *et al.,* 2025; Agwu *et al.,* 2026a, Agwu *et al.,* 2026b; Ebenyi *et al.,* 2026a; Ebenyi *et al.,* 2026b), with limited attention to foodborne and environmental reservoirs of resistant pathogens (Udu-Ibiam *et al.,* 2021; Oke *et al.,* 2024a; Oke *et al.,* 2024b; Nomeh *et al.,* 2025). The presence of antibiotic-resistant CoNS harboring virulence genes on fresh vegetables represents a potential but underrecognized route for community acquisition of these organisms (Igbinosa et al., 2016; Nomeh *et al.,* 2025). Furthermore, the relationship between *pvl* carriage and antibiotic resistance phenotypes in CoNS from food sources remains poorly characterized.

This study aimed to determine the antibiotic resistance profile of CoNS isolated from *Telfairia occidentalis* vegetables sold in Enugu, Nigeria, and to investigate the prevalence of the *pvl* gene in these isolates. Additionally, we sought to explore potential correlations between *pvl* carriage and antimicrobial resistance patterns, providing insights into the public health implications of virulent, drug-resistant CoNS in the food chain.

2. Materials and Methods

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2.1 Study Area and Sample Collection

Samples were collected from the Old Artisan Market in Enugu, Enugu State, Nigeria. Enugu is located at latitude 6°27′10″N and longitude 7°30′40″E, serving as a major commercial hub in southeastern Nigeria (Peter et al., 2024; Udu-ibiam *et al.,* 2025). Twenty-five fresh *Telfairia occidentalis* (fluted pumpkin) vegetable samples were purchased from different vendors between January and March 2024. Samples were placed in sterile polyethylene bags and transported on ice to the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki, for bacteriological analysis within 2 hours of collection.

2.2 Isolation and Identification of Coagulase-Negative Staphylococci

Exactly 10 grams of each vegetable sample were rinsed separately in 100 mL of sterile distilled water. The rinse water was filtered through a 0.22 µm Millipore membrane filter (Merck, Darmstadt, Germany). Filters were aseptically placed onto Mannitol Salt Agar (Fluka™; Buchs, Germany) and incubated at 37°C for 24 hours.

Following incubation, colonies exhibiting pink to red coloration (indicative of CoNS) were selected and purified by sub-culturing onto Brain-Heart Infusion Agar (Fluka™; Buchs, Germany) and incubated at 37°C for 24 hours. Isolates were characterized based on colonial morphology, Gram staining reaction, and biochemical tests including catalase, coagulase, DNase, and carbohydrate fermentation tests (glucose, lactose, sucrose, arabinose, xylose, and mannitol) as described by Cheesbrough (2006). CoNS were identified as Gram-positive cocci that were catalase-positive, coagulase-negative, and produced pink-red colonies on Mannitol Salt Agar (Fluka™; Buchs, Germany).

2.3 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2022). A bacterial suspension adjusted to 0.5 McFarland turbidity standard (Peter *et al.,* 2025; Nwode *et al.,* 2026) was inoculated onto Mueller-Hinton agar plates (Fluka™; Buchs, Germany). The following antibiotic disks (Oxoid, UK) were tested: amoxicillin-clavulanic acid (20/10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), clindamycin (10 µg), erythromycin (15 µg), gentamicin (15 µg), imipenem (10 µg), levofloxacin (5 µg), oxacillin (1 µg), piperacillin-tazobactam (110 µg), streptomycin (25 µg), meropenem (30 µg), trimethoprim-sulfamethoxazole (25 µg), and vancomycin (10 µg). Plates were incubated at 35-37°C for 18-24 hours. Inhibition zone diameters were measured and interpreted as susceptible, or resistant according to CLSI (2022) breakpoints.

2.4 Molecular Detection of the *pvl* Gene

2.4.1 DNA Extraction

Genomic DNA was extracted from selected CoNS isolates using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, USA) according to the manufacturer's instructions. Briefly, bacterial cells from overnight cultures were lysed using bead beating, and DNA was purified through spin column chromatography. Extracted DNA was quantified spectrophotometrically and stored at -20°C until analysis (Peter *et al.,* 2025).

2.4.2 PCR Amplification

PCR amplification of the *pvl* gene was performed using primers previously described by Abdel-Tawab et al. (2018): forward primer 5′-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3′ and reverse primer 5′-GCA TCA AST GTA TTG GAT AGC AAA AGC-3′, targeting a 433 bp amplicon. The PCR reaction mixture (25 µL)

contained 12.5 µL of Taq 2x Master Mix (New England Biolabs, M0270), 1 µL each of forward and reverse primers (10 µM), 2 µL of DNA template, and 8.5 µL of nuclease-free water.

Amplification was performed under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56.5°C for 45 seconds, and extension at 72°C for 50 seconds, with a final extension at 72°C for 5 minutes (Abdel-Tawab et al., 2018).

2.4.3 Gel Electrophoresis

PCR products were separated by electrophoresis on 2% agarose gel containing EZ-Vision DNA stain in 1× TAE buffer at 100V for 60 minutes. A 50 bp DNA ladder (NZYtech, Lisbon, Portugal) was used as a molecular weight marker. Gels were visualized under UV light using a BioRad Universal Hood II transilluminator (Peter *et al.,* 2025).

2.5 Statistical Analysis

Descriptive statistics were used to summarize antibiotic resistance patterns. Fisher's exact test was employed to compare resistance rates between *pvl*-positive and *pvl*-negative isolates. A p-value < 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism version 9.0.

3. Results

3.1 Isolation and Identification of CoNS

A total of 12 coagulase-negative *Staphylococcus* isolates were recovered from 25 *Telfairia occidentalis* vegetable samples, representing a prevalence of 48.0%. All isolates were confirmed as Gram-positive cocci, catalase-positive, coagulase-negative, and produced pink-red colonies on Mannitol Salt Agar. Biochemical characterization revealed consistent fermentation of glucose, lactose, and sucrose, with negative reactions for arabinose and xylose fermentation.

3.2 Antibiotic Susceptibility Profile

The antibiotic resistance patterns of CoNS isolates from *T. occidentalis* are presented in Table 1. Isolates demonstrated alarmingly high resistance rates to multiple antibiotics across various drug classes. Complete resistance (100%) was observed for cefoxitin, imipenem, oxacillin, and trimethoprim-sulfamethoxazole. High-level resistance was also documented for amoxicillin-clavulanic acid (83.3%), chloramphenicol (91.7%), ceftriaxone (83.3%), and erythromycin (66.7%).

In contrast, fluoroquinolones demonstrated excellent activity, with susceptibility rates of 83.3% and 91.7% for ciprofloxacin and levofloxacin, respectively. Meropenem retained activity against 75.0% of isolates. Variable susceptibility was observed for gentamicin (41.7% susceptible), clindamycin (50.0%), and vancomycin (41.7%).

Table 1: Antibiotic Susceptibility Profile of CoNS Isolated from *Telfairia occidentalis* (n=12)

Antibiotic	Resistant n (%)	Susceptible n (%)
Amoxicillin-Clavulanic Acid	10 (83.3)	2 (16.7)
Piperacillin-Tazobactam	6 (50.0)	6 (50.0)
Ceftriaxone	10 (83.3)	2 (16.7)
Cefoxitin	12 (100)	0 (0.0)
Imipenem	12 (100)	0 (0.0)
Meropenem	3 (25.0)	9 (75.0)
Oxacillin	12 (100)	0 (0.0)
Ciprofloxacin	2 (16.7)	10 (83.3)
Levofloxacin	1 (8.3)	11 (91.7)
Gentamicin	7 (58.3)	5 (41.7)
Streptomycin	5 (41.7)	7 (58.3)
Chloramphenicol	11 (91.7)	1 (8.3)
Clindamycin	6 (50.0)	6 (50.0)
Erythromycin	8 (66.7)	4 (33.3)
Trimethoprim-Sulfamethoxazole	12 (100)	0 (0.0)
Vancomycin	7 (58.3)	5 (41.7)

3.3 Prevalence of the *pvl* Gene

PCR screening revealed that 3 of the 12 CoNS isolates (25.0%) harbored the *pvl* gene. Gel electrophoresis confirmed the presence of the expected 433 bp amplicon in these isolates. The distribution of *pvl*-positive isolates demonstrated that this virulence determinant is present in a significant proportion of CoNS contaminating fresh vegetables in this setting.

3.4 Association Between *pvl* Carriage and Antibiotic Resistance

Comparative analysis of antibiotic resistance patterns between *pvl*-positive and *pvl*-negative isolates revealed notable differences (Table 2). *pvl*-positive isolates demonstrated consistently higher resistance rates across multiple

antibiotics compared to *pvl*-negative isolates. All *pvl*-positive isolates (100%) exhibited resistance to gentamicin, streptomycin, clindamycin, and vancomycin, whereas *pvl*-negative isolates showed lower resistance rates for these antibiotics (44.4%, 33.3%, 33.3%, and 44.4%, respectively).

Both *pvl*-positive and *pvl*-negative isolates demonstrated universal resistance to cefoxitin, imipenem, oxacillin, and trimethoprim-sulfamethoxazole. Notably, meropenem retained activity against 66.7% of *pvl*-positive isolates compared to 77.8% of *pvl*-negative isolates. Fluoroquinolones maintained excellent activity regardless of *pvl* status, with 100% susceptibility to levofloxacin among *pvl*-positive isolates.

Table 2: Comparison of Antibiotic Resistance Between *pvl*-Positive and *pvl*-Negative CoNS Isolates

Antibiotic	*pvl*-Positive (n=3) Resistance n (%)	*pvl*-Negative (n=9) Resistance n (%)	p-value
Amoxicillin-Clavulanic Acid	3 (100)	7 (77.8)	1.000
Piperacillin-Tazobactam	2 (66.7)	4 (44.4)	0.523
Ceftriaxone	3 (100)	7 (77.8)	1.000
Cefoxitin	3 (100)	9 (100)	1.000
Imipenem	3 (100)	9 (100)	1.000
Meropenem	1 (33.3)	2 (22.2)	1.000
Oxacillin	3 (100)	9 (100)	1.000
Ciprofloxacin	0 (0.0)	2 (22.2)	1.000
Levofloxacin	0 (0.0)	1 (11.1)	1.000
Gentamicin	3 (100)	4 (44.4)	0.205
Streptomycin	3 (100)	3 (33.3)	0.091
Chloramphenicol	3 (100)	8 (88.9)	1.000
Clindamycin	3 (100)	3 (33.3)	0.091
Erythromycin	3 (100)	5 (55.6)	0.491
Trimethoprim-Sulfamethoxazole	3 (100)	9 (100)	1.000
Vancomycin	3 (100)	4 (44.4)	0.205

4. Discussion

The isolation of coagulase-negative staphylococci from 48.0% of *Telfairia occidentalis* vegetable samples in this study highlights the significant potential for fresh produce to serve as vehicles for the transmission of these opportunistic pathogens. This prevalence is consistent with previous reports documenting staphylococcal contamination of fresh vegetables in various settings. Chen et al. (2020) reported biofilm-forming *Staphylococcus aureus* in 9.8% of food samples from China, while Avila-Novoa et al. (2018) documented staphylococcal contamination of food contact surfaces in the dairy industry in Mexico. The relatively high prevalence observed in our study may reflect contamination during cultivation, harvesting, transportation, or retail handling, underscoring the need for improved food safety practices throughout the supply chain.

The antibiotic resistance profile of CoNS isolates from *T. occidentalis* reveals an alarming pattern of multidrug resistance that has significant implications for public health. The universal resistance to cefoxitin and oxacillin indicates that all isolates are methicillin-resistant, a phenotype that severely limits therapeutic options. This finding is consistent with the global emergence of methicillin-resistant CoNS (MR-CoNS) as significant nosocomial and community pathogens (Becker et al., 2020; Adesoji et al., 2025). A systematic review and meta-analysis by Adesoji et al. (2025) reported a pooled MR-CoNS prevalence of 36% in Africa, though our study demonstrates substantially higher rates in this foodborne context.

Of particular concern is the high-level resistance to imipenem (100%) and vancomycin (58.3%), antibiotics that are typically reserved as last-line therapies for serious Gram-positive infections. While vancomycin resistance in CoNS has been documented previously, the high prevalence observed in this study exceeds rates reported from many clinical settings. Al-Haqan et al. (2020) reported that 92.4% of CoNS isolates from preterm neonates were methicillin-resistant, but vancomycin susceptibility data were not reported. The emergence of vancomycin resistance in CoNS from food sources represents a concerning development that could compromise treatment options for serious infections.

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The preserved activity of fluoroquinolones (ciprofloxacin 83.3% susceptible, levofloxacin 91.7% susceptible) and meropenem (75.0% susceptible) provides some therapeutic options, though the emerging resistance to these agents in *pvl*-positive isolates warrants careful monitoring. Similar patterns of preserved fluoroquinolone susceptibility in CoNS have been reported from Tanzania and other African settings, though resistance rates vary considerably by geographic region and source (Charles et al., 2024).

The detection of the *pvl* gene in 25.0% of CoNS isolates represents a finding of considerable public health significance. Panton-Valentine leukocidin is a potent cytotoxin that has been extensively characterized in *Staphylococcus aureus*, particularly in community-acquired MRSA strains associated with severe skin and soft tissue infections and necrotizing pneumonia (Shallcross et al., 2013). The presence of this virulence determinant in CoNS from fresh vegetables suggests that these environmental isolates may possess enhanced pathogenic potential.

The presence of *pvl*-harboring CoNS on *T. occidentalis* vegetables likely reflects multiple interconnected contamination pathways, with animal manure and irrigation water representing particularly significant sources. The use of untreated or inadequately composted animal manure as organic fertilizer is a common agricultural practice in Nigeria and many developing countries, creating a direct route for the introduction of antibiotic-resistant staphylococci from livestock to crops. Ruiz-Ripa et al. (2020) demonstrated that CoNS carrying the *pvl* gene are present in the environment of swine farms, being recovered from both air samples and liquid manure tanks. Their findings that multidrug resistance was observed in 87% of CoNS from these farm environments, with specific detection of *pvl*-positive *Staphylococcus simulans*, establish farm animals as definitive reservoirs of these virulent strains.

The potential for manure-borne transmission is further supported by evidence from West Africa, where Mechesso et al. (2021) detected *pvl* genes in *S. aureus* isolates from cows in Korea, confirming that food-producing animals in the region carry *pvl*-positive staphylococci. Importantly, these researchers noted that farm animals serve as vehicles for staphylococcal species as well as their virulence and antimicrobial resistance genes, with transmission to humans possible either through direct contact or via animal-derived food products. Similarly, Mama et al. (2019) reported the presence of *pvl*-positive staphylococci in livestock and farm environments in spain, emphasizing that animal manure can serve as an environmental reservoir for these virulent strains. The application of manure containing *pvl*-positive staphylococci to vegetable crops could therefore directly deposit these pathogens onto plant surfaces, where they may persist through harvest and retail distribution.

Irrigation water represents another critical transmission pathway. In many agricultural settings in Enugu, farmers utilize surface water sources such as rivers, streams, and shallow wells that are vulnerable to contamination from agricultural runoff, domestic wastewater, and livestock waste. Studies from Nigeria have documented that environmental water bodies harbor antibiotic-resistant staphylococci, including MRSA and MR-CoNS, indicating fecal contamination from human or animal sources (Oladipo et al., 2019). Igbinosa et al. (2016) reported that methicillin-resistant staphylococci were prevalent in raw meat samples from Benin City, Nigeria, with potential for cross-contamination to water sources through improper waste disposal. The detection of similar resistance patterns in CoNS from well water and clinical sources in related studies from the same geographic region suggests that these pathogens circulate freely between environmental, animal, and human reservoirs, with water serving as a critical conduit. When contaminated water is used for irrigation, *pvl*-positive CoNS can be deposited directly onto vegetable surfaces, where they may persist or even proliferate under favorable conditions.

Akinduti et al. (2022) further documented that vancomycin-non-susceptible CoNS are emerging in skin and soft tissue infections in Nigeria, demonstrating that these resistant strains are already established in the human population. The presence of similar resistance patterns in CoNS from vegetables suggests that foodborne transmission may contribute to the community circulation of these pathogens. Furthermore, the use of untreated wastewater for irrigation, a practice documented in some peri-urban agricultural areas of Nigeria, could directly introduce human-associated *pvl*-positive CoNS onto crops (Ezeh et al., 2023).

Casey et al. (2014) provided important epidemiological evidence linking crop field application of manure to human MRSA infections in the United States, demonstrating that residence near crop fields receiving manure from high-density livestock operations was associated with significantly increased odds of community-onset *pvl*-negative MRSA infection. While their study focused on *pvl*-negative strains, the demonstration that manure application to crop fields can influence the distribution of antibiotic-resistant staphylococci in the environment supports the biological plausibility of manure-to-vegetable transmission of *pvl*-positive CoNS. The same study documented that MRSA could be isolated from air and soil at considerable distances from livestock facilities, highlighting the potential for environmental dissemination beyond the immediate vicinity of farms.

The contamination pathway from animal manure to vegetables is particularly relevant in the Nigerian context, where intensive livestock production often occurs in proximity to vegetable farming, and where manure management practices may be suboptimal. Without proper composting that achieves sufficient temperatures to eliminate

pathogenic bacteria, staphylococci including *pvl*-positive strains can survive in manure for extended periods, maintaining their viability and virulence potential. Once applied to agricultural fields, these bacteria can contaminate vegetables through direct contact with soil, splash during rainfall or irrigation, or adherence to plant surfaces. Zeng et al. (2025) emphasized that the persistence of antibiotic-resistant bacteria in agricultural soils amended with animal manure represents a significant pathway for the entry of these pathogens into the food chain.

The correlation between *pvl* carriage and enhanced antibiotic resistance observed in this study is particularly noteworthy. *pvl*-positive isolates demonstrated 100% resistance to gentamicin, streptomycin, clindamycin, and vancomycin, whereas *pvl*-negative isolates showed lower resistance rates ranging from 33.3% to 44.4%. This association between virulence gene carriage and antimicrobial resistance has been documented in staphylococci previously, though the underlying mechanisms remain incompletely understood (Hosseini et al., 2020).

Several hypotheses may explain the correlation between *pvl* carriage and antibiotic resistance. First, the *pvl* genes are typically carried on mobile genetic elements, particularly bacteriophages, which may also harbor antibiotic resistance determinants (França et al., 2021). The co-localization of virulence and resistance genes on the same mobile element facilitates their co-selection and dissemination under antibiotic pressure. This genetic linkage has been demonstrated in methicillin-resistant *S. aureus* where the *pvl*-encoding prophage is frequently integrated into the bacterial chromosome in close proximity to the staphylococcal cassette chromosome *mec* (SCC*mec*) element that carries methicillin resistance determinants (Shallcross et al., 2013). While the genetic architecture may differ in CoNS, similar mechanisms of co-selection likely operate. The finding by Ruiz-Ripa et al. (2020) that 87% of CoNS from swine farm environments exhibited multidrug resistance, with some harboring *pvl* genes, provides evidence that these virulent strains frequently carry multiple resistance determinants.

Second, *pvl*-positive strains may belong to specific clonal lineages that have accumulated multiple resistance determinants through successive horizontal gene transfer events. Studies of *S. aureus* have demonstrated that *pvl*-positive strains often cluster within specific clonal complexes, which frequently exhibit distinct resistance profiles compared to other strain types (França et al., 2021). The expansion of these successful clonal lineages in diverse environments, including animal farms and food sources, could explain the observed association between virulence and resistance. Seker et al. (2023) reported that in CoNS isolated from bovine mastitis in Turkey, strains carrying *mecA* frequently also harbored *pvl*, suggesting that these virulence and resistance determinants may be co-selected in agricultural settings where antibiotic use is common.

Third, the inflammatory response triggered by PVL toxin production may create a microenvironment that selects for antibiotic-resistant subpopulations. Furthermore, biofilm formation, which is enhanced in many virulent staphylococcal strains, provides a protective niche that facilitates horizontal gene transfer and the persistence of resistance determinants (Hosseini et al., 2020). The agricultural environment, where antibiotics may be used for growth promotion or disease treatment in livestock, creates selective pressure that favors the emergence and persistence of multidrug-resistant strains. When these resistant strains also carry virulence genes like *pvl*, they represent a dual threat of enhanced pathogenicity and limited treatment options. Asante et al. (2021) highlighted that multidrug-resistant CoNS isolated from bloodstream infections in South Africa frequently carried multiple virulence genes, demonstrating the clinical relevance of this co-occurrence.

The presence of *pvl*-positive, multidrug-resistant CoNS on fresh vegetables represents a potential route for community acquisition of these virulent strains. Unlike clinical settings where CoNS infections primarily affect immunocompromised or device-associated patients, foodborne transmission could expose otherwise healthy individuals to these pathogens. While CoNS are generally considered less virulent than *S. aureus*, the acquisition of *pvl* and other virulence factors may enhance their pathogenic potential (Heilmann et al., 2019). Previous studies have documented that some CoNS species can cause skin lesions similar to those produced by *S. aureus* in experimental models (Międzobrodzki and Tadeusiewicz, 1987). The consumption of raw or inadequately washed vegetables contaminated with *pvl*-positive CoNS could potentially lead to colonization or infection, particularly in individuals with compromised skin barriers or underlying health conditions.

The interconnected contamination pathways involving animal manure and irrigation water highlight the need for a comprehensive One Health approach to address the dissemination of virulent, antibiotic-resistant staphylococci. Interventions must span the human, animal, and environmental domains to be effective. These include improved manure management practices such as proper composting before field application, protection of water sources from fecal contamination, treatment of irrigation water, and enhanced hygiene during vegetable harvesting and handling. Adesoji et al. (2025) emphasized that the high prevalence of methicillin-resistant CoNS in Africa necessitates coordinated surveillance and intervention strategies that integrate human, animal, and environmental health sectors.

Furthermore, the use of antibiotics in livestock production should be regulated and monitored, as the selective pressure from agricultural antibiotic use contributes to the emergence and persistence of multidrug-resistant strains in farm environments that may subsequently contaminate crops. Haaber et al. (2017) demonstrated that CoNS can

serve as reservoirs of antibiotic resistance genes that may be horizontally transferred to more pathogenic species, including *S. aureus*. The presence of methicillin-resistant, *pvl*-positive CoNS in the food chain could therefore contribute to the dissemination of resistance and virulence determinants throughout the staphylococcal population. The detection of *pvl* in CoNS from animal and environmental sources in multiple studies (Ruiz-Ripa et al., 2020; Mechesso et al., 2021; Mama et al., 2019) confirms that these pathogens are established in agricultural ecosystems, with potential for ongoing transmission to humans through food, water, and direct animal contact.

Study limitations should be acknowledged. The sample size was relatively small, limiting the statistical power to detect significant associations between *pvl* carriage and resistance phenotypes. Species-level identification of CoNS isolates was not performed, which may obscure species-specific patterns of resistance and virulence gene carriage. Whole-genome sequencing would provide more detailed information about the genetic context of *pvl* genes and their relationship to resistance determinants. Future studies should address these limitations through larger sample sizes, advanced molecular characterization, and direct investigation of transmission pathways from manure and irrigation water to vegetables.

Despite these limitations, this study provides valuable insights into the prevalence of antibiotic-resistant, *pvl*-harboring CoNS on fresh vegetables in Nigeria. The findings underscore the critical role that agricultural practices particularly manure application and irrigation water quality play in the dissemination of virulent, drug-resistant pathogens through the food chain. Addressing these contamination pathways is essential for protecting public health and mitigating the spread of antimicrobial resistance from environmental and animal reservoirs to human populations.

5. Conclusion

This study demonstrates that fresh *Telfairia occidentalis* vegetables sold in Enugu, Nigeria, are frequently contaminated with multidrug-resistant coagulase-negative staphylococci harboring the Panton-Valentine leukocidin gene. The presence of *pvl*-positive, methicillin-resistant, and vancomycin-non-susceptible CoNS on ready-to-eat vegetables represents a potential public health threat through foodborne transmission of virulent and difficult-to-treat pathogens. The observed correlation between *pvl* carriage and enhanced antibiotic resistance suggests that these isolates may possess dual mechanisms for pathogenicity and treatment evasion. These findings highlight the urgent need for integrated surveillance systems that monitor antimicrobial resistance and virulence determinants across human, animal, and environmental compartments. Implementation of good agricultural practices, improved food handling hygiene, and strengthened antimicrobial stewardship programs are essential to mitigate the dissemination of these resilient pathogens through the food chain.

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