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Phenotypic Virulence Characteristics, Virulence Gene Profiles and ERIC-PCR Diversity of Urinary Enterococcus faecalis Isolates from Pregnant Women in the Federal Capital Territory, Nigeria

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Abstract

Background

Enterococcus faecalis is an adaptable opportunistic uropathogen whose adhesion, biofilm formation, extracellular enzyme production and cytolysin-associated traits may promote urinary persistence. The pathogen carries important risks for both mothers and unborn children. However, molecular data describing virulence-gene carriage and strain diversity among urinary isolates from pregnant women in Nigeria remain limited.

Methods

A cross-sectional study was conducted from October 2025 to January 2026 among pregnant women attending antenatal clinics at selected hospitals across the six Area Councils of the Federal Capital Territory, Nigeria. Clean-catch midstream urine was cultured using standard methods. Twenty-seven presumptive *E. faecalis* isolates were evaluated for Congo red agar biofilm formation, gelatinase, protease and haemolytic activities. Ten selected isolates were confirmed by PCR targeting the species-specific *ddlE* gene, quantified for biofilm biomass by crystal-violet microtitre assay, screened for *esp, asa1, cylA, hyl and ace*, and fingerprinted by ERIC-PCR. Phenotypic associations were assessed using phi coefficients and Fisher's exact test with Holm adjustment. Spearman correlation assessed the relationship between virulence gene burden and biofilm biomass.

Results

Among 27 isolates, 17 (63.0%) were Congo red agar biofilm positive, 22 (81.5%) produced gelatinase, 19 (70.4%) produced protease and 14 (51.9%) exhibited beta-haemolysis. Gelatinase and protease were strongly associated ($\varphi = 0.735$; Fisher's exact $p = 0.0007$; Holm-adjusted $p = 0.0042$); other pairwise associations were not significant after correction. All ten selected isolates yielded the expected 941-bp *ddlE* amplicon. By microtitre assay, nine of ten isolates produced biofilm: five strong, three moderate and one weak producer. *esp* and *asa1* were each detected in five isolates, *cylA* in four, and *hyl* and *ace* in one isolate each. Virulence gene burden showed a moderate but non-significant positive correlation with OD_{570} (Spearman $\rho = 0.547$, $p = 0.102$). ERIC-PCR separated the ten isolates into four clusters and nine fingerprint variants.

Conclusion

Urinary *E. faecalis* isolates from pregnant women in the study setting displayed frequent biofilm and extracellular enzyme phenotypes, heterogeneous virulence gene profiles and substantial ERIC-PCR

diversity. These findings indicate multiple circulating strain backgrounds with variable pathogenic potential and support larger, prospectively designed molecular surveillance studies.

1. Introduction

Urinary tract infection during pregnancy is clinically important because physiological urinary stasis, ureteric dilatation and altered bladder dynamics can facilitate bacterial persistence and ascending infection [1]. Although Gram-negative organisms predominate in many settings, enterococci are increasingly recognised among urinary pathogens [2]. *Enterococcus faecalis* is particularly relevant because it combines environmental hardiness with the ability to adhere to host tissues, form biofilms and express extracellular products that may damage tissue or modify host defence [3, 4]. During vaginal delivery, newborns can also acquire *E. faecalis* from maternal tract, and the bacteria can occasionally cause serious neonatal urogenital infections, bacteremia, pneumonia, or sepsis [5]. These characteristics make the bacteria an important study among vulnerable pregnant women.

The pathogenicity of *E. faecalis* arises from the combined action of several virulence determinants. Enterococcal surface protein, encoded by the *esp* gene, facilitates initial surface attachment and contributes to biofilm maturation and persistence in *Enterococcus faecalis* [6, 7]. Aggregation substances, encoded by *asa1*, promotes cell-to-cell contact, adhesion and conjugative transfer [8]. The collagen-binding adhesin Ace can support interaction with extracellular matrix, while hyaluronidase-associated determinants may facilitate tissue spread. Cytolysin-associated genes, including *cylA*, contribute to maturation and activation of the cytolysin system and may be associated with haemolytic activity [9]. In addition, gelatinase and other proteolytic activities can degrade host substrates and modify biofilm architecture [10].

Characterizing the phenotypic virulence and specific genes profile of *E. faecalis* is crucial to the diagnosis and treatment of the bacterial infection. While several methods exist in diagnosing the bacteria, phenotypic detection such as agar-based and microtitre assays demonstrate observable activity under defined laboratory conditions, whereas conventional PCR demonstrates carriage of selected genetic determinants without establishing transcription, protein production or disease severity. Examining both levels is therefore useful, particularly because genotype-phenotype discordance may arise through regulatory variation, incomplete operons, growth conditions or unmeasured virulence determinants [11, 12].

Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) provides a more tailored approach for *E. faecalis* strain typing and adds an epidemiological dimension to the diagnosis. The ERIC-PCR generates banding profiles from repetitive genomic regions and can provide a rapid, relatively inexpensive estimate of relatedness where sequencing is not routinely available [13]. Distinct fingerprints suggest heterogeneous strain backgrounds, whereas highly similar profiles may indicate related isolates [14].

Despite the advances and several research on *E. faecalis* characterization in clinical settings, there is limited integrated phenotypic and molecular evidence on urinary *E. faecalis* from pregnant women in

Nigeria. Nevertheless, some Nigerian studies have identified virulence-associated traits, biofilm formation, and multidrug resistance among enterococci recovered from clinical, animal, food, and environmental sources [15.16.17].

Comparable findings have been reported elsewhere in Africa. In Egypt, *E. faecalis* isolates carried multiple virulence genes, including *gelE*, *ace*, *cylA*, *asa1*, *esp*, and *hyl*, with greater virulence-gene carriage reported among isolates of human origin than among food isolates [18]. Genomic analysis of South African *E. faecalis* isolates also identified genes associated with adhesion, biofilm formation, oxidative-stress survival, and tissue invasion [19]. These observations support the circulation of genetically diverse *E. faecalis* strains with multiple colonisation and persistence determinants across African settings.

To expand the surveillance of *E. faecalis* beyond food and environmental sources, we carried out phenotypic and molecular characterization of *E. faecalis* from urine samples of selected pregnant women attending selected hospitals in Abuja, Nigeria. We have recently reviewed pathogenic mechanisms, diagnosis, treatment and therapeutic challenges, and alternative strategies for *E. faecalis*-induced UTIs during pregnancy [20]. In this study, we experimentally determined the *in vitro* biofilm, gelatinase, protease and haemolytic activities of *E. faecalis* isolates and our results showed phenotypic variation in the bacterial strains detected. We also confirmed the virulence-associated genes in the isolates and their genetic diversity were confirmed using ERIC-PCR fingerprinting. Our work identifies virulent genes from *E. faecalis* among the pregnant women cohort and provides a need for follow up studies to characterize the bacteria in a larger population as part of a national surveillance study.

2. Materials and Methods

2.1 Study design, setting and participants

This cross-sectional study was conducted from October 2025 to January 2026 at selected general hospitals in Abaji, Kubwa, Kuje, Kwali, University of Abuja Teaching Hospital and Nyanya, serving the six Area Councils of the Federal Capital Territory, Nigeria. The target population comprised pregnant women aged 18-50 years attending antenatal clinics. Eligible participants provided informed consent and confirmed they had not used antibiotics two weeks before the sample collection. Clean-catch midstream urine was collected consecutively from consenting participants. Of 600 specimens collected, 578 were available for laboratory analysis after 22 specimens were lost through spillage during transport or bench handling.

2.2 Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of the Federal Capital Territory (FHREC/2024/01/401/31-12-24). Written informed consent was obtained before enrolment, and

participant information was coded and handled confidentially. The study was conducted in accordance with applicable institutional and national ethical requirements.

2.3 Urine culture and presumptive identification

Approximately 10 mL of clean-catch midstream urine was collected into sterile leak-proof containers and transported to study laboratory. Specimens were inoculated onto cystine-lactose-electrolyte-deficient agar and incubated aerobically at 37°C for 24 h. culture plates with significant bacteriuria (≥10^5 CFU/mL) were subcultured to obtain pure colonies. Presumptive enterococci were characterised by colony morphology, Gram staining, catalase reaction, bile-esculin hydrolysis and growth in 6.5% NaCl. Mannitol fermentation was used as differential medium to differentiate *E. faecalis* from *E. faecium* before molecular confirmation.

2.4 Phenotypic virulence assays

Ten isolates were selected based on phenotypic virulence characterisation across the six hospitals. Qualitative biofilm formation was assessed on Congo red agar prepared from brain-heart infusion medium supplemented with sucrose and Congo red. Plates were incubated at 37°C for 24-48 h. Black, dry, crystalline colonies were interpreted as positive, whereas red or pink colonies were interpreted as weak or negative [21].

Gelatinase activity was evaluated in nutrient gelatin medium. Following incubation, tubes were refrigerated for 15-20mins while persistent liquefaction was interpreted as gelatin hydrolysis. Protease activity was assessed on skim-milk agar. Overnight cultures standardised to 0.5 McFarland were spot-inoculated and incubated at 37°C for 24-48 h. A clear de-opacified zone around growth was recorded as positive casein hydrolysis.

Haemolysis was evaluated on blood agar supplemented with 5% defibrinated sheep blood. After incubation, reactions were classified as alpha, beta or gamma haemolysis using standard visual criteria. Complete clearing around colonies was recorded as beta-haemolysis and treated as the positive cytolysin-associated phenotype for analysis.

2.5 Quantitative microtitre-plate biofilm assay

The randomly ten selected isolates were grown in tryptic soy broth supplemented with 0.25% glucose at 37°C for 24 h. Inocula were adjusted to approximately 10^{-8}CFU/mL and 200 µL was dispensed into four replicate wells of a polystyrene 96-well plate. Broth-only wells served as negative controls. After static overnight incubation at 37°C, wells were washed three times with phosphate-buffered saline, fixed with absolute methanol for 15 min, stained with 1% crystal violet for 20 min, rinsed, air-dried and destained with 33% acetic acid. Absorbance was measured at 570 nm and the mean of four wells was calculated.

The cut-off optical density was defined as the mean negative-control OD plus three standard deviations: ODc = 0.050 + 3(0.010) = 0.080. Isolates were classified as non-producers (OD ≤ 0.080), weak (>0.080–0.160), moderate (>0.160–0.320) or strong producers (>0.320).

2.6 DNA extraction, quantification and species confirmation

Genomic DNA was prepared by heat lysis. Colonies from overnight culture were suspended, washed and heated at 95°C; clarified supernatants containing DNA were stored at -20°C. DNA concentration and purity were assessed using a NanoDrop 1000 spectrophotometer. Species confirmation targeted the *E. faecalis*-specific *ddlE* gene using forward primer 5'-ATCAAGTACAGTTAGTCT-3' and reverse primer 5'-ACGATTCAAAGCTAACTG-3', with an expected product of 941 bp. Reactions were performed in 25 µL using 2× DreamTaq master mix, primers and template DNA. Cycling comprised initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 60 s, 45°C for 45 s and 72°C for 50 s; and final extension at 72°C for 5 min. A no-template control was included.

2.7 Detection of virulence-associated genes

Conventional PCR screened the confirmed isolates for *esp, asa1, cylA, hyl* and *ace*. Amplification comprised initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 40 s, gene-specific annealing for 40 s and 72°C for 1 min; and final extension at 72°C for 10 min. Products of the expected size were interpreted as gene detection. PCR detection was not interpreted as evidence of gene expression or confirmed pathogenicity. The primers and the assay conditions are highlighted in Table 1.

Table 1. PCR targets and assay conditions.

Target	Primer sequence (5'–3')	Product (bp)	Annealing (°C)
ddlE	F: ATCAAGTACAGTTAGTCT R: ACGATTCAAAGCTAACTG	941	45
Esp	F: GATTTCATCTTTGATTCTTGG R: ATTTGATTCTTTAGCATCTGG	510	55
asa1	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375	58
cylA	F: ACTCGGGGATTGATAGGC R: GCTGCTAAAGCTGCGCTT	688	60
Hyl	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	276	57
Ace	5'-AAAGTAGAATTAGATCCACAC-3' 5'-TCTATCACATTCGGTTGCG-3'	616	56
ERIC	ERIC2: AAGTAAGTGACTGGGGTGAGCG ERIC1: ATGTAAGCTCCTGGGGATTCAC	50–2000	54

2.8 ERIC-PCR fingerprinting and electrophoresis

ERIC-PCR reactions contained 13.5 µL of 2× master mix, 2 µL of template DNA, 1 µL each of ERIC1 and ERIC2 primers, and nuclease-free water to 25 µL. Cycling comprised 94°C for 5 min; 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 2 min; and 72°C for 8 min. PCR products were resolved on 1% agarose in TBE buffer, stained with ethidium bromide, electrophoresed for approximately 1 h and visualised under ultraviolet illumination. Bands were scored manually as present (1) or absent (0), producing a binary matrix. The genetic relatedness of *Enterococcus faecalis* isolates was assessed from ERIC-PCR fingerprints using the Dice similarity coefficient. Cluster analysis was performed by the UPGMA algorithm in PAST software, and the resulting dendrogram was used to determine strain diversity and clustering patterns among the isolates.

2.9 Statistical analysis

Descriptive results were reported as counts and percentages. Wilson 95% confidence intervals were calculated for phenotypic positivity. Pairwise relationships among binary phenotypes were summarised using phi coefficients and tested using Fisher's exact test; Holm correction-controlled family-wise error across multiple comparisons. For the ten molecularly characterised isolates, virulence gene burden was defined as the number of detected genes among *esp, asa1, cylA, hyl* and *ace*. Spearman rank correlation assessed its relationship with OD_{570}. Two-sided $p < 0.05$ was considered statistically significant. Analyses were performed using IBM SPSS Statistics version 25 and Python 3.9.

3. Results

3.1 Isolate flow and molecular confirmation

We collected and analysed urine specimens of 578 pregnant women, out of which 27 presumptive *E. faecalis* isolates were recovered. We then evaluated the 27 isolates in the phenotypic virulence assays. Ten selected isolates out of the 27 based on the phenotypic virulence characterization underwent quantitative biofilm testing and molecular analysis using PCR. All ten produced the expected 941-bp *ddlE* amplicon, while the no-template control showed no amplification, confirming these selected isolates as *E. faecalis*.

3.2 Phenotypic virulence characteristics

We confirmed gelatinase as the most frequent phenotype, detected in 22 of 27 isolates (81.5%), followed by protease activity in 19 (70.4%), Congo red agar biofilm positivity in 17 (63.0%) and beta-haemolysis in 14 (51.9%) (Table 2). The haemolysis distribution was 29.6% alpha, 51.9% beta and 18.5% gamma. Gelatinase and protease showed a strong positive association ($\varphi = 0.735$; Fisher's exact $p = 0.0007$; Holm-adjusted $p = 0.0042$). No other pairwise phenotypic association remained significant after multiplicity correction ($|\varphi| \leq 0.335$; Holm-adjusted $p \geq 0.6006$).

Table 2. Phenotypic virulence characteristics of urinary *E. faecalis* isolates (n = 27).

Phenotype	Positive, n/N	Positive (%)	Wilson 95% CI
Biofilm on Congo red agar	17/27	63.0	44.2–78.5
Gelatinase	22/27	81.5	63.3–91.8
Protease	19/27	70.4	51.5–84.1
Beta-haemolysis	14/27	51.9	34.0–69.3

3.3 Quantitative biofilm formation

Quantify the biofilm formation of the isolates, we measured their absorbance at $OD_{570,}$ and the values ranged from 0.067 to 0.721. Using these values, we classified five isolates as strong producers, three as moderate producers, one as a weak producer, and one as a non-producer (Figure 1). Thus, nine of ten isolates (90.0%) produced measurable biofilm above the assay cut-off. We also noted that isolate 1D has the highest biomass ($OD_{570} = 0.721$), whereas isolate 1A was below the cut-off ($OD_{570} = 0.067$).

3.4 Virulence gene distribution

Then, we proceeded to conduct conventional PCR to screen the confirmed isolates for specific genes (Table 3). The *esp* and *asa1* genes were each detected in five of ten isolates (50.0%), *cylA* in four (40.0%), and *hyl* and *ace* in one isolate each (10.0%). Nine isolates carried at least one screened gene, while isolate 4B carried none. AD carried *esp, asa1* and *cylA*; 1D carried *esp, asa1 and hyl*. Isolate 6C carried *esp and cylA* genes.

Table 3. Frequency of virulence-associated genes among molecularly confirmed isolates (n = 10).

Gene	Functional association	Positive, n/N	Positive (%)
esp	Surface adhesion and biofilm-associated persistence	5/10	50.0
asa1	Aggregation, adhesion and conjugative transfer	5/10	50.0
cylA	Cytolysin maturation/activation; haemolytic potential	4/10	40.0
hyl	Hyaluronidase-associated tissue dissemination	1/10	10.0
ace	Collagen-binding adhesion	1/10	10.0

3.5 ERIC-PCR diversity

Then ERIC-PCR we used generated heterogeneous banding profiles. The ten isolates were assigned to four clusters comprising nine polymorphic variants. Cluster I contained isolates 6C, 2A and AD; Cluster II contained 5E and 1D; Cluster III contained 3C, 4B and 3B; and Cluster IV contained 2C and 1A (Table 4). Three isolates in Cluster I had distinct variants, while Clusters II and IV each contained two variants. Cluster III contained three isolates represented by two variants. The nine variants among ten isolates indicate marked fingerprint diversity and do not support a single dominant clonal pattern.

Table 4. Biofilm biomass, virulence-gene profiles and ERIC-PCR clusters of molecularly confirmed isolates.

Isolate	Detected genes	Gene burden	ERIC cluster
6C	*esp, cylA*	2	I
2A	*asa1*	1	I
AD	*esp, asa1, cylA*	3	I
5E	*esp, asa1*	2	II
1D	*esp, asa1, hyl*	3	II
3C	*asa1, ace*	2	III
4B	None detected	0	III
3B	*cylA*	1	III
2C	*cylA*	1	IV
1A	*esp*	1	IV

Virulence gene burden showed a moderate positive correlation with biofilm biomass, but the relationship was not statistically significant (Spearman ρ = 0.547, p = 0.102).

4. Discussion

In this study, we integrated phenotypic virulence assays, species-specific confirmation, virulence gene detection, quantitative biofilm measurement and ERIC-PCR fingerprinting of urinary *E. faecalis* recovered from selected pregnant women in the Federal Capital Territory, Nigeria. Our key findings were the phenotypic characterization by frequent gelatinase, protease and biofilm confirmation, detection of adhesion, aggregation, and cytolysin-associated genes, and substantial fingerprint heterogeneity among the PCR analysed isolates.

Gelatinase and protease were the most common phenotypic activities and were strongly associated with correction for multiple testing. This is clinically important because *E. faecalis* extracellular proteases, including GelE, hydrolyse gelatin and other protein substrates and can influence tissue interactions and biofilm structure [22]. We also found out that Congo red agar identified 63.0% of the 27 isolates as biofilm positive, whereas the microtitre assay classified nine of ten selected isolates as producers. Congo red agar primarily detects colony-associated exopolysaccharide characteristics, while the crystal-violet method measures total attached biomass on polystyrene. Our microtitre result is related to the high overall biofilm prevalence reported among clinical *E. faecalis* in India, although the distribution of strong, moderate and weak producers varies across studies [23]. Khalil *et al*. [24] likewise reported widespread moderate-to-high biofilm production among urinary enterococci in Saudi Arabia. By contrast, a study conducted in Lagos, Nigeria reported a lower overall frequency among mixed enterococcal species and clinical sources, which might be the influence of isolate source, species composition and assay protocol [25].

The β-haemolysis prevalence we observed in this study (51.9%) exceeded the 35.0% reported among urinary *Enterococcus faecalis* isolates in Türkiye [26]. This variation may reflect differences in strain distribution and laboratory methods because erythrocyte source, medium composition, and assay format influence haemolysis detection [27]. In Iraq, *cylLL* was detected in 92.0% of urinary *E. faecalis* isolates, demonstrating that cytolysin-gene carriage may exceed phenotypic expression under routine culture conditions [28]. Cytolysin is a two-component, pore-forming toxin with haemolytic and bacteriocin activities that contribute to host-cell injury and bacterial competition [29, 30]. After Holm correction, β-haemolysis was not significantly associated with resistance to individual antibiotics or the multiple antibiotic resistance index, consistent with findings from Türkiye [26]. However, our limited sample size may have reduced the statistical power to detect small or moderate associations, suggesting a need for larger sample size

We also carried out PCR assays as part of the gold standard techniques used in infection diseases surveillance. Five virulence-associated genes, *esp*, *asa1*, *cylA*, *hyl*, and *ace*, were also detected among the 10 *Enterococcus faecalis* isolates. The most frequent genes we found were *esp* and *asa1*, each found in 50.0% of the selected isolates. Both genes can contribute to adhesion and biofilm architecture, while aggregation substances promote bacterial aggregation, host-cell interaction and plasmid transfer [4]. Previous studies have similarly associated *esp* with moderate-to-strong biofilm formation among clinical *E. faecalis* isolates [7, 10, 31].The presence of the two genes in isolates AD, 5E and 1D therefore suggests a genetic repertoire potentially favourable to colonisation and persistence. Cytolysin-associated gene *cylA* encodes an extracellular serine protease required for maturation and activation of the cytolysin peptides and the cytolysin system may contribute to host-cell injury, bacterial competition, and increased virulence [31]. Our study detected *cylA* in 40% of the isolates, suggesting that those isolates possess cytolysin-associated genetic potential in addition to adhesion-related determinants [7]. However, detection of *cylA* alone does not confirm the presence of an intact cytolysin operon, gene expression, toxin production, or haemolytic activity. We suggest that further experiment would require to assess additional *cyl* genes, transcriptional analysis, or a functional haemolysis assay in al lager study set.

Detection of *hyl* and *ace* in only one isolate each suggests that these targets were uncommon in the screened subset. Although the the low frequencies may be because of the low numbers of samples we analysed, other studies have reported variable frequencies of these genes across clinical sources and regions, underlining the influence of clonal population structure and assay design [7, 18, 32]. The *hyl* gene is associated with hyaluronidase production and may facilitate tissue invasion through the degradation of extracellular matrix components [33], while the *ace* gene encodes the adhesin to collagen of *E. faecalis*, which mediates attachment to collagen-rich host tissues and may support colonisation [34]. The low occurrence of *hyl* gene in our study presents consistency with the 1.92% prevalence reported by Mubarak *et al*. [18] but differed from findings from Olanrewaju *et al*. [19], where genomic analysis identified hyaluronidase-associated genes in several *E. faecalis* strains. Likewise, occurrence of *ace* gene in the our study is lower than the 30.95% reported by Ghazvinian *et al.* [32] and the 51.92% reported by Mubarak *et al*. [18]. Differences among studies may reflect variation in isolate source,

geographical distribution, circulating lineages, sample size, clinical populations, and detection method. On the detection, whole-genome sequencing can be employed to identify a broader range of virulence-associated sequences than targeted polymerase chain reaction assays.

We also used ERIC-PCR to assess the genetic diversity and relatedness of the ten randomly selected confirmed *Enterococcus faecalis* isolates. This fingerprinting method amplifies genomic regions between enterobacterial repetitive intergenic consensus sequences, generating banding patterns that permit strain differentiation. Similar fingerprints results indicated greater genetic relatedness, whereas distinct patterns indicate genetic heterogeneity [35, 36, 37]. For example, Hassoun and Mohammed [35] differentiated 20 clinical *E. faecalis* isolates into five clusters and 13 polymorphic variants, demonstrating the discriminatory capacity of ERIC-PCR. In our study, we classified the 10 isolates into nine ERIC-PCR variants distributed across four major clusters, indicating substantial genetic heterogeneity. The predominance of unique fingerprint patterns suggests that the isolates represented multiple genotypes rather than the clonal dissemination of a single strain. This finding is consistent with reports of extensive ERIC-PCR diversity among clinical *E. faecalis* isolates [31, 35, 37]. A recent Iranian study similarly identified 35 ERIC types among 42 clinical isolates, further demonstrating considerable genetic diversity within clinical *E. faecalis* populations [38].

Another critical inference from our study is the support for other finding on the prevalence and genomic evidence of *E. faecalis* in Nigeria. Different studies in Nigeria have reported the detection of *E. faecalis* in different sample sources ranging from food to the environment [15, 16, 17]. For example, Salami *et al*. [17] detected *gelE* in 39.1% of multidrug-resistant enterococcal isolates from different sources across Nigeria. Although the genes investigated differed from those examined in our study, the findings collectively indicate that Nigerian enterococcal populations may harbour clinically relevant virulence determinants. Also, Osunla *et al*. [16] used whole-genome sequencing to identify sequence types ST477 and ST16 of *E. faecalis* among retail meat isolates from Akungba-Akoko and demonstrated distinct phylogenomic lineages. Although these findings support the circulation of multiple *E. faecalis* lineages in Nigeria the limited availability of recent Nigerian ERIC-PCR studies involving clinical urinary isolates restricts direct comparison especially among pregnant women.

5. Conclusion

Urinary *E. faecalis* isolates recovered from pregnant women in the Federal Capital Territory exhibited frequent gelatinase, protease, biofilm and beta-haemolytic phenotypes. Among ten molecularly confirmed isolates, *esp* and *asa1* were the most frequent screened genes. Most isolates produced measurable biofilm, and ERIC-PCR demonstrated marked fingerprint diversity. The data indicates heterogeneous *E. faecalis* populations with variable virulence-associated characteristics. The detection of the virulent genes therefore establishes the presence of virulence-associated determinants but does not independently demonstrate their expression or contribution to clinical severity. Thus, larger studies should be carried out to include molecular characterisation for all recovered isolates, incorporate gene-

expression assays and sequence-based typing, and link microbiological and phenotypical findings with prospectively defined clinical outcomes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was received for this study from the Federal Capital Territory Health Research Ethics Committee with approval number FHREC/2024/01/401/31-12-24. All authors declare that ‘written informed consent was obtained from the participants of this study. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

No competing interests in this manuscript

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Authors' contributions

J.T - Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing -original draft. B.P - Investigation. A.J.B. - Formal analysis, Writing - review & editing. A.P. - Writing - review & editing. B.A - Writing - review & editing. J.A. - Supervision, Writing - review & editing. All authors read and approved of the final manuscript.

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Figures

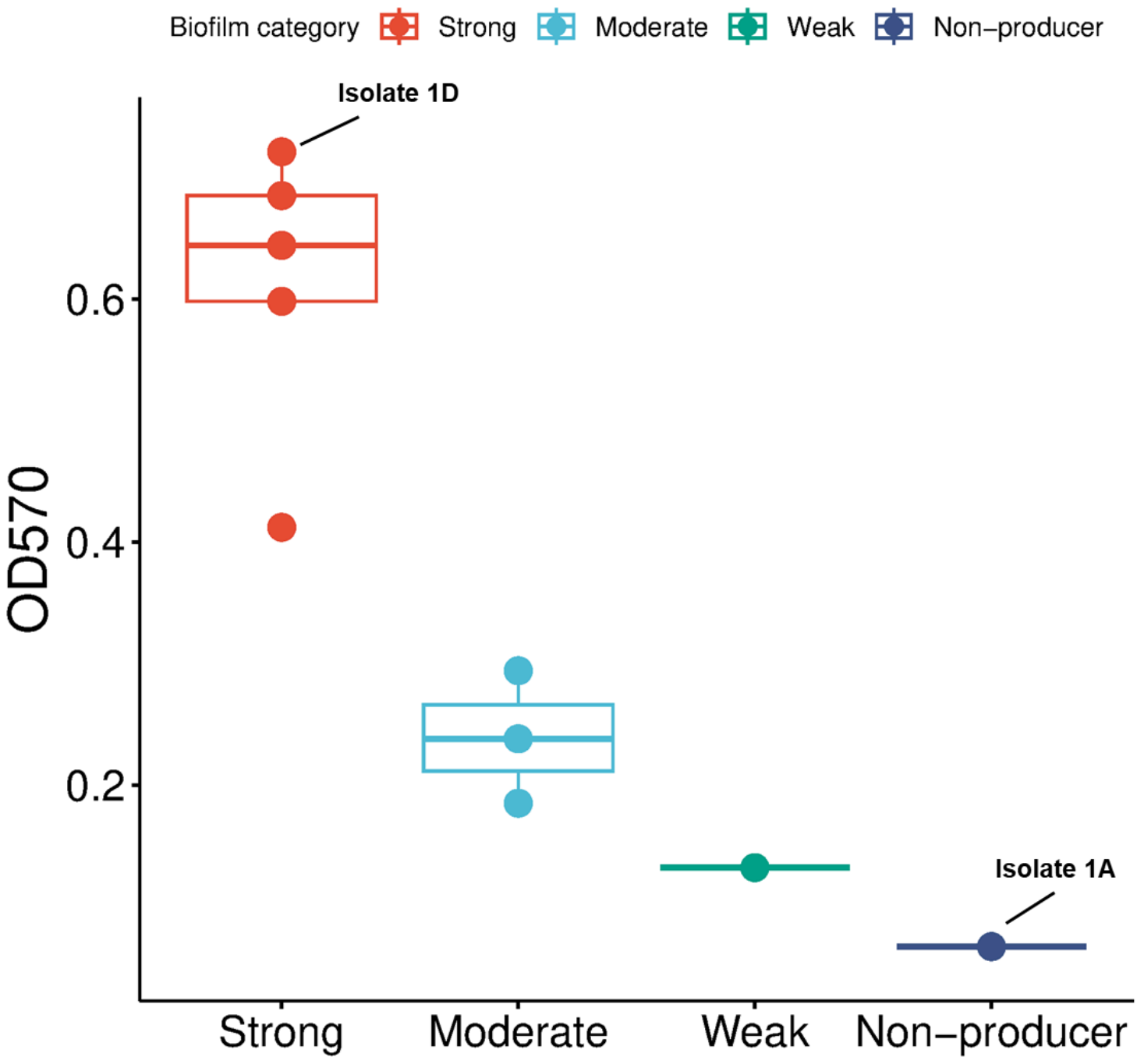


Figure 1

Biofilm classification of the *E. faecalis* isolates according to their biomass.